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Original Article

Detection of *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* in Cerebrospinal Fluid by Multiplex PCR for Diagnosis of Acute Bacterial Meningitis in Paediatric Population

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Abstract

The present study was done to evaluate a multiplex PCR based method for simultaneous detection of *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae* in CSF. A cross sectional study was carried out with 140 children (2 months to 12 years of age) with clinical suspicion of acute meningitis during July 2010 to June 2011. Three species-specific primers were used along with universal primers of bacterial gene 16S rRNA, in a two-stage PCR assay for diagnosis of acute bacterial meningitis.Among 140 patients, 42 (30%) cases were diagnosed as bacterial meningitis and other 98 (70%) as viral meningitis by clinical and cyto-biochemical criteria. Out of 42 bacterial meningitis cases, 9 (21.43%) were positive by Gram stain.These 9 cases were also positive by bacterial culture and PCR. Again, 15 (35.71%) were positive by bacterial culture which were also PCR positive. In 27 cases (out of 42), the etiologic diagnosis was not possible using routine bacteriological methods; in 11 of these patients, the etiologic agents were identified by PCR. In addition, PCR recognized 5 more cases whose etiologic diagnosis was not possible, as they were identified by universal primer of 16S rRNA. Hence, among 31 (73.81%) PCR positive cases, 12 (38.71%) were *S. pneumoniae*, 10 (32.26%) were *H. influenzae*, 4 (12.9%) were *N. meningitidis* and 5 (16.13%) were other bacteria.Among the antibiotic users, bacterial meningitis case detection by PCR was higher (65.52%) than that of culture (10.34%) and Gram staining (6.90%). The overall sensitivity and specificity of PCR assay was 100% and 66% respectively when bacterial culture was considered as gold standard. PCR can be used as a valuable supplementary diagnostic technique in routine clinical practice for diagnosis of acute bacterial meningitis in hospital setting.

Key words: Polymerase chain reaction (PCR), bacterial meningitis, cerebrospinal fluid (CSF)

Introduction:

Bacterial meningitis is a common disease worldwide, with high morbidity and mortality. In Bangladesh bacterial meningitis constitutes 24% cases among all age groups. *N. meningitidis, S. pneumoniaeand H. influenzae* type b (Hib) are responsible for 18%, 3% and 3% of cases respectively and the case-fatalityratios are 10% for *N. meningitidis,* 22% for *S. pneumoniae*, and 24% for *H. influenzae* type b (Hib) in Bangladesh.[1] In Bangladesh, laboratory diagnosis of bacterial meningitis is mainly made on the basis of cyto-biochemical analysis, Gram staining and culture of CSF, but their sensitivity is low. Moreover, culture requires 24 to 48 hours or longer to get the result if the number of viable organisms in the CSF is low. Multiplex PCR is a rapid, sensitive and specific method than bacteriological methods which can detect DNA of bacteria within few hours. The present study was carried out to identify the DNA of *N. meningitidis*, *S. pneumoniae* and *H. influenzae* from CSFby multipex PCR and to compare the result of PCR with bacteriological methods.

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Materials And Methods:

This Cross-sectional study was carried out in the department of Microbiology, Dhaka Medical College (DMC), Bangladesh from July 2010 to June 2011. Sampling technique was purposive and the study population was clinicallysuspected cases of meningitis in the age group of 2 months to 12 years (irrespective of sex). The targeted sample size was 140 and the patients were selected from the paediatric in patient departments of Dhaka Medical College Hospital (DMCH) and Dhaka Shishu Hospital (DSH). The clinical data and detail information were collected from each respondent by use of interview schedule, measured parameters and investigations and recorded systematically in a pre-designed data sheet.

All the meningitis cases were diagnosed clinically by physicians andhistory of taking antimicrobial therapy at the time of clinical diagnosis was recorded accordingly. Cerebrospinal fluid (CSF) was collected by lumbar puncture (LP) and the decision of doing LP was taken by the physicians of the respective wards. Informed consent was taken from the patient's attendants or

guardians before sample collection.After all aseptic precautions, 3 ml of CSF were collected and poured 1 ml in each of three separate sterile screw capped tubes. One tube was used for PCR, one for culture and Gram staining and one for cytological and biochemical testing. Inoculation of the culture plates (blood agar and chocolate agar) with CSF were done at bed side for better isolation of *N. meningitidis, S. pneumoniae* and *H. influenzae*. Then the inoculated culture plates and CSF containing tubes were transported to the microbiology laboratory as quickly as possible to carry out further tests. In the laboratory, blood agar and chocolate agar plates were placed within the candle jar. MacConkey agar plate was also inoculated. Thereafter MacConkey agar plate and candle jar were incubated aerobically at 37ºC for 24 to 48 hours.

Bacterial meningitis was diagnosed when WBC count of CSF ≥100/mm^3 with mostly polymorphs and elevated protein level (>50 mg/dl), decreased glucose level (<40 mg/dl) with or without positive bacterial CSF culture and/or positive Gram stain.[2,3] Viral meningitis was diagnosed bymoderately high WBC count of CSF (>5 to 500/mm^3) with mostly lymphocytes and elevated protein level (>50 mg/dl), normal glucose level with negative bacterial CSF culture and/or negative Gram stain.[3,4]

Non bacterial meningitis control group: Twenty (20) viral (i.e. non-bacterial) meningitis patients were taken randomly as control. PCR was performed for these control samples (20) with universal primers of 16S rRNA. These 20 control samples did not show any band.

Cytological Examination of CSF:
The specimens were examined with naked eye for the presence of turbidity. Purulent or cloudy CSF indicated presence of pus cells, suggestive of acute pyogenic bacterial meningitis. Clear CSF was regarded as aseptic meningitis. Then the specimens were examined by cell count and Gram film.

Biochemical Tests of CSF:
Biochemical tests included the measurement of glucose and protein in CSFwhich was doneby using commercially available colorimetric reagent methods. It was done as per manufacturers instructions. The supernatant fluid from centrifuged CSF or uncentrifuged CSF in case of clear sample was used to measure glucose and protein.

Bacteriological Methods:
Staining and microscopic examination:
CSF samples for Gram staining were centrifuged at 3000 rpm for 10 minutes. Then pellet was used to prepare smears on clean and dry glass slides. The smears were air dried and fixed with methanol. Then the fixed smears were stained with Gram staining technique and examined under microscope, using oil immersion lens.

Examination of the inoculated culture plates:
All the inoculated culture media were examined for any growth of bacteria after 18 to 24 hours of incubation. If there was no growth, plates were reincubated for another day. Suspected colonies of *Streptococcus pneumoniae* and *Neisseria meningitidis* were subcultured on blood agar media and *Haemophilus influenzae* on chocolate agar media. Then inoculated culture plates were incubated in a candle extinction jar at 37ºC for 24 hours and then again inspected for growth.

Identification of bacteria:
All the organisms isolated were identified by their colony morphology, Gram staining characters and relevant biochemical tests.

Molecular detection of the organisms by PCR:
Multiplex PCR technique was applied for simultaneous detection of *N. meningitidis, H. influenzae*, and *S. pneumoniae*. Species-specific primers were used along with universal primers of bacterial gene 16S rRNA, in a two-stage PCR assay. In the first stage, amplification was performed using three specific primers- one for each type of bacterium, generating species-specific amplicons with different molecular weights. In the second stage universal primers were used which amplified a region of bacterial gene 16S rRNA. A generic bacterial amplicon was generated, not specific to the type of bacterium present in the specimen.

DNA extraction:
i) Bacterial strains: Clinical isolates of *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis* used were obtained from the respective culture plates. DNA was extracted from bacterial pelletthat was prepared from overnight cultures of respective organisms grown on blood agar and chocolate agar at 37˚C in 5% CO_2. Bacterial pellet was suspended in 300µl of sterile deionized water in a microcentrifuge tube. Then the suspensions were mixed well by vortex and boiled in a heat blocker for 15 minutes. The tubes were then kept immediately on ice for 5 minutes and then were centrifuged at 4˚C at 10,000 rpm (or 12,000x*g*) for 10 minutes to precipitate the cell debris and proteins. The supernatant was used as a PCR template and inoculated into the PCR reaction mix for amplification of DNA.[5]

ii) Clinical samples: CSF specimens from culture proven and suspected cases of meningitis were used for DNA

extraction. At first, CSF samples (200µl) were heated for 10 to 20 minutes at 100°C and centrifuged at 4°C at 10,000 rpm (or 12,000Xg) for 5to10 minutes. Supernatants from boiled extracts were used directly in the PCR reaction mixes.[5,6,7]

PCR Primers:

Selection of primers: The primers were selected on the basis of different published works such as primer sets used for detection of the meningococcus-specific insertion sequence IS *1106*[8], the psaAgene encoding the pneumococcal surface adhesin A (PsaA) protein[9], *bexA* gene encoding the capsulation-associated Bex A protein of *H. influenzae*[10] and universal primers for detection of bacterial gene 16S rRNA.[11]

Table-I: Summary of the characteristics of the PCR primers

Target gene	Primer	Primer sequence (5′ -3′)	Amplicon size(s) (bp)	Primers for
IS1106	M1 (F) M2 (R)	ATT ATTCAG ACC GCC GGC AG TGC CGT CCT GCA ACT GAT GT	331	*N. meningitidis*
psaA	P1 (F) P2 (R)	CTTTCTGCAATCATTCTTG GCCTTCTTTACCTTGTTCTGC	838	*S. pneumoniae*
bexA	H1 (F) H2 (R)	CGTTTGTATGATGTTGATCCAGA TGTCCATGTCTTCAAAATGATG	343	*H. influenzae*
16S rRNA	16S(F) 16S(R)	AGA GTT TGA TCA TGG CTC AG GGA CTA CCA GGG TAT CTA AT	798	Universal primers for bacteria

PCR Procedure:

All PCRs were performed using a Master-cycler gradient thermocycler. After DNA extraction, three microliter of the supernatant was used as the template in each PCR. Reactions was run on a final volume of 25µl, containing 12.5µl of Master mix (that contained PCR buffer with $MgCl_2$, dNTPs, PCR water and Taq DNA polymerase), 3µl of primers, 6.5µl of nuclease free water and 3µl of extracted DNA.

DNA amplification was performed in a two-stage PCR assay. In the first amplification stage, three species specific primers were used. The final reaction volume of multiplex PCR was 25µl including the primer of *Neisseria meningitidis* (0.5µl forward primer + 0.5µl reverse primer), *Haemophilus influenzae* (0.5µl forward primer + 0.5µl reverse primer) and *Streptococcus pneumoniae* (0.5µl forward primer + 0.5µl reverse primer) that generated species-specific amplicons with different molecular weights. And in the second stage, 3µl of universal primers (1.5µl forward primer + 1.5µl reverse primer) were used which generated a generic bacterial amplicon (798 bp), not specific to the type of bacterium present in the specimen. Some procedures were followed to avoid contamination, such as wearing a pair of sterile hand gloves prior to preparation of reagent mixture, using aerosol resistant tips and sterile microcentrifuge tubes. A negative control (sample with no DNA added) was also included in all PCR reactions.

The cycling parameters were consisting of an initial denaturationat 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 45 seconds and elongation at 72°C for 2 min with a final extension step of 10 min at 72°C.

Gel electrophoresis and visualization:

Following amplification, PCR products were visualized by electrophoresis in 1.5% agarose gel. Here, at first 4µl of amplified DNA from each PCR reaction was mixed with 1µl of loading dye and then loaded into an individual well of the gel and thereafter run in horizontal gel electrophoresis at 100 volt (50mA) for 30 minutes. Then, the gel was stained by immersing it in a solution of ethidium bromide (0.05µg/ml) in deionized water for 30 min, followed by de-staining with deionized water for 15 min and finally visualized using UV transilluminator in a dark room. Specific *N. meningitidis* (331 bp), *S. pneumoniae* (838 bp) and *H. influenzae* (343 bp) bands were compared to the molecular weight markers (Figure-I).

Evaluation of operational characteristics of multiplex PCR in CSF for bacterial meningitis: The operational characteristics of the multiplex PCR in CSF (index test) for acute bacterial meningitis were evaluated using positive CSF cultures (definite bacterial meningitis) and/or a probable acute bacterial meningitis sample, defined by cytological and biochemical CSF characteristics as the reference (gold) standard.

The following performance parameters were evaluated:
Sensitivityof PCR assay= A/(A+C)
Specificity of PCR assay= D/(B+D)

		Culture Result + ve	Culture Result - ve
PCR result	+ ve	A	B
	- ve	C	D

[+ ve = Positive - ve = NegativePCR = Polymerasechainreaction]
[A= True Positives, B= False Positives, C= False Negatives, D= True Negatives]

Statistical analysis:
The results of the study were recorded systematically. Data analysis was done by using computer Microsoft office excel 2007 and SPSS (Statistical package for data analysis) version 16.0 program and according to the objective of the study. Results were presented in the form of tables and figures. The test of significance was calculated by using χ^2 (chi-square) and p value <0.05 was taken as level of significance.

Results:
A total of 140 clinically diagnosed meningitis cases were studied. Among them 42 (30%) were bacterial meningitis cases and other 98 (70%) were viral meningitis cases on the basis of biochemical and cytological findings of CSF (Table-II).

Table-II: Categories of meningitis cases on the basis of cyto-biochemical analysis (n=140)

Categories of meningitis	Number	Percentage
Bacterial meningitis	42	30
Viral meningitis	98	70
Total	140	100

Table-III: Positivity of bacterial meningitis cases by Gram stain, Culture and PCR (n=42).

Gram stain	Culture	PCR	No. ofCases (%)
+ ve	+ ve	+ ve	9 (21.43)
- ve	+ ve	+ ve	6 (14.29)
- ve	- ve	+ ve	16 (38.10)
- ve	- ve	- ve	11 (26.19)
9	15	31	42 (100.0)

[+ ve = Positive; - ve = Negative; PCR =Polymerase chain reaction]

Out of 42 bacterial meningitis cases, 9 (21.43%) were Gram stain positive and 15 (35.71%) were culture positive. These nine Gram stain positive cases were also culture positive and PCR positive.Another 6 cases were both culture and PCR positive. On the other hand, 16 cases were only PCR positive. Eleven cases were negative by Gram stain, culture and PCR (Table-III). These 11 cases were diagnosed on the basis of clinical findings such as fever, deteriorated level of consciousness, signs of meningeal irritation along with other features of CSF such as haziness, neutrophilic pleocytosis, high protein and low glucose contents.

Table-IV: Bacterial isolates among the CSF culture positive cases (n=15) and rate of PCR positivity (n=31)

Bacterial isolates	Culture positive cases		PCRpositive cases	
	N	(%)	No.	(%)
S. pneumoniae	10	(66.67)	12	(38.71)
H. influenzae	3	(20.00)	10	(32.26)
N. meningitidis	2	(13.33)	4	(12.90)
Other bacteria	0	(0)	5	(16.13)
Total	15	(100.0)	31	(100.0)

Table-IV shows that among 15 culture positive cases, theprevalent isolate was *Streptococcus pneumoniae* (66.67%), followed by *Haemophilus influenzae* (20%) and *Neisseria meningitidis* (13.33%). On the other hand, among 42 bacterial meningitis cases, 31 (73.81%) were PCR positive. Out of 31 PCR positive cases, 12 (38.71%) were *S. pneumoniae*, 10 (32.26%) were *H. influenzae*, 4 (12.9%) were *N. meningitidis* and 5 (16.13%) were other bacteria (Table-IV).

Table-V: Results of CSF Gram stain, Culture and PCR between the antibiotic users and non users group among the bacterial meningitis cases (n= 42)

Categories of Patients	Positive results by Gram stain, Culture and PCR		
	Gram stain	Culture	PCR
Antibiotic users (n = 29)	2 (6.90)	3 (10.34)	19 (65.52)
Antibiotic non users (n = 13)	7 (53.85)	12 (92.31)	12 (92.31)
Total = 42	9 (21.43)	15 (35.71)	31 (73.81)

[Figures within parenthesis indicate percentage]

$p < 0.01$: Significantly higher rate of positive case detection by Gram stain in antibiotic non users group. (here, $\chi^2 = 14.71$)

$p < 0.01$: Significantly higher rate of culture positive cases in antibiotic non users group. (here, $\chi^2 = 29.96$)

$p > 0.05$: No significant differences were found between antibiotic users group and non-users group by PCR. (here, $\chi^2 = 0.08$)

Table-V shows that out of 29 bacterial meningitis patients who received antibiotics, CSF Gram stain was positive in 2 (6.90%) cases, while 7 (53.85%) of the 13 bacterial meningitis patients who did not take antibiotics, were positive by Gram stain. The difference was statistically significant ($p<0.01$). Three (10.34%) of 29 bacterial meningitis patients who received antibiotics, were CSF culture positive, whereas 12 (92.31%) of 13 bacterial meningitis patients who did not take antibiotics were culture positive. The difference was statistically significant ($p<0.01$). Similarly, among 29 bacterial meningitis patients who received antibiotics, PCR was positive in 19 (65.52%) cases, while 12 (92.31%) of 13 patients who did not take antibiotics, were PCR positive. The difference was not statistically significant ($p>0.05$).

Table-VI: Sensitivity and specificity of PCR test compared to CSF culturein 62 cases [Bacterial meningitis group (n=42) and control group (n=20)]

		Culture Result + ve	Culture Result - ve	
PCR result	+ ve	A (15)	B (16)	31
	- ve	C (0)	D (31)	31
		15	47	62

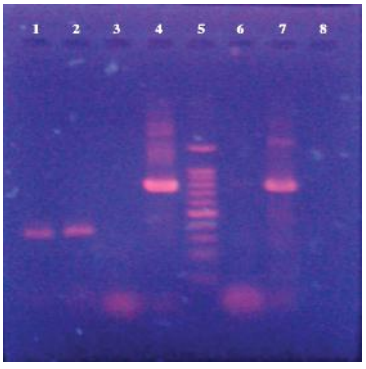
[+ ve = Positive - ve = NegativePCR = Polymerasechain reaction]

[A= True Positives, B= False Positives, C= False Negatives, D= True Negatives]

[Sensitivityof PCR= A/(A+C)= 100%; Specificityof PCR= D/(B+D)= 66%]

Out of 98 viral meningitis cases, PCR was performed for 20 samples with universal primers of 16S rRNA to see whether it was bacterial or not. These 20 control samples (i.e. cases of non-bacterial meningitis) did not show any band.The overall sensitivity and specificity of PCR assay was 100% and 66% respectively (Table-VI) when bacterial culture was considered as gold standard.

Figure-I: Photograph of gel electrophoresis of amplified DNA.



Lane 1, 2: *H. influenzae* (343 bp)

Lane 4, 7: *S. pneumoniae* (838 bp) C

Lane 5: 100 bp molecular weight marker

Lane 8: Negative control

Discussion:

Bacterial meningitis is a serious and sometimes fatal infection affecting the central nervous system (CNS). About two-thirds of all bacterial meningitis cases occur among children and 80 to 90% of the cases are caused by three major pathogens- *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis*.[12] A rapid and precise diagnosis of bacterial meningitis is essential to determine accurate treatment which significantly reduces mortality and the risk for long term sequelae.[13] Among the routine tests used for the etiologic diagnosis of bacterial meningitis, culture is considered the gold standard. However, it is a time-consuming technique, which requires viable microorganisms for cultivation and its sensitivity is directly affected by the start of antibiotic treatment before CSF collection.[14] In recent years, molecular biology techniques, such as PCR, have been widely used to amplify and detect microbial DNA in CSF. The present study was carried out in order to assess the simultaneous detection of *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis* by multiplex PCR for the diagnosis of bacterial meningitis.

In this study, 140 CSF specimens of clinically suspected meningitis patients were collected. On the basis of cytological and biochemical findings of CSF, the study population was categorized into 2 groups. Fourty two (30%) cases were diagnosed as bacterial meningitis and other 98 (70%) cases were diagnosed as viral meningitis. The results are consistent with findings of other studies conducted in Bangladesh.[1,15,16] Mazed (2002) isolated 25.62% bacterial meningitis cases[15] and Moony (2005) detected 25.34% bacterial meningitis cases in paediatric population.[16] Gurley *et al*. (2009) revealed in their study that 189 (24%) patients, representing all age groups were diagnosed as bacterialmeningitis.[1]

Microorganisms were detected in CSF by Gram stain in 9 (21.43%) cases out of 42 specimens in the present study. The result of Gram staining reported by Salih et al, (1995) was positive in 30.4% cases[17], which was almost similar in comparison to this study. On the other hand, Chakrabarti et al, (2009) of India found 6.7% Gram stain positive cases[18], which was less in comparison to this study.

In this study, culture yielded growth of bacteria in 15 (35.71%) of the 42 bacterial meningitis patients. This finding closely relates with those of Sarookhani et al. (2010)[7] and Salih et al. (1995)[17] who found 36% and 33.9% culture positive cases respectively in their study. This low number of culture positivity in this study might be due to pretreatment of patients with antibiotic therapy prior to lumbar puncture. The present study also

revealed that among 15 culture positive cases, the most prevalent isolate was *Streptococcus pneumoniae* (66.67%), followed by *Haemophilus influenzae* (20%) and *Neisseria meningitidis* (13.33%).

In the present study out of 42 bacterial meningitis cases, 31 (73.81%) were diagnosed by PCR. The rest 11 cases were diagnosed on the basis of clinical findings such as fever, deteriorated level of consciousness, signs of meningeal irritation along with certain features of CSF such as haziness, neutrophilic pleocytosis, high protein and low glucose contents. Several factors may interfere with the recovery of microorganisms from CSF, such as previous antibiotic treatment, inadequate collection and storage of culture specimens, and reduced number of bacteria in CSF.

Again, detection of bacterial DNA in the CSF sample by PCR not only depends on the number of CFU (colony forming unit), but also on the different concentrations of PCR inhibitors and different efficacies of extraction methods of DNA from bacteria. Reports from various studies indicate that in a significant proportion of CSF samples, amplification process is problematic due to presence of PCR inhibitors. Ratnamohan et al. (1998)[19] described that the inhibitory effects may be due to increased levels of proteins and increased cell numbers, but not due to cellular DNA. The findings of the present study also supported this observation as CSF protein and cell count were high in 11 PCR negative samples.

On the other hand, PCR was able to detect organisms in 16 more cases where conventional techniques i.e. Gram stain and/or culture were negative but other laboratory data like peripheral leukocytosis, elevated CSF white cell count with gross neutrophilia as well as high CSF protein and low CSF glucose inclined towards bacterial meningitis. Here, the cell counts and CSF biochemistry suggested probable bacterial meningitis that was confirmed by PCR. Moreover, all these 16 samples were re-tested with appropriate control to rule out contamination during PCR process.The present study also revealed that among 31 PCR positive cases, 12 (38.71%) were *S. pneumoniae*, 10 (32.26%) were *H. influenzae*, 4 (12.9%) were *N. meningitidis* and 5 (16.13%) were other bacteria and this result is consistent with findings of other studies.[20,21,22]

This study revealed that 38.71% of bacterial meningitis was due to *S. pneumoniae*. Similar findings was reported by Moony (2005)[16], Mazed (2002)[15] and Saha *et al*, (1997)[22] of Bangladesh, who found 37.50%, 29.04% and 32% of bacterial meningitis in paediatric patients due to *S. pneumoniae* in their studies.

In this study, *H. influenzae* was the 2nd causative agent causing 32.26% of bacterial meningitis in children. Similar finding was reported by Saha et al. (2005a)[23] and Chowdhury et al. (1992)[24] of Bangladesh, who found 35% and 36.54% of bacterial meningitis in paediatric patients due to *H. influenzae* in their studies.

In the present study, although *N. meningitidis* was not the most common causative agent of bacterial meningitis in children, it was responsible for 12.9% of cases detected among children. Similar findings were reported by Moony (2005)[16], Mazed (2002)[15] and Saha et al, (1997)[22] of Bangladesh, who found 12.5%, 19.35% and 18% of bacterial meningitis in paediatric patients due to *N. meningitidis* in their studies.

Following clinical diagnosis of meningitis empirical antimicrobial therapy is usually given to the patients to reduce the mortality and morbidity. The present study showed that out of 29 bacterial meningitis patients who received antibiotics, CSF Gram stain was positive in 2 (6.90%) cases, while 7 (53.85%) of the 13 bacterial meningitis patients who did not take antibiotics, were positive by Gram stain. The difference was statistically significant ($p<0.01$). Three (10.34%) of 29 bacterial meningitis patients who received antibiotics, were CSF culture positive, whereas 12 (92.31%) of 13 bacterial meningitis patients who did not take antibiotics were culture positive. The difference was statistically significant ($p<0.01$). Moony (2005)[16] of Bangladesh found that among the patients who received antibiotics, 28.57% and 55.26% bacterial meningitis cases were detected by culture and microscopy respectively. The present study also revealed that among 29 bacterial meningitis patients who received antibiotics,PCR was positive in 19 (65.52%) cases, while 12 (92.31%) of 13 patients who did not take antibiotics, were PCR positive. The difference was not statistically significant ($p>0.05$).

In this study, out of 98 viral meningitis cases, PCR was performed for 20 samples with universal primers of 16S rRNA to see whether it was bacterial or not. These 20 control samples (i.e. cases of non-bacterial meningitis) did not show any band. As broad-range 16S rRNA PCR indeed was negative for all control samples, it indicates that false positivity of PCR is not common. For evaluation of performance of the PCR assay, results of PCR were compared with CSF culture as gold standard. According to the data of the present study, the overall sensitivity and specificity of PCR assay was 100% and 66% respectively. This finding nearly similar with results of Sarookhani et al. (2010)[7] in Iran who found, out of 100 evaluated CSF samples, 36 were culture positive and 74 were PCR positive and sensitivity of PCR was 100% and specificity was 40.6%.

Conclusion:

Multiplex PCR is a helpful technique for rapid diagnosis of etiological agents of bacterial meningitis directly from CSF especially in pretreated cases. Moreover, PCR has a very high sensitivity and specificity as well as it is the only test that is capable of identifying different etiological agents even in culture negative CSF samples. Regarding the advantages and disadvantage in performance of the conventional CSF culture and CSF PCR, it is recommended to use both methods in clinical practice.

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