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rmpM GENE AS A GENETIC MARKER FOR HUMAN BACTERIAL MENINGITIS

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Abstract

Meningitis is a bacterial, viral or fungal infection of the protective membrane meninges covering the brain and spinal cord. Viral and other forms of meningitis are mild and get cured within one or two week without any treatment. Whereas, bacterial meningitis can prove lethal if not being diagnosed or treated in time. Meningitis is a contagious infection and can spread from one person to another through coughing, sneezing or close contact. Usually the disease is diagnosed from cerebrospinal fluid (CSF) of the patients using culture, PCR, immunological and biochemical tests. All these methods suffer from one or more limitations. Our lab has developed a quick PCR based detection of *Neisseria meningitidis* (bacterial meningitis) directly from the patient CSF samples using specific primers of virulent *rmpM* gene. The overall analysis completes in 80 min for confirmation of the disease. Amplicon of 308 bp of *rmpM* gene does not show homology with other organisms and can be used as a genetic marker for human bacterial meningitis caused by *Neisseria meningitidis*.

Key words: Bacterial meningitis, Genetic marker, *N. meningitidis, rmpM* gene.

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INTRODUCTION

Meningitis is a serious disease that causes inflammation of the meninges in the brain and spinal cord of the patients. Bacterial meningitis is a contagious disease which spreads very rapidly among people through coughing, sneezing and close contact. Therefore, a quick and accurate diagnostic method is necessary for early-stage diagnosis and treatment of the patient to prevent the outbreak of the disease (13, 8). The bacterial meningitis is usually detected by biochemical tests (15), Gram staining (9), latex agglutination test (31), PCR (17, 1, 20, 5), RT-PCR (7, 23, 29), microarray (33, 14, 21, 27) and biosensor (25, 26).

PCR has been routinely used as a diagnostic tool for diagnosis of many diseases (2, 11). Kotilainen *et al.* (1998) used broad-range PCR based amplification of genes that code for 16s and 23s RNA of *N. meningitidis* to detect bacterial meningitis. But the proteolysis step in this method requires more than 12 h of incubation at 56 °C (17). Seward and Towner (2000) used multiplex PCR amplification of rDNA from *N. meningitidis* for detection of bacterial meningitis (28) and the same method was used by Mohamed *et al.* (2003) for detection of bacterial meningitis during an epidemic outbreak of meningitis at Sudan (22). This method requires purification of RNA and synthesis of cDNA which is time consuming and showed 88.5% sensitivity in agarose gel electrophoresis. Taha *et al.* (2000) and Lewis and Clarke (2003) reported cumbersome and time consuming detection of different serogroups of *N.* meningitidis by analysis of PCR amplified *crgA* and *saiD* gene sequence, respectively (32, 20). Baethgen *et al.* (2003) and Bronska *et al.* (2006) reported PCR based detection of bacterial meningitis using purified genomic DNA as template (1, 5). Boving *et al.* (2009) used eight-plex PCR for simultaneous detection of *N. meningitidis*, *S. pneumoniae*, *E. coli*, *S. aureus*, *L. monocytogenes*, *S. agalactiae*, herpes simplex virus (types 1, 2) and varicella-zoster virus (4). They used purified DNA as PCR template and detected the PCR amplicon through array and microsphere coupling method, which require about a day for diagnosis of bacterial meningitis. Fraisier *et al.* (2009) used a single multiplex PCR for detection of bacterial meningitis based on multiple genes, which was complicated and expensive (12). Recently, Kumar *et al.* (2011) reported *opc* gene as a genetic marker for detection of bacterial meningitis based on 304 bp amplicon of opacity associated gene (18, 19).

rmpM (reduction-modifiable protein M) is a virulence-associated outer membrane protein of *N. meningitidis* (16, 34). rmpM has two interlinking domains one of which bind with peptidoglycan layer of bacteria while the other binds to outer membrane proteins (30, 3). *rmpM* gene plays an essential role for stability and viability of the bacteria (10). All reported methods are time consuming, expensive and non-confirmatory due to certain limitations. Here, we report a specific genetic marker based on virulence *rmpM* gene for quick detection of bacterial meningitis.

MATERIALS AND METHODS

Chemicals and patient samples

The PCR chemicals, Taq polymerase and agarose were obtained from Bangalore GeNei, India. Tris and EDTA were obtained from Sigma-Aldrich, USA. DNA purification kit was purchased from Biochem Life Sciences, India. Primers were synthesized from The Centre of Genomic Application (TCGA), India. The bacterial culture and patient CSF samples were obtained from National Centre for Disease Control (NCDC), India.

Sample preparation

Patient CSF (0.5 ml) was taken in an eppendorf tube and centrifuged at 6000xg for 5 min. The supernatant was discarded and the remaining pellet was suspended in 25 µl of PCR mix containing 1X PCR buffer (1.5mM $MgCl_2$;

0.01M Tris-HCl, pH 8.3, 0.05M KCl, 0.01% gelatin), 0.4 mM dNTP mix (0.1mM of each nucleotide), 0.4 µM of each forward and reverse primers of *rmpM* gene of *N. meningitidis*, 0.75U of Taq polymerase and water.

PCR amplification

The PCR was carried out in PCR tubes using a MJ Mini (Bio-Rad) thermal cycler with the following steps: initial denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 4s, annealing at 60 °C for 5 s, extension at 72 °C for 1 s and final extension of 3 min at 72 °C after the last cycle. The amplicon was analyzed by gel electrophoresis (1.5% agarose gel). The PCR amplicon was purified using GFX column (35) and sequenced at TCGA, India.

RESULTS AND DISCUSSION

rmpM is a unique virulent gene of *N. meningitidis* which does not show homology with any other gene of pathogens found in CSF of human. Primers used in our study are specific and amplify only specific (308 bp) sequence of *rmpM* gene (Fig. 1A) and the amplicon of 308 bp was purified using GFX column and sequenced at TCGA (Fig. 1B). The ClustalW (multiple sequence alignment computer programs) alignment of 308 bp *rmpM* gene showed changes in nucleotide bases of already reported sequence in NCBI GenBank. The new sequence was submitted to NCBI and obtained an accession No. HQ712170 (6). Here, we have used PCR for quick detection of *N. meningitidis* in 80 min (including gel electrophoresis) directly from patient CSF without isolating genomic DNA. The diagnosis of control and suspected bacterial meningitis patients (28 samples) were carried out using present available methods (Table 1) as well as PCR using 308 bp *rmpM* as genetic marker (Fig. 2). Controls (sample 1 and 2) were made positive with *N. meningitidis*. Sample 10, 18 and 26 were confirmed positive by PCR whereas it was shown negative by biochemical tests (sample 18) and catalse test (sample 10) due to their limitations. Presence of *E. coli* and other bacteria including limitations of the test may lead to false results with the other sample using present available methods. PCR using 308 bp *rmpM* as genetic marker can be used for confirmation of the disease. For validation of the results, PCR was also carried out using bacterial culture of *E. coli* and *S. pyogenes* (Table 1 sample 29 and 30). The results suggest that *rmpM* gene is only amplified in *N. meningitidis* bacteria but not in others (Fig. 2). Therefore, *rmpM* gene of 308 bp can be used as specific genetic marker for quick diagnosis of bacterial meningitis. The *rmpM* gene may be used in future for development of an electrochemical DNA sensor for detection of bacterial meningitis due to its virulence in nature.

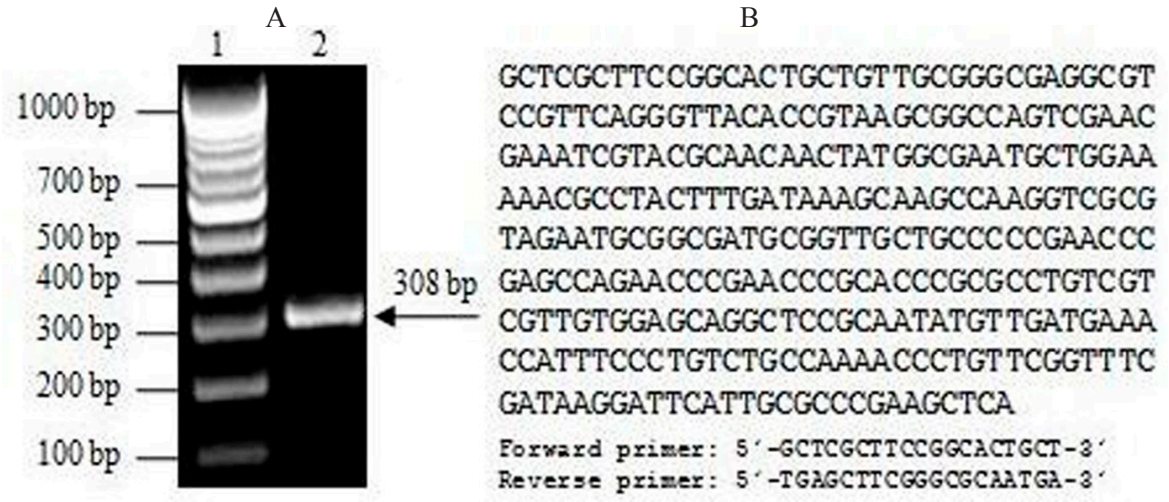

Figure 1. (A) Agarose gel electrophoresis (1.5%) of PCR product, Lane1: DNA Ladder 100bp; Lane2: PCR amplicon of *rmpM* gene (308 bp). (B) Gene sequence of PCR amplicon using reverse primer.

Figure 2. Agarose gel electrophoresis (1.5%) of suspected patients of bacterial meningitis. Lane 1 and 2 (control) and Lane 10, 18 and 26 showed single band corresponding to 308 bp of DNA marker (bacterial meningitis infected patients) whereas, Lane 3-9, 11-17, 19-25 and 27-28 showed no bands (Normal persons). Lane 29 and 30 also showed no bands (*E. coli and S. pyogenes*) used for validation of *rmpM* gene.

Table 1. Diagnosis of suspected patients of bacterial meningitis using present available methods and PCR with *rmpM* gene as genetic marker.

Sample No.	Present methods of diagnosis (24)						PCR with marker *rmpM gene* (308 bp)	Normal/ Infected patient
	Microscopic	Enzyme test		Immunological test	Biochemical test			
	Gram (-ve)	Oxidase	Catalase	Latex agglutination	Glucose	Sucrose		
1. Control	+	+	+	+	+	-	+	C
2. Control	+	+	+	+	+	-	+	C
3	-	-	-	-	-	-	-	N
4	-	-	-	-	-	-	-	N
5	-	-	-	-	-	-	-	N
6	-	-	-	-	-	-	-	N
7	+	-	-	-	-	-	-	N
8	-	-	-	-	-	-	-	N
9	-	-	-	-	-	-	-	N
10	+	+	-	+	+	-	+	P
11	-	-	-	-	-	-	-	N
12	-	-	-	+	-	-	-	N
13	-	-	+	-	-	-	-	N
14	-	-	-	-	-	-	-	N
15	+	+	-	-	-	-	-	N
16	-	-	-	-	-	-	-	N
17	-	-	-	-	-	-	-	N
18	+	+	+	+	+	+	+	P
19	-	-	-	-	-	-	-	N
20	-	-	-	-	-	-	-	N
21	-	-	-	-	+	-	-	N
22	-	-	-	-	-	-	-	N
23	-	-	-	-	-	+	-	N
24	-	-	+	-	-	-	-	N
25	-	-	-	-	-	-	-	N
26	+	+	+	+	+	+	+	P
27	-	-	-	-	-	-	-	N
28	-	-	-	-	-	-	-	N
29. *E. coli*	+	-	+	+	+	-	-	N
30. *S. pyogenes*	-	-	-	+	-	-	-	N

Normal healthy individuals (controls) = N
N. meningitidis infected patients = P

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Other articles in this theme issue include references (36-63).

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