



Universal Primers Used for Detection of Bacterial Meningitis

Mohammad Reza Pourmand^{*}, Hooman Sadighian, Ramina Mahboubi,
Elaheh Salimi

Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

ARTICLE INFO

Article type:
Original Article

Article history:

Received: 29 Dec 2012
Revised: 17 Feb 2013
Accepted: 10 Mar 2013

Keywords:

Meningitis
Bacterial Infection
Meningitis, Bacterial

ABSTRACT

Background: Acute bacterial meningitis is among serious infections of the central nervous system (CNS). The early diagnosis and empiric antibiotic treatments have led to a reduction in morbidity and mortality rates. PCR and the enzymatic digestion of 16S rDNA fragment following the PCR by universal primers led up fast and sensitive determination. The aims of the present study was to improve our previous method for rapid and specific detection of common bacteria causing acute meningitis.

Methods: According to the gene encoding 16S rDNA found in all bacteria, a set of primers was designed. Then the universal PCR was performed for bacterial agents of meningitis by employing broad-range DNA extraction method. The amplicons were digested with restriction enzymes to identify bacterial species.

Results: By the enzymatic digestion of the amplicons of each standard strain, specific patterns were achieved. These specific patterns may be used for comparison in CSF examination. The analytical sensitivity of the assay was approximately 1.5×10^2 CFU/ml of CSF even in samples with high amount of proteins.

Conclusion: The universal PCR coupled with enzymatic digestion can be used to detect and identify bacterial pathogens in clinical specimens rapidly and accurately. Molecular diagnostic of bacterial meningitis, though expensive and labor-intensive, but is valuable and critical in patient management.

- **Please cite this paper as:** Pourmand MR, Sadighian H, Mahboubi R, Salimi E. Universal Primers Used for Detection of Bacterial Meningitis. *J Med Bacteriol.* 2013; **2** (1, 2): pp. 54-59.

^{*} *Corresponding Authors:* Mohammad Reza Pourmand, PhD., Department of Pathobiology, School of Public Health, and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, IR Iran. *Tel:* +98 912 5168520, *E-mail:* mpourmand@tums.ac.ir

Introduction

Acute bacterial meningitis is among the most serious infectious diseases in central nervous system (CNS). According to the statistics, incidence rate is three to five cases in one hundred thousand people per year and the mortality rate is between 6% to 26% in the United States (1, 2). Rate of infection and associated mortality are significantly higher in developing countries. For instance, in the “Meningitis Belt” of sub-Saharan Africa, bacterial CNS infections cause tens of thousands cases; as a result, thousands of deaths occur during epidemic years (3). Permanent life threatening neurologic complications include deafness, seizures, and mental retardation. Moreover, behavioral changes may be present in up to one-third of survivors (4).

Neisseria meningitidis and *Streptococcus pneumoniae* are responsible for approximately 80% of cases in acute bacterial meningitis in countries with general vaccination against *Haemophilus influenzae* type (B). In addition, several other bacteria can cause meningitis, such as *Streptococcus agalactiae* (Group B streptococci; GBS), *Listeria monocytogenes* and *H. influenzae*. On the other hand, infections by *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococci* spp., *salmonella* spp. and *enterococci* spp. may rarely lead to meningitis (5).

In order to detect bacterial meningitis, the culture remains the gold standard among the various methods currently used in clinical laboratories. The down side in this method is that it takes at least 24 hours to obtain results (6). Consequently, there is substantial need to develop new and rapid diagnostic methods that aid clinical decision-making.

Such methods are particularly useful in an adjunctive assay in acute care settings.

Polymerase chain reaction (PCR) assays rely on amplification of small amounts of target DNA. This method is currently used in clinical settings for definitive identification of viral CNS infections such as Enterovirus meningitis, Herpes simplex virus meningitis, and for slow-growing bacterial CNS infections such as infections caused by *Mycobacterium tuberculosis* (7).

The ability to early identify bacterial species in developing countries facilitates early treatment with effective therapies and decreases high mortality rate associated with CNS infections. Ribosomal RNA genes are vital for survival of all organisms. They are highly conserved in the bacterial kingdoms (7- 9). Therefore, they seem to be promising target for rapid detection of bacteria.

The aim of this experiment is to improve previous methods for rapid and specific detection of bacteria causing acute meningitis (10).

The method was developed and optimized by designing new universal primers and following fast restriction enzyme. Finally, to show results agarose gel electrophoresis was used instead of polyacrylamide gel electrophoresis. In designing new universal primers, the concern was the size of fragments obtained from enzymatic digestion of PCR amplicons. The result would give societies the ability to use agarose gel electrophoresis and makes it more practical in clinical laboratories.

Material and Methods

Bacterial strains

Bacterial species were obtained from American Type Culture Collection (ATCC) (Table 1). The sensitivity of the primer sets were evaluated by using samples from culture-confirmed cases of meningitis and PCR-confirmed cases of viral meningitis (25 CSF samples).

Strains	Source or reference
<i>Haemophilus influenzae</i>	ATCC 33930
<i>Neisseria meningitidis</i>	ATCC 10377
<i>Streptococcus pneumoniae</i>	ATCC 49619
<i>Streptococcus agalactiae</i>	ATCC 13813
<i>Escherichia coli</i>	ATCC 35218
<i>Mycobacterium tuberculosis</i>	ATCC 25618
<i>Staphylococcus aureus</i>	ATCC 51299
<i>Staphylococcus epidermidis</i>	ATCC 12228

Extraction of DNA for PCR

As directed by the manufacturer, DNA was extracted and isolated from 200- μ l aliquot of bacterial suspension and 500- μ l of each CSF sample by using the DNeasy Blood and tissue kit (QIAGEN, Germany). Eluates were stored at -20°C.

Design of Primers

The target site within the 16S rRNA gene (highly conserved region) is chosen. Primers were designed based on 16SrRNA sequence data obtained from GenBank (National Institutes of Health, Washington, DC). All sequences (16S rRNA sequences of all bacteria causing meningitis) were aligned by MEGA4 software. GENERUNR and Oligo6 software were used to design primers. Theoretical specificity of all designed

primers was further analyzed by using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST) program (Table 2).

Name	Sequence
TEHF-2	5'-AGACACGGTCCAGACTCCTAC-3'
TEHR-2	5'-CGGGCGGTGTGTACAAG-3'

PCR amplification

According to kit manufacturer (HotStarTaq Plus Master Mix Kit, QIAGEN, Germany). Each PCR procedure was performed in 50 μ l total volume, which was composed of 25 μ l of PCR master mix and 2 μ l of extracted DNA (50 ng), as sample input, 5 μ l coral load and 0.5 μ M of each of primer sets. The PCR mixture was subjected to denaturation at 94°C for 45 sec, annealing at 57°C for 30 sec, extension at 72°C for 90 sec, followed by 30 cycles. Initial activation and final extension were 5 min, 95°C and 10 min, 72°C respectively. DNA-free water for negative controls and DNA for positive controls were used.

Enzymatic digestion

To improve digestion results, PCR products were purified by cleanup kit (QIAGEN, Germany). Each of PCR products are digested by fast restriction enzyme (*Bbv*I) in 15 min as advised by the manufacturers (fermentase, Denmark, Copenhagen). To show a digestion patterns, 1.5% agarose gel was used.

Results

All ATCC species of bacteria showed one band by PCR using universal primers. PCR amplicons sizes of all bacteria by these

primer sets are about 1100 bp. Positive CSF samples (by gram staining and culture) showed the same results. Fragments obtained from digestion of PCR amplicons by *BbvI* showed different digestion patterns (Figure 1). Size of fragments achieved from digestion are shown in Table 3. According to notable fragment of each bacterium (showed in bold in table 3), it is possible to detect bacterial species very easily and rapidly on gel agarose electrophoresis.

Table 3. Size of fragments obtained from enzymatic digestion

Organism	Size of fragment
<i>N. meningitidis</i>	170,210,294, 367
<i>H. influenzae</i>	130,155,210,213, 292
<i>S. pneumoniae</i>	105,171, 766
<i>S. agalactiae</i>	105 ,171,337, 431
<i>E. coli</i>	130,368, 504
<i>M. tuberculosis</i>	111,122, 710
<i>S. aureus</i>	138,170,310, 398
<i>S. epidermidis</i>	170,310, 536

Discussion

Our principle goal in this experiment was to improve the method, presented in the previous research (10). The universal nature of the 16S rRNA gene and its extremely conserved regions of nucleotides are variable between species; in contrast, they are constant within a species that make it an ideal target for a diagnostic PCR involving all the commonly occurring bacterial infections (11).

In the present experiment we were able to obtain an early diagnosis of bacterial meningitis by DNA-based methods using universal primers that targeted conserved regions in many species of bacteria on 16S

ribosomal RNA and enzymatic digestion to produce specific fragments for each PCR products. In current study, each PCR products for bacterial targets produces a significant fragment that causes the detection process to be done easily for any bacteria according to digestion patterns (Table 3). PCR by universal primers showed bands in 8 species that were examined in present experiment as causing bacterial meningitis. This method could be applied directly in low amount of CSF and it is performed in an easier and rapid format.

To compare with other research projects there are some studies in which universal primers and restriction enzymes were used to identify bacterial species. LU *et al* applied five different restriction enzymes for identification the species followed universal PCR (12) and Pandit *et al* utilized three different enzymes (13), but it makes confusing and increases the cost and the time of examination. In our previous study (10), three restriction enzymes were used to detect bacteria, while by current method this could be carried out by using just one restriction enzyme.

In the previous experiment, polyacrylamide gel electrophoresis was utilized (10). The technique for using restriction enzymes and polyacrylamide gel electrophoresis are time-consuming process and complex. Therefore, to solve these problems we developed a method to use universal primer pairs. Solely one restriction enzyme and gel agarose electrophoresis were used. In this way, duration of test and cost of examination were significantly decreased. In designing primers the concern was size of fragments obtained from enzymatic digestion. In this research, fragments were simply identified on agarose gel.

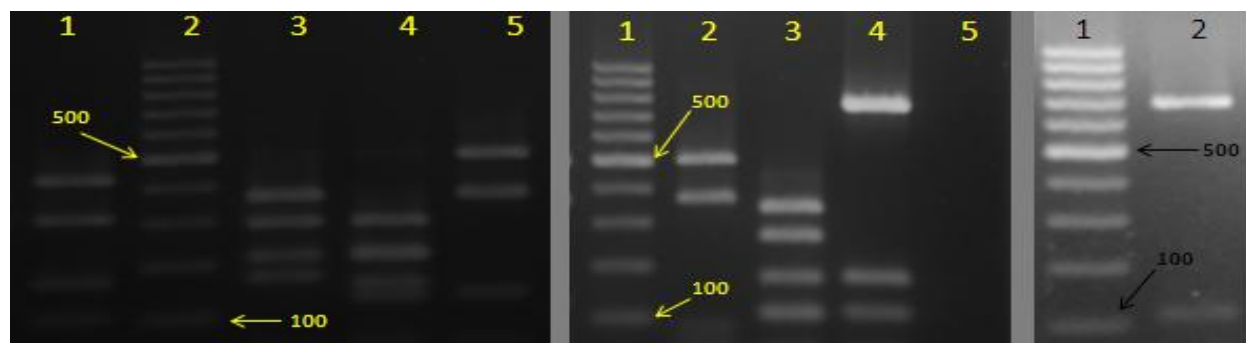


Figure 1. Electrophoretic analysis of PCR amplicons by universal primers after enzymatic digestion by *BbvI* on an agarose gel

A.

Lane 1-*S. aureus*
Lane 2-Molecular marker (100bp plus)
Lane 3- *N. meningitidis*
Lane 4- *H. influenzae*
Lane 5- *E. coli*

B.

Lane 1-Molecular marker (100bp plus)

Lane 2-*E. coli*

Lane 3- *S. agalactiae*

Lane 4- *S. pneumoniae*

Lane 5- Negative control

C.

Lane 1-Molecular marker (100bp plus)

Lane 2-*M. tuberculosis*

Other scientists used different methods such as sequencing (14), Real-Time PCR (15), and microarrays (16). The employed methods are sophisticated, complex and expensive therefore, it is not possible to use them as routine tests for symptomatic patients with meningitis especially in developing countries.

Conclusion

Based on the result it may be concluded that this method seems to be applicable for the detection of bacteria from cerebrospinal fluid specimens. Simultaneously, there is a need to continue to test this procedure as part of well designed clinical studies.

Acknowledgement

None declared.

Conflict of Interest

None declared conflicts of interest.

References

1. Swartz MN. History of medicine: on bacterial meningitis. *N Engl J Med* 2004; **351**: 1826.
2. Schuchat A, Robinson K, Wenger JD, et al. Bacterial meningitis in the United States in 1995. Active Surveillance Team. *N Engl J Med* 1997; **337**: 970-6.
3. World Health Organization. Meningococcal meningitis. Available at: <http://www.who.int/mediacentre/factsheets/fs141/en/>. Accessed Apr 12, 2010.
4. Singhi P, Bansal A, Geeta P, et al. Predictors of long term neurological outcome in bacterial meningitis. *Indian J Pediatr* 2007; **74**: 369-74.

5. van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M. Clinical features and prognostic factors in adults with bacterial meningitis. *N Engl J Med* 2004; **351**: 1849-59.
6. Winn W, Allen S, Janda W, et al. 2006. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins, P. 92-6.
7. Liu Y, Han J, Huang H, Zhu B. Development and evaluation of 16S rDNA microarray for detecting bacterial pathogens in cerebrospinal fluid. *Exp Biol Med* 2005; **230**: 587-91.
8. Sawada N, Iwamura Y, Shimizu T, et al. Detection and identification of pathogenic bacteria by polymerase chain reaction with primers from DNA sequence of ribosomal RNA. *Nippon Saikingaku Zasshi* 1992; **47**: 607-16 (in Japanese).
9. Tatsukawa T, Chikumi H, Matsumoto Y. A method to detect and identify bacteria in the whole blood targeting bacterial 16S rRNA gene. *Yonago Igaku Zasshi* 1998; **49**: 168-77.
10. Sadighian H, Pourmand MR. Molecular Detection of Common Bacterial Pathogens Causing Meningitis. *Iranian J Publ Health* 2009; **38** (1): 60-8.
11. Radstrom P, Backman A, Qian N, et al. Detection of bacterial DNA in cerebrospinal fluid by an assay for simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and streptococci using a seminested PCR strategy. *J Clin Microbiol* 1994; **32**: 2738-44.
12. Lu JJ, Perng CL, Lee SY, et al. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. *J Clin Microbiol* 2000; **38** (6): 2076-80.
13. Pandit L, Kumar S, Karunasagar I. Diagnosis of partially treated culture-negative bacterial meningitis using 16S rRNA universal primers and restriction endonuclease digestion. *J Med Microbiol* 2005; **54** (6): 539-42.
14. Schuurman T, de Boer RF, Kooistra-Smid AM, et al. Prospective study of use of PCR amplification and sequencing of 16S ribosomal DNA from cerebrospinal fluid for diagnosis of bacterial meningitis in a clinical setting. *J Clin Microbiol* 2004; **42** (2): 734-40.
15. Corless CE, Guiver M, Borrow R, et al. Simultaneous Detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in Suspected Cases of Meningitis and Septicemia Using Real-Time PCR. *J Clin Microbiol* 2001; **39** (4): 1553-8.
16. Desantis TZ, Brodie El, Moberq JP, et al. High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. *Microb Ecol* 2007; **53** (3): 371-8.