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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

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Primers specific to conserved and variable regions in the 16S rRNA sequence were selected from the partially sequenced 16S rRNA genes of *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. agalactiae*, and *Staphylococcus epidermidis*. The PCR assay was divided into two DNA amplifications. The first resulted in a general bacterial amplicon, and the second resulted in a species-specific amplicon. The high specificity of the PCR assay was documented after testing bacteria of 28 different species (133 strains). A total of 304 clinical cerebrospinal fluid samples, including 125 samples from patients with bacterial meningitis, were assayed to investigate the diagnostic sensitivity and specificity for bacterial meningitis. The assay showed high sensitivity (0.94) and specificity (0.96) with the clinical samples, although some false results were obtained, the reasons for which are discussed. With agarose gel electrophoresis for detection of the PCR products, the detection limit for meningococci in cerebrospinal fluid was 3×10^2 CFU/ml.

Acute bacterial meningitis is a rapidly progressing, potentially lethal condition. Common causative agents of acute bacterial meningitis are *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *S. agalactiae*. Adequate treatment requires rapid detection and identification of the bacteria in the cerebrospinal fluid (CSF).

The diagnosis of acute bacterial meningitis is based on direct microscopy, which is quick but unspecific and has a low sensitivity, and culture of CSF or blood which, however, takes at least 12 to 24 h and may show negative results due to antibiotic treatment prior to sampling. Various laboratory investigations of CSF have been developed for the rapid and specific diagnosis of acute bacterial meningitis, mainly through detection of bacterial antigens by immunological methods (1, 6, 13, 19). Nevertheless, none of these tests alone, or in combination, is entirely satisfactory because of limited sensitivity and specificity (12).

In recent years, molecular diagnostic techniques, such as the PCR, have constituted the most substantial technical advances in the field of rapid detection of bacteria in different types of samples (4, 15). Some difficulties are, however, associated with PCR, such as false-positive results due to contaminating nucleic acids and inhibition of PCR by complex samples (14). In a previous paper, a PCR assay for the diagnosis of a single agent causing bacterial meningitis was reported (8). The detection method was based on primers corresponding to sequences flanking the chromosomal dihydropteroate synthase (*dhps*) gene in *N. meningitidis* (17) and showed that PCR is a technique which rapidly demonstrates the presence of meningococcal DNA, even in culture-negative CSF. Moreover, a CSF specimen can be used directly in the PCR mixture without any pretreatment of the sample other than heat (23). The observation that the *dhps* gene and many other chromosomal genes are involved in horizontal transfer among naturally transformable bacteria, such as meningococci and streptococci (17), motivated us to construct primers from the nucleotide sequence encoding the small-subunit rRNA, an approach which was also chosen by Greisen et al. (7). The reasons for using the 16S rRNA gene as a distinct signature for bacteria are the universal nature of the gene and the fact that it contains extremely conserved regions of nucleotides alternating with regions that are variable between species but constant within a species (5, 26). This makes 16S rRNA sequences convenient priming targets for oligodeoxynucleotide primers (10).

In the present report, we describe a PCR strategy for the simultaneous detection, in CSF, of *N. meningitidis*, *H. influenzae*, *S. pneumoniae*, *S. agalactiae*, and eubacteria in general. The three genus- or species-specific primers were used together with primers complementary to universal regions U3 and U8 of the 16S rRNA molecule (5, 27) in a two-step PCR assay. The first step consisted of amplification with the universal primers. This reaction amplified a general bacterial amplicon independently of which bacterial species was present in the PCR mixture. The second step in the assay, based on a dilution of the first amplification, was a PCR with four primers that amplified species-specific amplicons of different sizes. One of the main aims of the study was to evaluate the PCR assay in the diagnosis of bacterial meningitis by examination of CSF from patients with and without meningitis.

MATERIALS AND METHODS

Bacterial strains and culture methods. Two different strains each of *N. meningitidis* (418/88 and MO035), *H. influenzae*, *S. pneumoniae*, and *S. agalactiae* and one strain of *Staphylococcus epidermidis* were used as sources for analysis of the 16S rRNA

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gene. Meningococcal strain 418/88 is a serogroup A, serotype 4/21 CSF isolate from Sudan (20). Strain MO035 is a serogroup B, serotype 15 isolate from a patient in Norway with meningococcal disease (9). The other strains, kindly provided by Gunnar Lindahl, were isolated from CSF specimens sent to the Laboratory of Clinical Microbiology, Lund University Hospital, Lund, Sweden. The 133 bacterial isolates listed in Table 2 were obtained from the Department of Clinical Bacteriology at Uppsala University Hospital, Uppsala, Sweden. *Escherichia coli* JM105 was used as a host for bacteriophages M13mp18 and M13mp19 to generate single-stranded templates for DNA sequencing (28). JM105 was grown in twofold-concentrated YT broth, and all of the other bacteria were grown on chocolate or blood agar plates. The strains were stored at −70°C. The sensitivity of the PCR was tested with meningococcal strain 11-01135/85, a serogroup B isolated from CSF, which was serially diluted in sterile CSF devoid of bacterial DNA. CFU counts were made to estimate the concentration of bacteria. Simultaneous dilutions were performed in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) to verify the CFU count in CSF.

Patients and CSFs. Patients with suspected bacterial meningitis in Örebro County are subjected to lumbar puncture, and the CSF is examined at the Department of Clinical Microbiology and Immunology, Örebro Medical Center Hospital, Örebro, Sweden. Any CSF remaining after direct microscopy, culture, and antigen detection assays from 1970 onward has been stored uncentrifuged at −20 or −70°C. These stored samples, not more than one per patient, remained frozen or were thawed not more than twice before the present investigation. A retrospective study of the patient records, without regard to the PCR results, enabled us to make a definite diagnosis of either bacterial meningitis (culture and/or antigen positive), possible bacterial contamination of CSF, viral meningitis, or no central nervous system or meningeal infection.

CSF samples from 125 patients with bacterial meningitis (diagnosed between 1970 and 1993) were divided into the following two groups: I, 98 samples that should give positive results with the species-specific PCR primers (4 had been culture negative for *N. meningitidis*, and 1 had been culture negative for *S. pneumoniae*); II, 27 samples containing other bacteria.

CSFs from patients without bacterial meningitis were grouped as follows: IV, 46 CSF samples from patients with no verified bacterial meningitis (sampled in 1992) but without further diagnostic information; V, 28 CSF samples from patients with viral meningitis (sampled in 1990 and 1991) and 10 CSF samples from patients with parotitis meningitis (sampled from 1980 to 1983); VI, 80 CSF samples without signs of meningeal inflammation (sampled from 1980 to 1986), including normal concentrations of proteins and leukocytes. Group III (15 samples) was thought to be bacterially contaminated.

Amplification, cloning, and DNA sequencing of the 16S rRNA gene. A 0.99-kb region of the 16S rRNA gene in *N. meningitidis*, *H. influenzae*, *S. pneumoniae*, *S. agalactiae*, and *S. epidermidis* was amplified by using the PCR technique (18) and sequenced by using the dideoxynucleotide chain termination method of Sanger et al. (22). The forward u3′ primer (see Table 1) was, except for one mismatch, complementary to the highly conserved U3 region in the 16S rRNA of *E. coli*, positions 515 to 534 (5). Nucleotide 6 in the u3′ primer contained a T instead of an A to create a useful *Pst*I cloning site. The ru8′ primer (see Table 1) was complementary to the end of the 16S rRNA gene (positions 1522 to 1541), except for one mismatch. The location of the primer corresponds to the conserved Shine-Dalgarno sequence within the U8 region (24). The mismatch base at position 8 in the ru8′ primer (G instead of T) formed a *Bam*HI site at the 3′ end of the amplified fragments.

The amplification procedure was performed as described earlier (17). Several independently amplified 16S rRNA-encoding DNA fragments from two strains each of *N. meningitidis*, *H. influenzae*, *S. pneumoniae*, and *S. agalactiae* and one strain of *S. epidermidis* were cloned with the help of the *Pst*I and *Bam*HI sites in both orientations in M13mp18 and M13mp19. The inserts were sequenced on both strands in accordance with the protocol for single-stranded templates given in the reagent kit for DNA sequencing with Sequenase version 2.0 (United States Biochemical Corp., Cleveland, Ohio). The labeling component was [α-^{35}S]dATP from Amersham International plc (Little Chalfont, United Kingdom). The −40 universal sequencing primer specific for bacteriophage M13 was used to determine the sequences at the flanks of the inserted fragments. The remaining parts of the cloned PCR products were sequenced by using primers complementary to conserved sequences within the 16S rRNA gene (Table 1). Sequence analysis was performed with the Genetics Computer Group software package (3).

Conditions in the PCR assay. A seminested PCR strategy divided into two independent PCR incubations was used. The first step in the assay consisted of amplification with primers u3 and ru8 (see Table 1). The PCR was performed with a 50- or 25-μl reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 20°C), 1.5 mM $MgCl_2$, 10 μg of gelatin, each primer at 0.5 μM, each of the deoxyribonucleotides at 0.2 mM, and 0.025 U of *Taq* DNA polymerase (Boehringer GmbH, Mannheim, Germany) per μl. The reaction mixture was overlaid with 1 or 2 drops of mineral oil and processed in a programmable DNA thermal cycler (Perkin-Elmer Cetus Corp., Norwalk, Conn.). The samples consisted of boiled solutions of isolated bacteria in a preservation medium (10% glycerol–0.15 M NaCl in 50 mM Tris-HCl [pH 7.0]) or boiled CSF. The CSF samples were boiled for 15 min. We used a positive control (5×10^3 *N. meningitidis* OR173/87 [20] bacteria per ml in water) and a negative control (water) for each PCR. To eliminate any contaminating DNA in the reagents, the reaction mixture was UV irradiated in the reaction tubes for 5 min on a Macrovue Transilluminator (302 nm) before addition of a 10-μl CSF sample. The reaction mixtures were subjected to PCR under the following conditions: heat denaturation at 94°C for 6 min, 28 cycles with heat denaturation at 94°C for 1 min, primer annealing at 54°C for 1 min, and DNA extension at 72°C for 2 min. Finally, the samples were maintained at 72°C for 7 min to complete the synthesis of all strands. The second step in the assay consisted of amplification with primers NM, HI, STREP, and ru8 (see Table 1) under conditions similar to those described above but with annealing at 55°C for 25 cycles. The samples added (2 to 50 μl or 1 to 25 μl) were 1:500 dilutions in double-distilled, deionized water from the first reaction. PCR products from the two steps were visualized by 1.0% agarose gel electrophoresis with 1× TBE buffer stained with ethidium bromide, as described by Sambrook et al. (21).

Nucleotide sequence accession numbers. The partial 16S rRNA sequences shown in Fig. 1 were submitted to the European Molecular Biology Laboratory Data Library and have been assigned accession numbers Z22776 (*N. meningitidis*), Z22806 (*H. influenzae*), Z22807 (*S. pneumoniae*), Z22808 (*S. agalactiae*), and Z22809 (*S. epidermidis*).

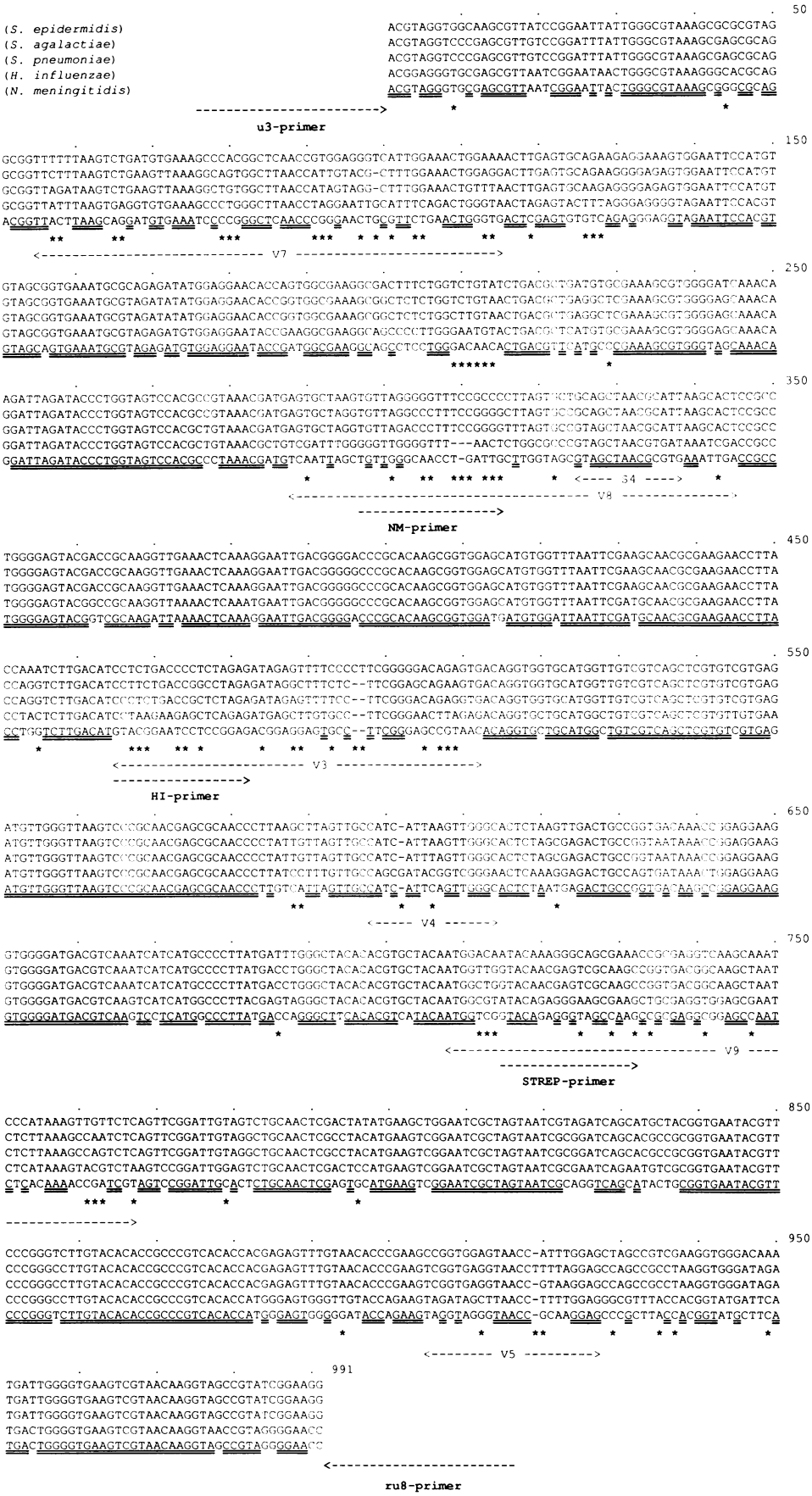
(S. epidermidis)
(S. agalactiae)
(S. pneumoniae)
(H. influenzae)
(N. meningitidis)
u3-primer
V7
V8
NM-primer
V3
HI-primer
V4
STREP-primer
V9
V5
ru8-primer

TABLE 1. Primers used in this study

Primer[a]	Position in 16S rRNA sequence of *E. coli*[b] (region)	Sequence[c] (5′ to 3′)	Length (nucleotides)	Use
u3′	515–534 (U3)	GTGCCTGCAGCCGCGGTAAT	20	PCR (*Pst*I site), cloning
u3	509–533 (U3)	AACT(C/A)CGTGCCAGCAGCCGCGGTAA	25	PCR, detection of eubacteria
u4′	793–810 (U4)	CTCGGTACCCTGGTAGTCCACGC	23	PCR (*Kpn*I site), cloning
NM	831–847 (V8)	TGTTGGGCAACCTGATTG	18	PCR, detection of *N. meningitidis*
u5′	907–926 (U5)	AAACTCAAATGAATTGACGG	20	Sequencing primer
ru5′	926–907 (U5)	CCGTCAATTCATTTGAGTTT	20	Sequencing primer
HI	998–1015 (V3)	CCTAAGAAGAGCTCAGAG	18	PCR, detection of *H. influenzae*
rs5′	1099–1083 (S5a)	CTCGTCGACGGGACTTAACCCAACA	25	PCR (*Sal*I site), cloning
u6′	1220–1240 (U6)	GGGCTACACACGTGCTACAAT	21	Sequencing primer
ru6′	1240–1220 (U6)	ATTGTAGCACGTGTGTAGCCC	21	Sequencing primer
STREP	1246–1263 (V9)	GTACAACGAGTCGCAAGC	18	PCR, detection of *Streptococcus* spp.
ru7′	1406–1392 (U7)	ACGGGCGGTGTGTAC	15	Sequencing primer
ru8′	1541–1522 (U8)	AAGGAGGGGATCCAACCGCA	20	PCR (*Bam*HI site), cloning
ru8	1541–1517 (U8)	AAGGAGGTGATCCA(G/A)CCGCA(G/C)(G/C)TTC	25	PCR, detection of eubacteria

[a] Primes denote primers used for cloning and sequencing work.
[b] Numbering is based on the corresponding positions in the secondary structure of the 16S rRNA from *E. coli* defined by Woese et al. (27). The universal (U), semiconserved (S), and variable (V) regions are adapted from Gray et al. (5).
[c] The u4′ and rS5′ primers have added bases at the 5′ end which lack corresponding locations in the 16S rRNA sequence. Alternated base positions are in parentheses.

RESULTS

Design of primers. Two-thirds of the 16S rRNA gene in two independently isolated strains each of *N. meningitidis*, *H. influenzae*, *S. pneumoniae*, and *S. agalactiae* was determined and aligned with the corresponding investigated sequence in *S. epidermidis* (Fig. 1). This sequence corresponds to the region between nucleotide positions 535 and 1521 in the 16S rRNA gene of *E. coli* (2). Comparison of the sequences in the two meningococcal strains showed three nucleotide differences in variable region V5 of the 16S rRNA model proposed by Gray et al. (5). The meningococcal strain isolated in Norway, MO035, contained an A instead of a G at positions 913 and 920 and a T instead of a C at position 928 compared with the sequence in strain 418/88 (Fig. 1), which is an isolate from Sudan. No nucleotide differences were found between the two strains of *H. influenzae* or the two strains of *S. pneumoniae* or the two strains of *S. agalactiae*. When the 16S rRNA sequences determined were compared with the corresponding sequences in the DNA database, minor nucleotide differences were found. The number of differences was in the same range as that of the differences found between the two meningococcal strains.

The percentages of homology between the 16S rRNA gene of *S. epidermidis* examined and the corresponding sequences in *S. agalactiae*, *S. pneumoniae*, *H. influenzae*, and *N. meningitidis* were 88.2, 87.4, 78.7, and 78.4%, respectively (Fig. 1). Four larger domains with greater variability were recognized throughout the alignment, at positions 57 to 128, 288 to 345, 465 to 512, and 712 to 767. These regions corresponded well to the locations of V7, V8, V3, and V9, which represent sequences that differ markedly in length and/or potential secondary structure and show a very low level of conservation, except in cases of very close relationship (5). The V8, V3, and V9 regions were selected for the design of species- and genus-specific primers (Table 1). A 0.7-kb fragment of the 16S rRNA gene in *N. meningitidis* was amplified with the primer set NM and ru8. The HI primer in combination with ru8 amplified a product of 0.5 kb when *H. influenzae* cells were used as the template. The design of the STREP primer was based on a sequence within the V9 domain that is common to both *S. pneumoniae* and *S. agalactiae* but different from that of *S. epidermidis*. A 0.3-kb fragment was obtained when the streptococci were amplified with the STREP and ru8 primers.

Specificity and sensitivity of the PCR assay. Different combinations of primers NM, HI, and STREP with ru8 showed no cross-reaction among the bacteria studied (data not shown). A total of 133 different bacteria belonging to 28 species were tested with the primer set u3 and ru8. All except three showed the expected general bacterial amplicon of 1.0 kb (Table 2). The sensitivity for bacterial detection in general was 0.98 (130 of 133). Some bacteria gave, in addition, faint products of incorrect sizes. The second step in the PCR assay for species-specific amplicons employed four primers (NM, HI, STREP, and ru8) and had a sensitivity of 0.94 (51 of 54). PCR products of incorrect sizes were also seen in some cases. A sensitivity of 0.90 (112 of 125) and a specificity of 0.96 (158 of 164) were reached when the PCR assay was used to detect the general bacterial amplicon in 304 clinical CSF samples (Table 3). These included samples from five patients with borreliosis (three CSF samples were positive) and three infected with *M. tuberculosis* (all positive). A species-specific amplicon was detected in 87 of 98 CSF samples (sensitivity, 0.89) from patients with proven meningitis due to *N. meningitidis*, *H. influenzae*, or streptococci (group I). No false-positive species-specific amplicons were seen. Altogether, eight CSF samples from patients with bacterial meningitis (groups I and II) were negative in both steps of the PCR assay, giving a sensitivity for the detection of bacteria of 0.94 (117 of 125). The detection limit for bacteria in CSF, estimated by using repeated serial

FIG. 1. Alignment of 16S rRNA sequences from *S. epidermidis* (989 bp), *S. agalactiae* (987 bp), *S. pneumoniae* (986 bp), *H. influenzae* (985 bp), and *N. meningitidis* (986 bp). Hyphens represent gaps introduced for maximum alignment. Arrows indicate the locations of variable regions (V) in accordance with the nomenclature of Gray et al. (5) and the positions of the primers used in the assay. Identical nucleotide positions are indicated by double underlining, and gaps or positions with three or more nucleotide differences are indicated by asterisks.

TABLE 2. PCR results obtained with 133 isolated bacteria suspended in preservation medium

Bacterial species	No. of strains	Amplicon detection	
		General bacterial	Species specific
Neisseria meningitidis	8	+	+
Haemophilus influenzae	23	+[c]	+[c]
Streptococcus pneumoniae	8	+[d]	+[d]
Streptococcus agalactiae	8	+	+
Streptococcus pyogenes	1	+	+
Streptococcus faecalis	4	+	+
Alpha streptococci	2	+	+
Listeria monocytogenes	4	+	
Listeria ivanovi	1	+	
Listeria innocua	1	+	
Staphylococcus epidermidis	39	+	
Escherichia coli	9	+	
Klebsiella pneumoniae	3	+[a]	—[b]
Enterobacter aerogenes	1	+	
Enterobacter cloacae	1	+	
Hafnia alvei	1	+	
Pseudomonas fluorescens	1	+	
Pseudomonas putida	1	+	
Salmonella typhimurium	1	+	
Salmonella enteritidis	1	+	
Salmonella dublin	1	+	
Salmonella thompson	1	+	
Borrelia burgdorferi	4	+	
Mycobacterium tuberculosis	3	+	
Mycobacterium paratuberculosis	1	+	
Diphtheroid rods	3	+	
Micrococcus luteus	1	+	
Lactococcus lactis subsp. *lactis*	1	+[a]	

[a] Additional fragments seen.
[b] —, fragments of incorrect size seen.
[c] One strain was PCR negative.
[d] Two strains were PCR negative.

dilutions of *N. meningitidis* 11-01135/85 in normal CSF, was 3 × 10^2 CFU/ml.

DISCUSSION

A two-step PCR assay for the diagnosis of acute bacterial meningitis was designed. The first PCR showed the presence of bacteria in general in the CSF. This amplification was found to be a good complement to the bacteria identified in the assay, since other organisms, such as *E. coli*, staphylococci, and *Listeria monocytogenes*, may cause acute meningitis. The second reaction in the assay, which was based on a dilution of the first amplified PCR mixture and consisted of an amplification with four primers complementary to sequences within the 1.0-kb PCR product, amplified species-specific amplicons. The PCR assay was evaluated with clinical CSF samples (Table 3). These results showed that bacteria could be detected with a sensitivity of 0.94 (117 of 125) in verified positive CSF, a sensitivity of 0.93 (112 of 120) when compared with culture-positive CSF, and a high specificity of 0.96 (158 of 164) with culture-negative CSF. The bacteria detected were correctly diagnosed as *N. meningitidis*, *H. influenzae*, and streptococci in 87 of 98 CSF samples (sensitivity, 0.89), and no false-specific bands were seen. Verification of PCR amplicons would probably be needed when working with unknown samples.

The primer set in the first reaction was found to amplify a 1.0-kb fragment in almost all of the bacterial species tested (Tables 2 and 3). However, some negative PCR results were found. The two PCR-negative *S. aureus* CSF samples were from patients with epidural abscesses and could be expected to have low concentrations of bacteria. This is probably also the case for the CSF samples classified as contaminated, which all came out as negative in the PCR assay. The assay was, however, sensitive enough to give positive results with five culture-negative CSF samples, four with *N. meningitidis* and one with *S. pneumoniae* verified by antigen detection, which all gave a 1.0-kb fragment and a species-specific fragment. A false-positive 1.0-kb fragment was seen in three culture-negative CSF samples and three viral meningitis CSF samples. The three culture-negative CSF samples contained macroscopic blood, and the three viral meningitis CSF samples had high cell counts. Such samples should not give a 1.0-kb PCR product per se, but a bacteremic stage could probably give enough bacteria in a sample with a mixture of CSF and blood. Contamination of the samples before, during, or after storage cannot be entirely ruled out.

The primers in the second PCR step were found to be specific for the bacterial dilutions that were tested (Table 3) and amplified a product in excess, even if the amount of the 1.0-kb fragment was too small to detect in agarose gels stained with ethidium bromide. Many of the CSF samples, containing, e.g., *Borrelia burgdorferi*, gave a 1.0-kb fragment in this second step of the PCR assay, sometimes together with other fragments of correct or incorrect sizes. This suggests that the first reaction is inhibited, and as the samples are diluted 500 times, a higher sensitivity is obtained in the second PCR. This also shows that sufficient amounts of primer u3 are evidently transferred to this step and that the additional 25 cycles increase the sensitivity of the PCR assay. Seven samples were negative with the species-specific primers and gave only the 1.0-kb fragment, which could be the effect of genetic differences between the primers and the 16S rRNA region.

The concentrations of *N. meningitidis* organisms that could be detected in CSF were determined to be approximately 3 × 10^5 CFU/ml in the first reaction after 28 cycles of amplification (data not shown) and 3 × 10^2 CFU/ml in the second reaction after 25 cycles. This gives a rough estimate of the detection level for bacteria in CSF. Differences between CSF samples do, however, exist, such as different numbers of CFU (13), different concentrations of PCR-inhibitory factors, and different efficacies of lysis of the bacteria and thus exposure of the DNA. Finally, the smaller amplicons are more difficult to detect than the larger ones with ethidium bromide staining on agarose gel. However, the larger PCR amplicons might also be amplified less efficiently than the smaller ones in the PCR assay. It has been reported that as few as five *H. influenzae* cells per PCR incubation were detected after 40 cycles of amplification with a primer set derived from outer membrane protein P6 (25). However, to eliminate false-positive reactions, the number of cycles was reduced from 40 to 35, which resulted in a higher level of *H. influenzae* detection (25). Another way of increasing the sensitivity is the nested-primer approach (11), which has been used to increase both specificity and sensitivity in the detection of cytomegalovirus (16). The strategy employing nested primers is similar to our approach. However, the nested approach increases the risk of contamination of the PCR with amplicons as reaction tubes have to be opened between the PCR steps. Furthermore, it makes it impossible to use commercially available anticontamination procedures. Problems associated with false-positive results, probably due to DNA in the *Taq* DNA polymerase purchased, were eliminated efficiently by UV irradiation. From our experience, this treatment did not affect the efficiency of the PCR assay.

In conclusion, this study demonstrates a PCR strategy which

TABLE 3. PCR results from CSF samples from 304 different patients with and without meningitis[a]

Group and etiology or diagnosis	No. of samples	No. of times amplicon detected				No. of times no amplicon detected
		0.7 kb	0.5 kb	0.3 kb	1.0 kb	
I PCR-verifiable bacteria						
Neisseria meningitidis	17	15			17 (2)[b]	
Haemophilus influenzae	51		45 (2)[c]		46 (3)[d]	3
Streptococcus pneumoniae	24			23 (3)[e]	20	1
Other streptococci	6			4	6 (2)[f]	
II Other bacteria						
Listeria monocytogenes	3				3	
Staphylococcus aureus	8				6	2
Staphylococcus epidermidis	4				4	
Escherichia coli	1				1	
Borrelia burgdorferi	5				3	2
Mycobacterium tuberculosis	3				3	
Acinetobacter species	1				1	
Bacillus sphaericus	1				1	
Pasteurella multocida	1				1	
III Probable contamination of CSF						
Streptococci	4					4
Staphylococcus aureus	3					3
Staphylococcus epidermidis	4					4
Escherichia coli	1					1
Klebsiella pneumoniae	1					1
Pseudomonas species	1					1
Diphtheroid rods	1					1
IV Culture-negative patients	46				3	43
V Viral meningitis	28				3	25
Parotitis meningitis	10					10
VI No CNS[g] or meningeal inflammation	80					80

[a] The PCR assay was designed to give a general bacterial amplicon of 1.0 kb and species-specific amplicons of 0.7 kb for *N. meningitidis*, 0.5 kb for *H. influenzae*, and 0.3 kb for streptococci.
[b] Of 17 positive results, 2 were positive only with the 1-kb amplicon and negative with the species-specific second PCR.
[c] Of 45, 2 were positive only with HI and negative for the 1-kb amplicon.
[d] Of 46 positive results, 3 were positive only with the 1-kb amplicon.
[e] Of 23, 3 were positive only with STREP and negative for the 1-kb amplicon.
[f] Of six positive results, two were positive only with the 1-kb amplicon.
[g] CNS, central nervous system.

can be used to identify more than one bacterial species with preserved sensitivity and specificity. The detection limit in the second reaction is better than that of most other specific, rapid tests used to examine CSF from patients suspected of having bacterial meningitis. PCR inhibitors seem to be the major cause of the few false results, and it would therefore be interesting to develop a method that separates the bacteria from the inhibitors.

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