JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1994, p. 2729–2737
0095-1137/94/$04.00+0

Vol. 32, No. 11

Development of PCR Assays To Detect Ampicillin Resistance Genes in Cerebrospinal Fluid Samples Containing *Haemophilus influenzae*

FRED C. TENOVER,[1]* MING BO HUANG,[1] J. KAMILE RASHEED,[1] AND DAVID H. PERSING[2]

Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333,[1] and Section of Microbiology, Department of Laboratory Medicine, Mayo Clinic, Rochester, Minnesota 55079[2]

Received 8 April 1994/Returned for modification 2 June 1994/Accepted 18 August 1994

We developed PCR primers specific for the bla_{TEM} and bla_{ROB} ampicillin resistance genes. The specificity of the primers was confirmed by testing a series of *Escherichia coli* isolates containing a variety of ampicillin resistance genes and a series of ampicillin-resistant and ampicillin-susceptible *Haemophilus influenzae* isolates. There was a perfect correlation between ampicillin MICs, the presence of β-lactamase (as determined by the nitrocefin test), and the results with the bla_{TEM} and bla_{ROB} primers. Isolates of *H. influenzae* and *Streptococcus pneumoniae* obtained from 25 frozen cerebrospinal fluid (CSF) specimens were also tested. Four of 14 *H. influenzae* isolates were positive with the bla_{TEM} primers; none were positive with the bla_{ROB} primers. Ampicillin MICs were determined for the *H. influenzae* isolates, and penicillin MICs were determined for the *S. pneumoniae* isolates. Only the four PCR-positive *H. influenzae* isolates had elevated MICs of ampicillin and were β-lactamase positive. None of the *H. influenzae* isolates contained the bla_{ROB} gene, and none of the *S. pneumoniae* isolates produced positive reactions with either primer set. We then used universal primers directed to conserved regions of rRNA and a *Haemophilus* detection probe to identify which of the 25 frozen samples of CSF contained *H. influenzae*. Fourteen of the 25 CSF specimens were positive for *H. influenzae*, which correlated with the number of organisms obtained by culture of the CSF samples. Four of the CSF samples were positive with the bla_{TEM} primer set, and these correlated with the four *H. influenzae* isolates that were positive when tested directly by PCR. The bla_{TEM} assay required the use of native *Taq* polymerase because Amplitaq preparations were contaminated with vector DNA that contained the bla_{TEM-1} gene.

PCR has been used to detect a wide variety of microorganisms directly in clinical specimens (24), including bacteria in blood, cerebrospinal fluid (CSF), and tissue (3, 10, 13, 15, 22, 25, 26, 30). PCR has also been used to characterize antimicrobial resistance genes in bacterial isolates (29), but the use of PCR to detect resistance genes directly in clinical samples has been limited. Telenti et al. and Hunt et al. used PCR to detect mutations associated with rifampin resistance in the *rpoB* gene of *Mycobacterium tuberculosis* (11, 28) directly in sputum samples, while Larder and Kemp used PCR to detect mutations associated with zidovudine resistance in human immunodeficiency virus thymidine kinase genes in blood samples (16).

In a similar fashion, PCR could be used to guide therapy in the early stages of bacterial meningitis by detecting resistance genes directly in CSF samples once a pathogen, such as *Haemophilus influenzae* (30) or *Neisseria meningitidis* (15, 22), has been identified. Algorithms linking various pathogens with the resistance genes that they may carry and that are most likely to compromise therapy may broaden the clinical utility of nucleic acid amplification assays.

The goals of this study were to develop PCR primer sets specific to the bla_{TEM} and bla_{ROB} genes, to detect and identify ampicillin resistance genes directly in CSF samples previously shown to contain ampicillin-resistant *H. influenzae*, and to validate the use of the universal primer set RW01 and DG74 (10) for detecting *H. influenzae* in CSF samples.

* Corresponding author. Mailing address: Nosocomial Pathogens Laboratory Branch, Hospital Infections Program (G08), Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333. Phone: (404) 639-3246. Fax: (404) 639-1381.

MATERIALS AND METHODS

Bacterial strains and CSF samples. *Escherichia coli* strains containing the bla_{TEM-1}, bla_{TEM-4}, bla_{TEM-9}, bla_{SHV-1}, bla_{SHV-3}, bla_{SHV-5}, bla_{CARB-2}, and bla_{CARB-4} β-lactamase genes (12) from the strain collection of the Centers for Disease Control and Prevention (CDC) were used as controls to test the specificity of the primer sets. Three strains of *H. influenzae*, each containing the bla_{ROB} gene (6) and kindly provided by Robert Daum, University of Chicago, were used as additional controls. The ampicillin MICs and β-lactamase test results for these strains are shown in Table 1. Twenty-five CSF specimens, previously shown by culture to contain either *H. influenzae* or *Streptococcus pneumoniae*, were recultured at the Mayo Clinic, coded, and shipped to CDC for analysis. The isolates recovered from the specimens, 14 *H. influenzae* and 11 *S. pneumoniae*, were also coded and sent separately to CDC. The MICs for these organisms are shown in Table 2. Organisms were reidentified by standard procedures (8, 14).

Antimicrobial susceptibility testing. Ampicillin MICs for the isolates were determined by broth microdilution with cation-adjusted Mueller-Hinton broth for *E. coli* isolates and cation-adjusted Mueller-Hinton broth containing 5% lysed horse blood for *H. influenzae* isolates (21). Penicillin MICs for the *S. pneumoniae* isolates were also determined in Mueller-Hinton broth containing 5% lysed horse blood (21). Breakpoints were those published by the National Committee for Clinical Laboratory Standards (21). Quality control organisms included *E. coli* ATCC 25922; *H. influenzae* ATCC 49247, ATCC 49766, and ATCC 10211; and *S. pneumoniae* ATCC 49619. β-Lactamase testing was performed with nitrocefin (Glaxo).

TABLE 1. Bacterial strains, MICs, and PCR results

Organism	β-Lactamase present[a]	Ampicillin MIC (μg/ml)	PCR result[b]	
			bla_{TEM}	bla_{ROB}
E. coli	+ (TEM-1)	>32	+	−
	+ (TEM-4)	>32	+	−
	+ (TEM-9)	>32	+	−
	+ (SHV-1)	>32	−	−
	+ (SHV-3)	>32	−	−
	+ (SHV-5)	>32	−	−
	+ (CARB-2)	>32	−	−
	+ (CARB-4)	>32	−	−
F50	−	4	−	−
F107	−	2	−	−
H. influenzae				
619	+ (TEM-1)	≥32	+	−
270	+ (TEM-1)	>32	+	−
358	+ (TEM-1)	>32	+	−
909	+ (TEM-1)	32	+	−
274	+ (TEM-1)	≥32	+	−
277	+ (TEM-1)	≥32	+	−
246	+ (TEM-1)	≥32	+	−
260	+ (TEM-1)	≥32	+	−
314	+ (TEM-1)	8	+	−
244	+ (TEM-1)	8	+	−
238	−	0.5	−	−
245	−	0.25	−	−
275	−	≤0.12	−	−
47	− (BLNAR)	4	−	−
51	− (BLNAR)	8	−	−
72	− (BLNAR)	8	−	−
36	− (BLNAR)	4	−	−
62	− (BLNAR)	8	−	−
888	+ (ROB-1)	>32	−	+
49	+ (ROB-1)	>32	−	+
109	+ (ROB-1)	8	−	+
1068	−	0.25	−	−
ATCC 49247	−	4	−	−
ATCC 49766	−	0.25	−	−
ATCC 10211	−	≤0.12	−	−

[a] +, β-lactamase activity present in strain; −, no β-lactamase activity. The type of β-lactamase is shown in parentheses.
[b] +, gene detected in strain; −, gene not detected in strain.

TABLE 2. MIC, β-lactamase, and PCR results for Mayo Clinic isolates

Organism	MIC (μg/ml)[c]	β-Lactamase present[a]	PCR results[b]	
			bla_{TEM}	bla_{ROB}
H. influenzae				
MAYO-1	<0.25	−	−	−
MAYO-2	<0.25	−	−	−
MAYO-3	<0.25	−	−	−
MAYO-4	<0.25	−	−	−
MAYO-5	32	+	+	−
MAYO-6	<0.25	−	−	−
MAYO-7	>32	+	+	−
MAYO-8	>32	+	+	−
MAYO-9	<0.25	−	−	−
MAYO-10	<0.25	−	−	−
MAYO-11	<0.25	−	−	−
MAYO-12	>32	+	+	−
MAYO-13	<0.25	−	−	−
MAYO-14	<0.25	−	−	−
S. pneumoniae				
MAYO-15	0.06	−	−	−
MAYO-16	0.06	−	−	−
MAYO-17	0.06	−	−	−
MAYO-18	0.06	−	−	−
MAYO-19	0.06	−	−	−
MAYO-20	<0.03	−	−	−
MAYO-21	<0.03	−	−	−
MAYO-22	0.06	−	−	−
MAYO-23	<0.03	−	−	−
MAYO-24	<0.03	−	−	−
MAYO-25	0.06	−	−	−

[a] +, β-lactamase activity present in strain; −, no β-lactamase activity.
[b] +, gene detected in strain; −, gene not detected in strain.
[c] For *H. influenzae*, MICs are of ampicillin; for *S. pneumoniae*, MICs are of penicillin.

DNA extraction. Initially, DNA was extracted from control strains with the Iso-Quick system (MicroProbe, Inc., Bothell, Wash.). Briefly, organisms were grown overnight on Mueller-Hinton chocolate agar at 37°C in 5% CO_2 for 24 h, suspended in buffer to a concentration of 5×10^5 CFU/ml, and extracted as described by the manufacturer. For *S. pneumoniae* isolates, the organisms were first treated with 1% deoxycholate for 20 min before suspending them in reagent A. After optimization of PCR conditions, the bacterial isolates recovered from CSF were lysed directly in the PCR mix. Briefly, organisms were grown overnight on Mueller-Hinton chocolate agar at 37°C in 5% CO_2. Two colonies were touched with an inoculating loop and suspended in the PCR mix, which was overlaid with mineral oil. Bacteria were lysed by heating the PCR mix at 95°C for 10 min. Samples were immediately cooled and kept on wet ice until PCR was initiated.

CSF samples were thawed, gently vortexed, and centrifuged for 30 min at 13,000 × *g* at 4°C. The supernatant was then decanted, and the pellet was extracted as described in the Iso-Quick package insert. Oyster glycogen (1 μg/ml; Sigma) was added as a carrier in the final precipitation step.

Primers, probes, and PCR conditions. The nucleotide sequences of the bla_{TEM}, bla_{ROB}, universal primers, and the *Haemophilus* detection probe are shown in Table 3. The universal primers and the *Haemophilus* probe are those described by Greisen et al. (10). The probe used to detect *H. influenzae* is complementary to a region of the 16S rRNA gene conserved among members of the family *Pasteurellaceae*. Specificity testing (10) showed that the probe detected 6 of 6 *H. influenzae* isolates and did not cross-react with 54 additional isolates representing 17 other species. However, the probe may cross-react with some *Actinobacillus* and *Pasteurella* strains. This probe is referred to as the *Haemophilus* detection probe. The 298-bp *Hin*cII-*Pst*I fragment of pBR322, which is derived from the 424-bp *Hin*cII-*Bgl*I probe previously shown by Levesque et al. to be specific for bla_{TEM} genes (18), was used as a bla_{TEM} probe in these studies. PCRs were carried out in either a Perkin-Elmer model 480 or 9600 thermal cycler. Amplitaq was obtained from Perkin-Elmer Cetus (Norwalk, Conn.), native *Taq* polymerase was from Boehringer Mannheim, and low-DNA *Taq* polymerase was provided by Roche Molecular Systems (Alameda, Calif.). The bla_{TEM} assays used a model 480 thermal cycler and the following cycling parameters: 94°C for 5 min; 30 cycles of 94°C for 2 min, 57°C for 1 min, and 72°C for 2 min; followed by 72°C for 10 min. The bla_{ROB} assays used a model 9600 thermal cycler and the following cycling parameters: 94°C for 5 min; 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s; followed by 72°C for 10 min. The cycling parameters for the universal primers, RW01 and DG74, with the model 9600 thermal cycler, were 95°C for 5 min; 30 cycles of 95°C for 1 s, 95°C for 1 min, 55°C for 1 s, and

TABLE 3. Oligonucleotide primers and probes

Primer[a]	Sequence	Size of PCR product (bp)
TEM(321)	5′ TGG GTG CAC GAG TGG GTT AC 3′	526
TEM(846)	5′ TTA TCC GCC TCC ATC CAG TC 3′	
ROB(419)	5′ ATC AGC CAC ACA AGC CAC CT 3′	692
ROB(1110)	5′ GTT TGC GAT TTG GTA TGC GA 3′	
RW01	5′ AAC TGG AGG AAG GTG GGG AT 3′	370
DG74	5′ AGG AGG TGA TCC AAC CGC A 3′	
Haemophilus detection probe (RDR125KG)	5′ GGA GTG GGT TGT ACC AGA AGT AGA T 3′	

[a] Primers include the universal primers RW01 and DG74 and the *Haemophilus* detection probe from Greisen et al. (10).

55°C for 1 min; followed by 72°C for 10 min. Amplification products were electrophoresed through 1.8% agarose gels containing molecular size standards (a *Hae*III digest of ϕX174 DNA with or without a *Hin*dIII digest of Lambda DNA; Gibco BRL). DNA from all gels was transferred to Zeta probe membranes (Bio-Rad, Hercules, Calif.) by the method of Southern (27) for DNA probe analysis with digoxigenin-labeled probes prepared at CDC.

The *Haemophilus* detection probe was synthesized with a 5′-end digoxigenin label (CDC Biotechnology Core) and de-

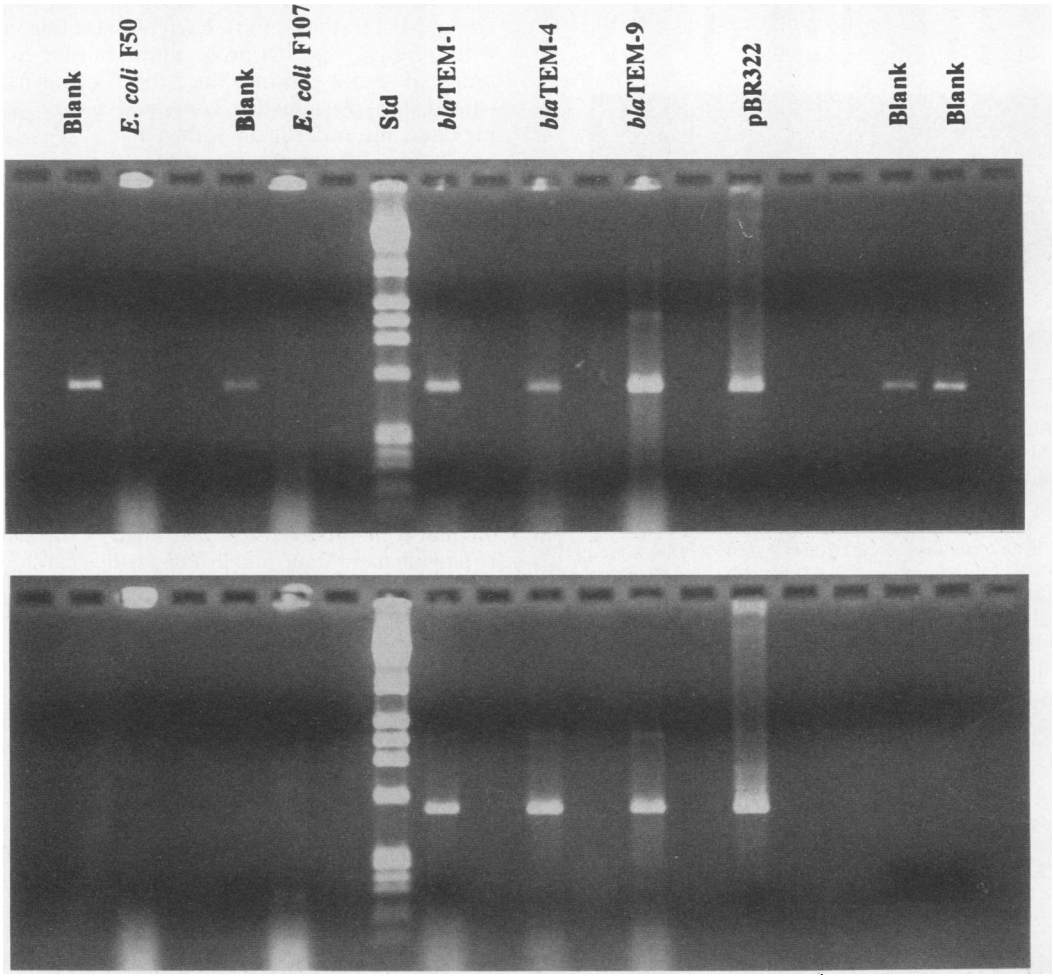

FIG. 1. (Top panel) Agarose gel showing amplification products of PCR assays for the bla_{TEM} gene by using Amplitaq polymerase. *E. coli* F50 and F107 are ampicillin-susceptible negative controls. Blanks are PCR assays with all reagents except template DNA. (Bottom panel) Same reactions with native *Taq* polymerase. The molecular size standard (Std) is *Hin*dIII-digested Lambda DNA plus ϕX174 DNA digested with *Hae*III. The fragment sizes in base pairs are 23,130, 9,416, 6,557, and 4,361 (seen as a large diffuse band at the top of the lane); 2,322 and 2,027 (as a doublet); 1,353, 1,078, 872, and 603 (as four distinct bands); 310 and 281 (large diffuse band); and 234, 194, 125, and 118. The 526-bp bla_{TEM} PCR product migrates between the 603- and 310-bp fragments of the ϕX174 standard.

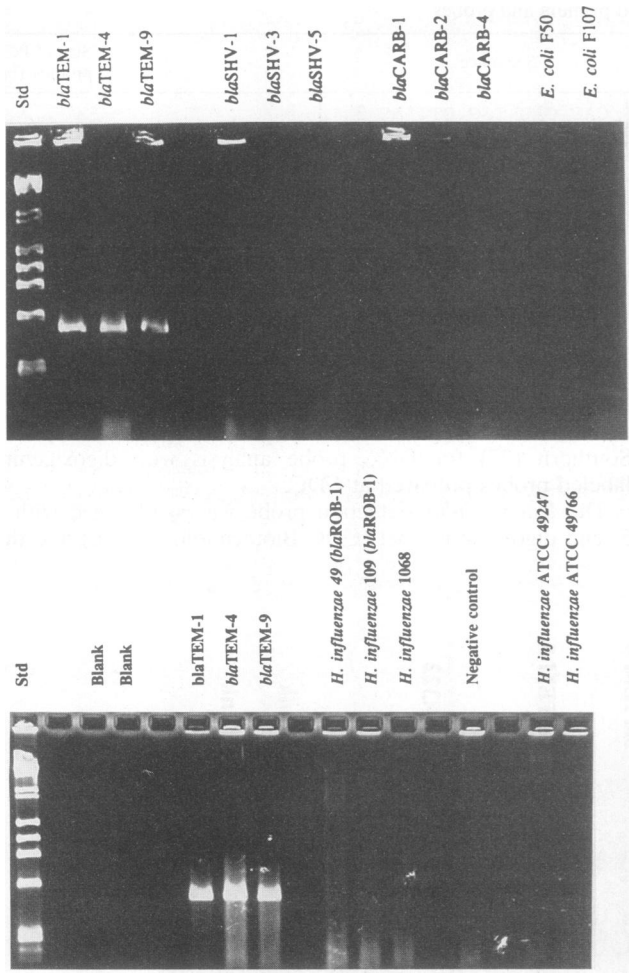

FIG. 2. (Top panel) Agarose gel showing amplification products obtained during specificity studies of bla_{TEM} PCR primers with *E. coli* containing the bla_{SHV} and bla_{CARB} genes as negative controls. *E. coli* F50 and F107 are ampicillin-susceptible negative controls. (Bottom panel) Additional specificity controls of the bla_{TEM} primers included *H. influenzae* bla_{ROB}-containing strains and *H. influenzae* ATCC 49247 (BLNAR) and ATCC 49766 (ampicillin susceptible). The negative control in the bottom panel is *E. coli* F50. The molecular size standard (Std) is *Hin*dIII-digested Lambda DNA plus ϕX174 DNA digested with *Hae*III. The fragment sizes in base pairs are 2,3130, 9,416, 6,557, and 4,361 (seen as a large diffuse band at the top of the lane); 2,322 and 2,027 (as a doublet); 1,353, 1,078, 872, and 603 (as four distinct bands); and 310 and 281 (as a doublet). The 526-bp bla_{TEM} PCR product migrates between the 603- and 310-bp fragments of the ϕX174 standard.

tected after hybridization by using the Genius kit (Boehringer Mannheim). Dot blots were prepared and hybridized with labeled probe as described by Gootz et al. (9).

RESULTS

Susceptibility testing of isolates. Ampicillin MICs were determined for the *E. coli* and *H. influenzae* control strains. The results are shown in Table 1. All *E. coli* β-lactamase-containing control strains were ampicillin resistant, showing ampicillin MICs of >32 μg/ml. Eighteen of the stock isolates of *H. influenzae* and the ATCC 49247 quality control strain were ampicillin resistant (MIC of ≥4 μg/ml). Of these, 13 were β-lactamase positive and 5 were β-lactamase-negative ampicillin-resistant (BLNAR) strains. The *H. influenzae* 1068 isolate, which was sent to CDC as a bla_{ROB} β-lactamase control, was ampicillin susceptible by MIC and devoid of plasmid DNA and thus was assumed to have lost the bla_{ROB} gene; it was also negative by PCR (see below). This isolate was carried throughout the study as a negative control.

Ampicillin MICs were also determined for the *H. influenzae* isolates from the CSF specimens, and penicillin MICs were determined for the *S. pneumoniae* CSF isolates (Table 2). Four of the *H. influenzae* CSF isolates had ampicillin MICs of ≥4.0 μg/ml and produced β-lactamase. None of the other *H. influenzae* isolates were ampicillin resistant, nor did they produce β-lactamase. All *S. pneumoniae* isolates were penicillin susceptible and β-lactamase negative.

Amplitaq versus native *Taq* polymerase. Primers for the bla_{TEM} gene were tested by PCR against a battery of *E. coli* isolates containing cloned β-lactamase genes. The three strains containing the bla_{TEM} gene each yielded an amplification product of the expected size (Fig. 1, top). Although no amplification products were observed from the negative control strains (*E. coli* F50 and F107) in the agarose gel, the reagent blanks that did not receive template DNA were positive, showing an amplification product with the expected size of 526 bp (Fig. 1, top). Each of these fragments hybridized with a bla_{TEM}-specific probe after transfer to a nylon membrane (data not shown). Since the *Taq* polymerase gene was cloned in a plasmid that contained a bla_{TEM} gene (17), we repeated the experiment with native *Taq* polymerase. When native *Taq* polymerase was used, the bla_{TEM} primers yielded the 526-bp product with only bla_{TEM}-containing strains; no amplification products were observed with the negative controls or the reagent blanks (Fig. 1, bottom). The specificity of the bla_{TEM} primers was then tested with *E. coli* isolates containing bla_{SHV} and bla_{CARB} β-lactamase genes, and *H. influenzae* isolates containing bla_{ROB} genes. Amplification products were observed only from bla_{TEM}-containing strains (Fig. 2).

Twenty-two stock strains of *H. influenzae* isolates were tested with the bla_{TEM} primer set. The 10 bla_{TEM}-containing isolates produced the 526-bp amplification product, while ampicillin-susceptible strains, the BLNAR strains, and the strains containing the bla_{ROB} gene did not (Table 1). The specificity of the amplification products was confirmed by transferring the DNA to a nylon membrane and probing with a bla_{TEM}-specific probe (data not shown). Similar experiments showed the bla_{ROB} primers to be specific for the bla_{ROB} gene (Table 1).

Next, the 25 isolates from the CSF samples (14 *H. influenzae* and 11 *S. pneumoniae*) were tested by PCR; four of the *H. influenzae* isolates were positive with the bla_{TEM} primers (Fig. 3A and C). The specificity of the amplification products was confirmed by Southern blot analysis with a bla_{TEM}-specific probe (Fig. 3B and D). Each of the positive CSF isolates corresponded to an *H. influenzae* isolate that was ampicillin resistant and β-lactamase positive. No false-positive reactions were noted. None of the *H. influenzae* isolates from the Mayo Clinic were positive with the bla_{ROB} primers, and none of the *S. pneumoniae* isolates were positive with the bla_{TEM} primers (Fig. 3) or the bla_{ROB} primers (data not shown).

Analysis of CSF samples. The conditions for using the bla_{TEM} and bla_{ROB} primers in CSF (as opposed to buffer) were optimized by testing four CSF control specimens. DNA was extracted from 200-μl samples, spiked with known concentrations of ampicillin-resistant *H. influenzae*, and tested with the bla_{TEM} and bla_{ROB} primers. Multiple bands were noticed for two of the spiked samples, suggesting that the annealing

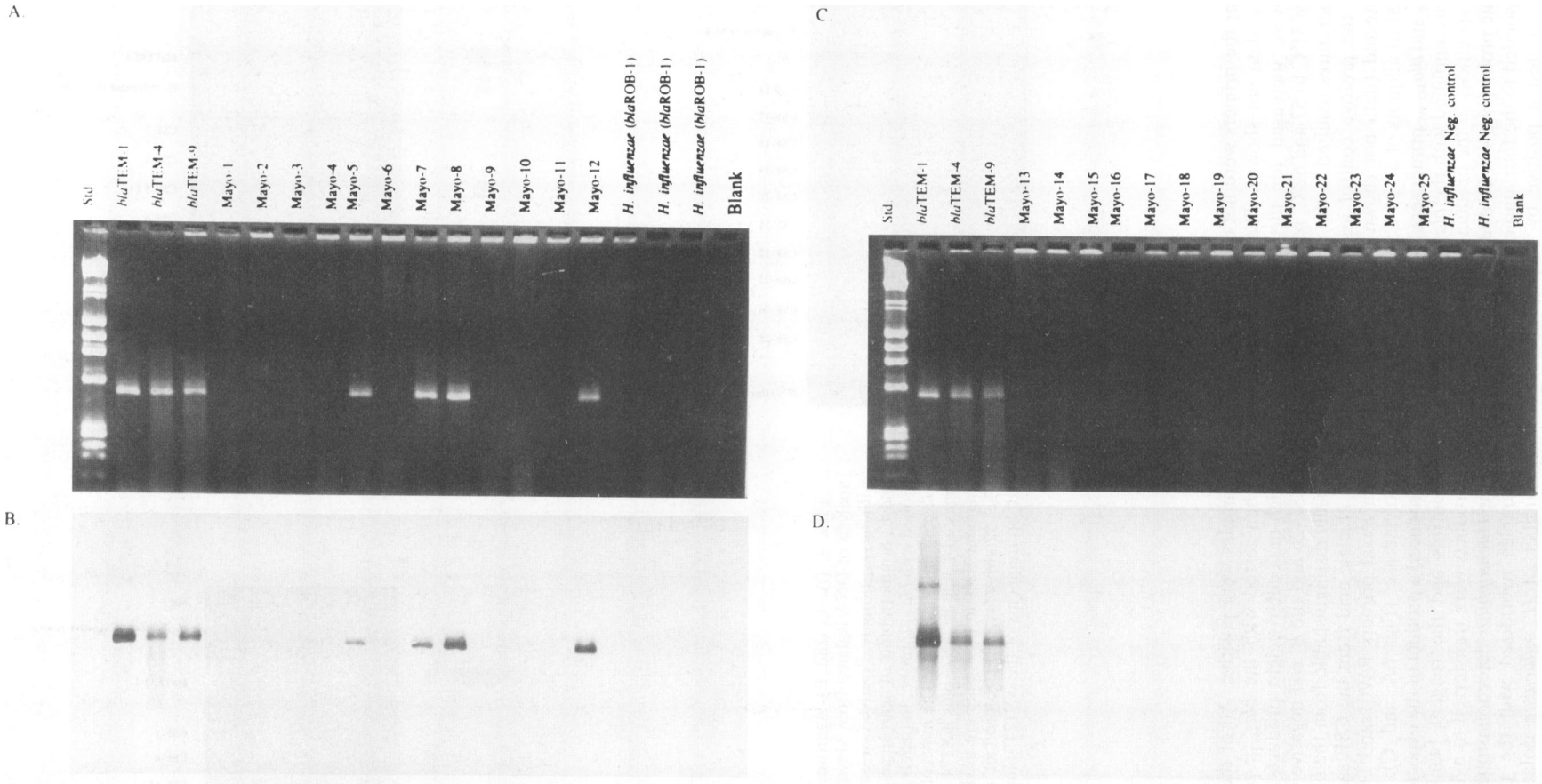

FIG. 3. (A and C) Agarose gel showing PCR results when template DNA from the 25 bacteria isolated from the CSF samples provided from the Mayo Clinic was tested with the bla_{TEM} primers. The negative controls in panel C are *H. influenzae* ATCC 49247 and ATCC 49766. (B and D) Southern blots of the agarose gels in panels A and C, respectively, probed with the 298-bp *Hin*cII-*Pst*I, bla_{TEM}-specific fragment of pBR322. The molecular size standard (Std.) is *Hin*dIII-digested Lambda DNA plus ϕX174 DNA digested with *Hae*III. The fragment sizes in base pairs are 23,130, 9,416, 6,557, and 4,361 (seen as a large diffuse band at the top of the lane); 2,322, 2,027, 1,353, 1,078, 872, and 603 (as six distinct bands); 310 and 281 (two diffuse bands); and 234, 194, and 125. The 526-bp bla_{TEM} PCR product migrates between the 603- and 310-bp fragments of the ϕX174 standard.

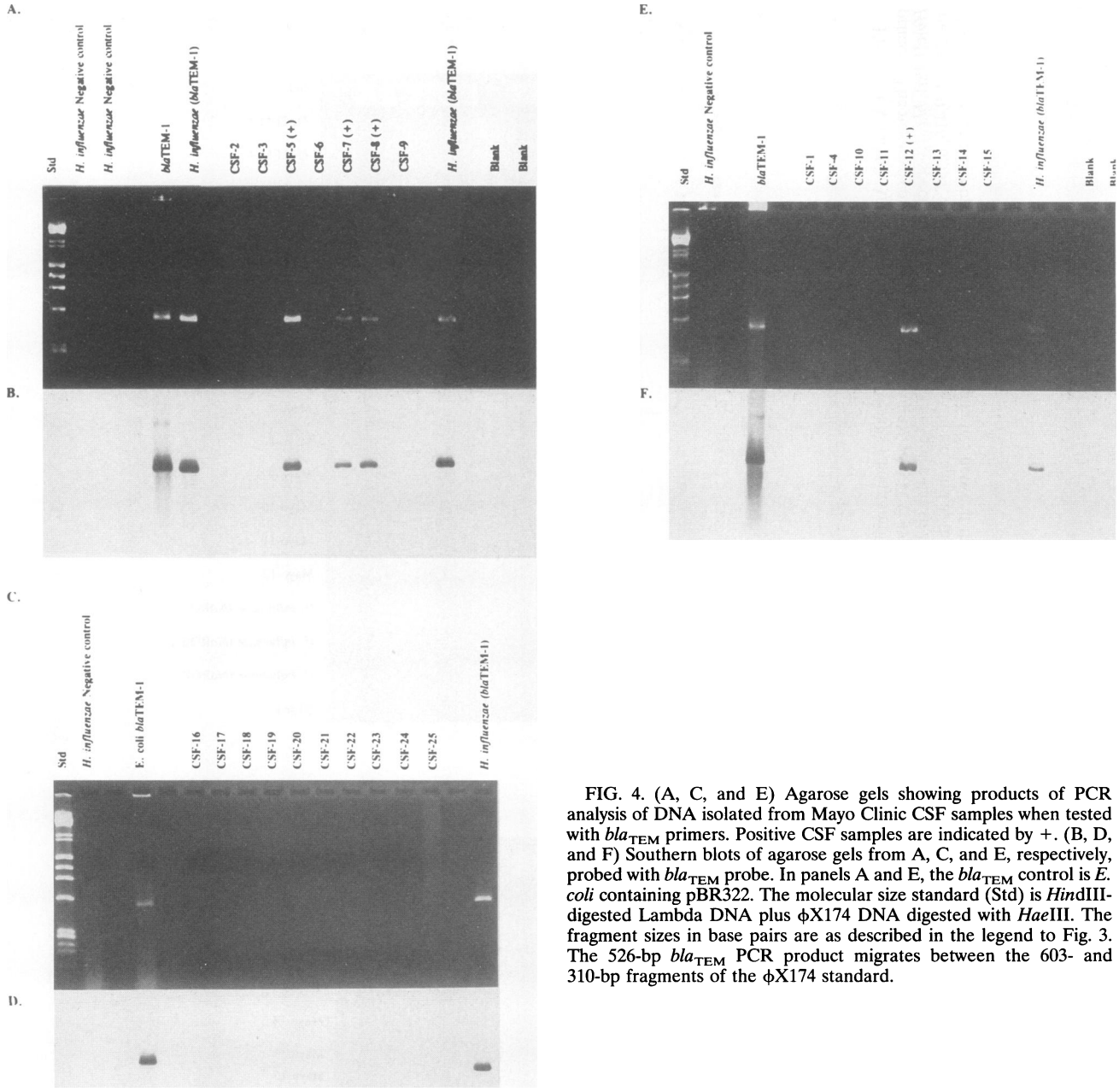

FIG. 4. (A, C, and E) Agarose gels showing products of PCR analysis of DNA isolated from Mayo Clinic CSF samples when tested with bla_{TEM} primers. Positive CSF samples are indicated by +. (B, D, and F) Southern blots of agarose gels from A, C, and E, respectively, probed with bla_{TEM} probe. In panels A and E, the bla_{TEM} control is *E. coli* containing pBR322. The molecular size standard (Std) is *Hin*dIII-digested Lambda DNA plus ϕX174 DNA digested with *Hae*III. The fragment sizes in base pairs are as described in the legend to Fig. 3. The 526-bp bla_{TEM} PCR product migrates between the 603- and 310-bp fragments of the ϕX174 standard.

temperatures for the primers were not optimized for use with clinical samples (data not shown). Therefore, the annealing temperatures were increased for the bla_{TEM} and bla_{ROB} primer sets from 51°C to 57 and 62°C, respectively. The higher annealing temperatures resulted in the production of a single band in all of the positive control assays; no false-positive bands were detected (data not shown). DNA was then extracted from 200-μl aliquots of all 25 CSF samples and tested with the bla_{TEM} and bla_{ROB} primer sets. DNA from four of the CSF samples was amplified with the bla_{TEM} primers (Fig. 4A, C, and E). The specificity of the products was determined with a bla_{TEM}-specific probe (Fig. 4B, D, and F). The positive CSF samples corresponded to the four ampicillin-resistant *H. influenzae* isolates that tested positive by PCR. No other CSF samples were positive. All of the CSF samples were negative when tested with the bla_{ROB} primer set (Fig. 5); although high-molecular-weight products were occasionally seen with CSF-25 and the *E. coli* negative controls, these products were easily distinguishable from the bla_{ROB}-specific products.

Nucleic acid from the CSF samples was amplified with the universal primers RW01 and DG74 by using low-DNA *Taq* polymerase (Fig. 6A and C). Each of the CSF samples produced a product of the expected size of 370 bp; the negative controls and the reagent blanks were negative. The PCR products were transferred to nylon filters and hybridized with the *Haemophilus* detection probe. DNA from 14 of the 25 CSF samples hybridized with the probe, corresponding to the number of *H. influenzae* isolates recovered from the CSF

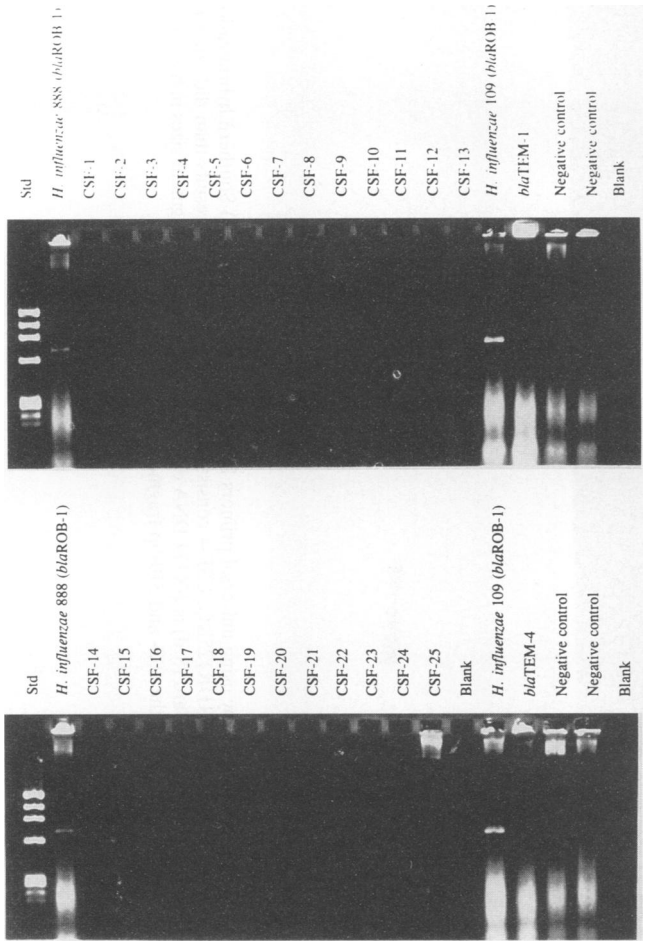

FIG. 5. Agarose gels showing amplification products of PCR assay of DNA isolated from CSF samples by using bla_{ROB} primers. The negative controls in both panels are *E. coli* F50 (left) and F107 (right). CSF-25, the negative control *E. coli* F50, and several other control strains occasionally yielded diffuse, nonspecific high-molecular-size bands. The molecular size standard (Std) is φX174 DNA digested with *Hae*III. The fragment sizes in base pairs are 1,353, 1,078, 872, and 603; 310 and 281 (diffuse band); and 234, 194, and 118. The 692-bp bla_{ROB} PCR product migrates between the 872- and 603-bp fragments of φX174.

samples by culture (Fig. 6B and D). The products from the universal primers from CSF yielding *S. pneumoniae* did not react with the *Haemophilus* detection probe (Fig. 6B and D). CSF-4 consistently (three times) yielded weaker results than did other *H. influenzae*-containing CSF samples. However, hybridization of the *Haemophilus* detection probe to the *H. influenzae* isolate that was cultured from this specimen yielded a strong positive result. This suggests that CSF-4 contains only low levels of *H. influenzae* DNA.

DISCUSSION

Although DNA probes have been used to detect the bla_{TEM} ampicillin resistance gene directly in *Neisseria gonorrhoeae* isolates in urethral samples from males (23) and in *E. coli* from urine (4), to the best of our knowledge PCR has not been used to detect the bla_{TEM} or bla_{ROB} genes in CSF samples. We chose to use PCR to detect ampicillin resistance genes and *H. influenzae* DNA in CSF as a model system for pairing sets of PCR primers for identification of bacteria and detection of antimicrobial resistance genes.

In this study, there was a perfect correlation between the detection of ampicillin-resistant *H. influenzae* by traditional MIC testing and the results of the direct PCR assay carried out on DNA extracted from CSF samples. Three key observations were made during this study. First, ampicillin resistance genes can be detected in CSF by PCR, but native *Taq* polymerase must be used to avoid false-positive results due to the contamination of Amplitaq preparations with cloning vector DNA (17). The bla_{TEM} gene may be the only resistance gene for which this presents a problem; nonetheless, it is an important consideration when setting up the assay. Second, universal primers can be used to detect *Haemophilus* spp. directly in CSF samples, provided that low-DNA *Taq* polymerase is used and the PCR products are confirmed with a specific DNA probe. The probe we used may be problematic in a clinical setting, because cross-reactions with DNA from *Actinobacillus* sp. and *Pasteurella* sp., although rare causes of meningitis, are possible (10). As Böttger has shown, *Taq* polymerase preparations are frequently contaminated with endogenous DNA that can lead to false-positive reactions (2). In lieu of using a low-DNA *Taq* preparation, incorporating isopsoralen-10 into the reagent mix followed by irradiation of the reaction vials with UV light can reduce false-positive reactions to a manageable level (19). This method, however, was not attempted in this study, but acceptable results were achieved with low-DNA *Taq* polymerase. Finally, it is critical that optimization of PCR assay conditions be conducted in the same milieu as the clinical samples to be tested, preferably in a clinical sample that is known to be negative for the analytes under investigation. After optimizing the PCR assay conditions for analysis of purified bacterial DNA, we found it necessary to raise the annealing temperature as much as 6°C to reduce nonspecific priming when testing CSF samples. Even with the change in temperature, we still noted some high-molecular-weight nonspecific products when using the bla_{ROB} primers on the CSF-25 sample and occasional nonspecific bands with the *E. coli* negative control strains.

In this study, all of the ampicillin-resistant *H. influenzae* isolates recovered from the CSF samples contained the bla_{TEM} gene (7); none contained the bla_{ROB} gene, and none were BLNAR strains. However, such strains are present in the United States (6, 20), and the BLNAR strains in particular pose potential problems for using a genetic approach to guide therapy since they would be misclassified as ampicillin susceptible by PCR. While we feel that strains of *H. influenzae* carrying either the bla_{TEM} or bla_{ROB} genes should be reported as ampicillin resistant, microbiologists in communities in which BLNAR strains have been isolated may choose to qualify their reports for strains that are negative by PCR for both genes with a statement such as "Lack of common resistance genes suggests that isolates are ampicillin susceptible." In most communities, however, the rare frequency of BLNAR isolates suggests that the isolates could be reported as ampicillin susceptible.

We have confirmed the utility of the universal primers RW01 and DG47 and the *Haemophilus* detection probe described by Greisen et al. (10) and confirmed the sensitivity of the primer systems. The variability of the signals produced after PCR, especially for sample CSF-4, suggests that the concentration of target DNA varies considerably from specimen to specimen. However, even the weak bands were detected by hybridization. We were also able to amplify *S. pneumoniae* DNA with the universal primers, but we did not achieve strong and reproducible hybridization signals with the pneumococcal probe suggested by Greisen and coworkers (10).

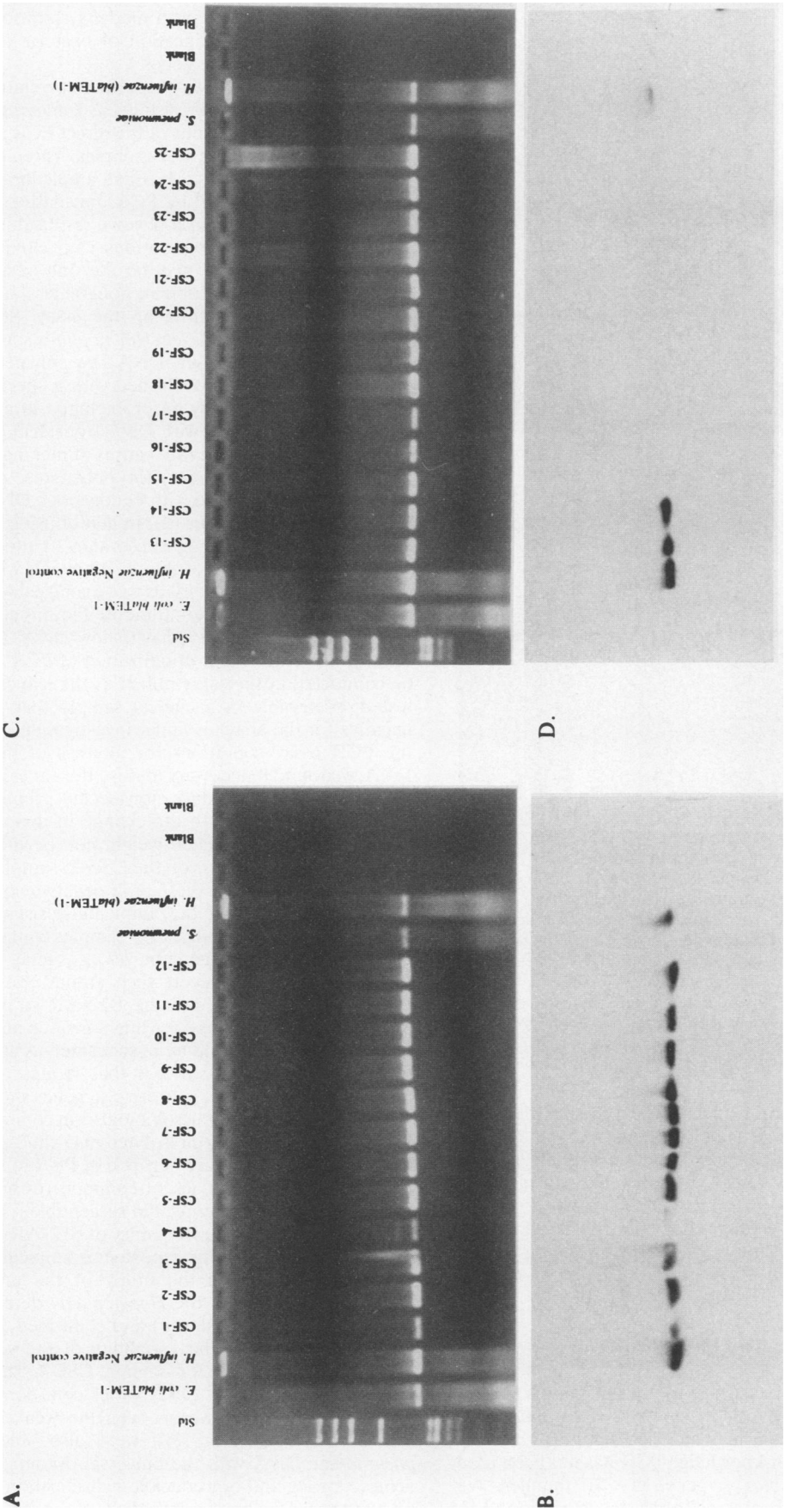

FIG. 6. (A and C) Agarose gels showing PCR amplification products of DNA obtained from CSF samples by using universal primers RW01 and DG74. (B and D) Southern hybridization blots of agarose gels in panels A and C, respectively, when hybridized with the *Haemophilus* detection probe RDR125KG. CSF-4 consistently yielded weaker PCR results than did other *H. influenzae*-containing samples. CSF-25 shows nonspecific high-molecular-size products. The molecular size standard (Std) is ϕX174 DNA digested with *Hae*III. The fragment sizes in base pairs are 1,353, 1,078, 872, 603, 310, 281, 234, 194, 118, and 72. The 370-bp rRNA PCR product migrates between the 603- and 310-bp fragments of the ϕX174 standard.

Thus, a more effective probe should be sought, given the importance of pneumococcal meningitis, especially in pediatric and elderly populations. While PCR primers have been described for pneumococcal penicillin-binding protein genes (5), analysis of the products requires the use of DNA sequencing gels or access to direct DNA sequencing, both of which are beyond the scope of all but a few clinical laboratories. Thus, there is no easy way to distinguish penicillin-resistant pneumococci from penicillin-susceptible strains by PCR. As PCR becomes more widely used in clinical laboratories, the value of using PCR to detect and characterize resistance genes carried by pathogens directly in clinical samples will likely receive more attention. Much in the same way Telenti et al. (28) and Hunt et al. (11) showed the value of using PCR on sputum samples to detect mutations associated with rifampin resistance in *M. tuberculosis* strains, so too our data show that PCR may be applied to aid in guiding treatment early in cases of bacterial meningitis. Although *H. influenzae* meningitis has decreased in the United States as a result of the widespread use of *H. influenzae* type b vaccine (1), the rates continue to be high in other parts of the globe.

ACKNOWLEDGMENT

We appreciate the help of Carolyn Baker, who contributed to the MIC studies.

REFERENCES

1. **Adams, W. G., K. A. Deaver, S. L. Cochi, B. D. Plikaytis, E. R. Zell, C. V. Broome, J. D. Wenger, and the *Haemophilus influenzae* Study Group.** 1993. Decline of childhood *Haemophilus influenzae* type b (Hib) disease in the Hib vaccine era. JAMA **269:**221–226.
2. **Böttger, E.** 1990. Frequent contamination of Taq polymerase with DNA. Clin. Chem. **36:**1258–1259.
3. **Brakstad, O. G., K. Aasbakk, and J. A. Maeland.** 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol. **30:**1654–1660.
4. **Carter, G. I., K. J. Towner, N. J. Pearson, and R. C. B. Slack.** 1989. Use of a nonradioactive hybridization assay for direct detection of gram-negative bacteria carrying TEM β-lactamase genes in infected urine. J. Med. Microbiol. **28:**113–117.
5. **Coffey, T. J., C. G. Dowson, M. Daniels, J. Zhou, C. Martin, B. G. Spratt, and J. M. Musser.** 1991. Horizontal transfer of multiple penicillin-binding protein genes, and capsular biosynthetic genes, in natural populations of *Streptococcus pneumoniae*. Mol. Microbiol. **5:**2255–2260.
6. **Daum, R. S., M. Murphy-Corb, E. Shapira, and S. Dipp.** 1988. Epidemiology of Rob β-lactamase among ampicillin-resistant *Haemophilus influenzae* in the United States. J. Infect. Dis. **157:** 450–455.
7. **Elwell, L. P., J. De Graaf, D. Seibert, and S. Falkow.** 1975. Plasmid-linked ampicillin resistance in *Haemophilus influenzae* type *b*. Infect. Immun. **12:**404–410.
8. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci, p. 238–257. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
9. **Gootz, T. D., F. C. Tenover, S. A. Young, K. P. Gordon, and J. J. Plorde.** 1985. Comparison of three DNA hybridization methods for detection of the aminoglycoside 2″-*O*-adenylyltransferase gene in clinical bacterial isolates. Antimicrob. Agents Chemother. **28:** 69–73.
10. **Greisen, K., M. Loeffelholz, A. Purohit, and D. Leong.** 1994. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. J. Clin. Microbiol. **32:**335–351.
11. **Hunt, J. M., G. D. Roberts, L. Stockman, T. A. Felmlee, and D. H. Persing.** 1994. Detection of a genetic locus encoding resistance to rifampin in mycobacterial cultures and in clinical specimens. Diagn. Microbiol. Infect. Dis. **18:**219–227.
12. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
13. **Kaneko, K., O. Onodera, T. Miyatake, and S. Tsuji.** 1990. Rapid diagnosis of tuberculosis meningitis by polymerase chain reaction (PCR). Neurology **40:**1617–1618.
14. **Kilian, M.** 1991. *Haemophilus*, p. 463–470. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
15. **Kristiansen, B.-E., E. Ask, A. Jekins, C. Fermer, P. Radstrom, and O. Skold.** 1991. Rapid diagnosis of meningococcal meningitis by polymerase chain reaction. Lancet **337:**1568–1569.
16. **Larder, B. A., and S. D. Kemp.** 1989. Multiple mutations in HIV-1 reverse transcriptase confer high levels of resistance to zidovudine (AZT). Science **246:**1155–1158.
17. **Lawyer, F. C., S. Stoffel, R. K. Saiki, K. Myambo, R. Drummond, and D. H. Gelfand.** 1989. Isolation, characterization, and expression in *Escherichia coli* of the DNA polymerase gene from *Thermus aquaticus*. J. Biol. Chem. **264:**6427–6435.
18. **Levesque, R. C., A. A. Medeiros, and G. A. Jacoby.** 1987. Molecular cloning and DNA homology of plasmid mediated β-lactamase genes. Mol. Gen. Genet. **206:**252–258.
19. **Meier, A., D. H. Persing, M. Finken, and E. C. Böttger.** 1993. Elimination of contaminating DNA within polymerase chain reaction reagents: implications for a general approach to detection of unusual pathogens. J. Clin. Microbiol. **31:**646–652.
20. **Mendelman, P., D. O. Chaffin, T. L. Stull, C. E. Rubens, K. D. Mack, and A. L. Smith.** 1984. Characterization of non-β-lactamase-mediated ampicillin resistance in *Haemophilus influenzae*. Antimicrob. Agents Chemother. **26:**235–244.
21. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed., vol. 13, no. 25. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
22. **Ni, H., A. I. Knight, K. Cartwright, W. H. Palmer, and J. McFadden.** 1992. Polymerase chain reaction for the diagnosis of meningococcal meningitis. Lancet **340:**1432–1434.
23. **Perine, P. L., P. A. Totten, K. K. Holmes, E. H. Sng, A. V. Ratman, R. Widy-Wersky, H. Nsanze, E. Habte-Gabr, and W. G. Westbrook.** 1985. Evaluation of a DNA hybridization method for detection of African and Asian strains of *Neisseria gonorrhoeae* in men with urethritis. J. Infect. Dis. **152:**59–63.
24. **Persing, D. H.** 1993. In vitro nucleic acid amplification techniques, p. 51–87. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. Principles and applications. American Society for Microbiology, Washington, D.C.
25. **Persing, D. H., D. Mathiesen, W. F. Marshall, S. R. Telford, A. Spielman, J. W. Thomford, and P. A. Conrad.** 1992. Detection of *Babesia microti* by polymerase chain reaction. J. Clin. Microbiol. **30:**2097–2103.
26. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N. Engl. J. Med. **323:**1573–1580.
27. **Southern, E.** 1975. Detection of species specific sequences among DNA fragments by gel electrophoresis. J. Mol. Biol. **98:**503–517.
28. **Telenti, A., P. Imboden, F. Marchesi, T. Schmidheini, and T. Bodmer.** 1993. Direct, automated detection of rifampin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single-strand confirmation polymorphism analysis. Antimicrob. Agents Chemother. **37:**2054–2058.
29. **Tenover, F. C., T. Popovic, and Ø. Olsvik.** 1993. Using molecular methods to detect antimicrobial resistance genes. Clin. Microbiol. Newsl. **15:**177–181.
30. **Van Ketel, R. J., B. De Wever, and L. Van Alphen.** 1990. Detection of *Haemophilus influenzae* in cerebrospinal fluids by polymerase chain reaction DNA amplification. J. Med. Microbiol. **33:**271–276.