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Evaluation of Polymerase Chain Reaction for Diagnosis of Pneumococcal Pneumonia

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To test the ability of the polymerase chain reaction (PCR) to detect *Streptococcus pneumoniae* in blood, we generated two sets of nested primers. The first defined 559-bp and 649-bp regions of the pneumolysin gene, and the second defined 445-bp and 553-bp regions of the autolysin gene. These nucleotide segments were detected in DNAs from isolates of all 20 pneumococcal serotypes tested, but they were not detected when used to test DNAs from 41 isolates of nonpneumococcal bacteria and fungi. The sensitivity was evaluated by using purified pneumococcal DNA. We were able to detect 10 fg of *S. pneumoniae* DNA, or 4.3 genome equivalents. Blood samples were obtained from 16 patients with culture-proven pneumococcal bacteremia and were subjected to PCR analysis. Of eight buffy coat fractions tested, six showed reactivity in the PCR with the pneumolysin primers, and five of the eight produced the expected products when tested with the autolysin primers (sensitivities, 75 and 63%, respectively). Of the eight whole-blood specimens tested, only three produced the expected products with either set of primers. Additionally, we tested 14 samples from patients with bacteremia that were culture positive for nonpneumococcal bacterial species, and 13 were negative (specificity, 93%). This combination of sensitivity and specificity may make detection of *S. pneumoniae* in blood by PCR in comparison with that by blood culture a very promising alternative for a means of definitive diagnosis.

Streptococcus pneumoniae remains a major cause of pneumonia, meningitis, sepsis, and otitis media worldwide. In Alaska, invasive pneumococcal disease persists as a common cause of illness and death among the Alaska Native population of all age groups, with yearly age-adjusted incidence rates of 75 cases per 100,000 people, in comparison with 13 cases per 100,000 people among non-Native Alaskans (8). Additionally, in some regions of the state, pneumococcal pneumonia and meningitis are more than 30 times more frequent in Alaska Native infants than in infants in "average" U.S. communities (8).

Currently, a definitive diagnosis of pneumococcal pneumonia requires the isolation of *S. pneumoniae* from blood or pleural fluid. Although these methods provide the most accurate diagnosis, cultures may be positive in less than 30% of patients with pneumococcal pneumonia (13, 33), they may be negative if the patient has received antibiotics before blood was taken, and they may take several days to yield results. An alternative to culture is antigen detection by using body fluids such as serum and urine. However, the methods now available, counterimmunoelectrophoresis and coagglutination, have failed to show uniform diagnostic sensitivity or specificity, even when applied to specimens collected from patients with culture-confirmed bacteremic pneumococcal pneumonia (5, 24, 31). A major problem for assays based on the immunologic detection of pneumococcal capsular polysaccharides is the necessity for the assay to detect 83 pneumococcal serotypes, each one of which has a different immunogenicity. Therefore, there remains a need for a species-specific method of detection that combines high sensitivity and high specificity and that would facilitate a definitive diagnosis of this disease.

Within the last decade, much progress has been made in the application of specific nucleic acid probes for the diagnosis of infectious disease pathogens. More recently, the development of the polymerase chain reaction (PCR) has made it possible to detect low numbers of infectious agents by selectively amplifying specific microbial genes or fragments of DNA more than 10^6-fold (26). Therefore, PCR has great potential for improving the ability of clinicians to diagnose infectious diseases caused by microorganisms that are fastidious or that cannot be cultured in vitro (2, 12, 18, 22, 25, 29).

In the study described here, we evaluated the PCR as a possible means of detecting pneumococcal DNA. Two sets of nested primers were constructed. One set amplified sequences within the cell wall autolysin protein gene (*lytA*), and the second set amplified sequences within the pneumolysin gene which codes for the hemolysin protein of *S. pneumoniae*. The specificities of our primer sets were evaluated with different nonpneumococcal bacteria, fungi, and 20 of the most common invasive pneumococcal serotypes. In addition, we evaluated the sensitivity of this technique for detecting purified pneumococcal DNA. We further report the results of the PCR assay as a diagnostic tool on samples of blood from patients with culture-proven pneumococcal bacteremia.

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TABLE 1. Bacterial strains used for specificity testing

Strain
Legionellae
L. pneumophila group 1
L. pneumophila group 6
L. micdadei
L. bozemanii
L. longbeachae
L. feeleii
L. dumoffii
Mycobacteria
M. tuberculosis
M. avium-M. intracellulare
Streptococci
S. bovis ATCC 9809
S. mitis C
S. intermedius (mitis I)
S. intermedius viridans group A
S. sanguis type IIC
S. faecalis 6056 (durans)
Group A streptococci types I DSC-491, II Lance T2/44/R64, ATCC 19615
Group B streptococci types 1a 090R, II V9, III D136C
Group C, *S. equisimilis*
Group F *Streptococcus* sp. strain I57
Group G streptococcus, Lancefield type H69C7
S. pneumoniae serotypes 1, 3, 4, 6B, 7F, 8, 9N, 9V, 10A, 11, 12F, 14, 17, 18C, 19A, 19F, 20, 22, 23F, 33
Chlamydiae
C. pneumoniae TW183 (Seattle)
C. pneumoniae CWL-029, 050 (CDC TWAR isolates)
C. trachomatis serovar D, L_2 (genital)
C. psittaci (two strains)
Fungus
Candida albicans
Miscellaneous
Staphylococcus aureus
Klebsiella pneumoniae
Listeria (three species listeriolysin positive)
Pseudomonas aeruginosa
Mycoplasma pneumoniae

MATERIALS AND METHODS

Bacterial species and blood samples. The bacterial species, *Chlamydia* species, and fungi used to evaluate the specificity of our PCR protocol were obtained from strain collections at the Centers for Disease Control and Prevention, Atlanta, and Anchorage, Alaska (Table 1). Whole-blood samples were obtained from 35 patients admitted to the Alaska Native Medical Center with radiographic evidence of community-acquired pneumonia and subsequent blood culture-confirmed bacteremia. Only blood remaining from the routine physician-directed diagnostic patient workups was obtained. Of these 35 blood samples, 16 were from patients with pneumococcal bacteremia and 14 were from patients with bacteremia; the 14 blood samples from bacteremic patients were culture positive for nonpneumococcal bacterial species. The five remaining blood samples were collected from patients diagnosed with pneumococcal pneumonia by antigen detection methods.

Preparation of samples for PCR analysis. (i) Bacterial species. Pneumococcal isolates representing common invasive serotypes (Table 1) were grown in Mueller-Hinton broth and were standardized to a concentration of approximately 1.5×10^8 cells per ml (0.5 McFarland standard). Aliquots of 1 ml were centrifuged at 8,832 × *g* for 2 min and were resuspended in 0.2 ml of 10 mM Tris-HCl (pH 8.1)–3 drops of 1.0% sodium deoxycholate. Suspensions were incubated at 37°C for 30 min and were then boiled for 5 min. These suspensions were centrifuged through 1.5-ml Sepharose CL6B columns (23), and the crude DNA was recovered as the eluate. A similar strategy was used for other streptococcal species, except that the cell wall structure was removed by resuspending the cell pellets in 0.2 ml of protoplast buffer (30% raffinose in 0.5 M K_2HPO_4 [pH 6.1], 50 μg of mutanolysin). After incubation of the suspension at 37°C for 30 min, protoplasts were recovered by centrifugation at 8,832 × *g* for 2 min, and the cell pellets were resuspended in lysis buffer (10 mM Tris-HCl [pH 8.1], 10 mM EDTA, 0.5% sodium dodecyl sulfate [SDS], 2 μl of proteinase K [10 mg/ml] per sample). Following incubation of the samples at 37°C for 60 min, the proteinase K was inactivated by incubating the samples at 95°C for 10 min. Free DNA was recovered by centrifuging the mixture through Sepharose CL6B columns. All samples were stored at −20°C until they were used.

For preparation of DNA from *Chlamydia* species, partially purified elementary bodies were thawed and sonicated; aliquots were then removed and centrifuged at 14,000 rpm in an Eppendorf microcentrifuge for 20 min. The supernatant was aspirated from each sample and 20 μl of 0.1 N NaOH was added. The samples were vortexed and incubated at 37°C for 30 min; 5 μl of 1 M Tris was then added. This volume represented a four- to eightfold concentration over the starting material, and 5 μl was used in the PCR immediately or following storage at −20°C.

For preparation of DNA from nonstreptococcal, nonchlamydial strains of bacteria, overnight cultures were adjusted to an optical density at 420 nm of 0.1 in phosphate-buffered saline, and then 0.500-ml aliquots were pelleted by centrifugation in a microcentrifuge for 10 min. Pellets were resuspended in 0.45% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.)–0.45% Tween 20–150 μg of proteinase K per ml and were then incubated for 1 h at 56°C and then 10 min at 95°C. Lysed samples were stored at 4°C until they were used for PCR tests.

(ii) Purification of pneumococcal DNA for sensitivity testing. Cells from an overnight growth of *S. pneumoniae* (serotype 3) were collected by centrifugation and were resuspended in 10 ml of 10 mM Tris-HCl (pH 8.0). Sodium deoxycholate was added to a final concentration of 0.2%, and the suspension was incubated at room temperature for 15 min. The lysate was extracted twice with an equal volume of phenol saturated with TE (Tris-EDTA) buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) and twice with chloroform. The DNA was then precipitated at −20°C overnight with 3 volumes of cold ethanol and was pelleted by centrifugation at 8,832 × *g* for 60 min. The precipitate was dried under vacuum, resuspended in 1.0 ml of TE buffer, and digested with 400 μg of RNase at 37°C for 60 min. The DNA was extracted again with buffer-saturated phenol and chloroform; this was followed by precipitation with ethanol as described above. Purified pneumococcal DNA was stored at −20°C.

(iii) Blood samples. Blood samples (1.5 to 7.5 ml) either were frozen at −20°C as whole-blood samples or were centrifuged at 1,407 × *g* for 20 min to facilitate buffy coat removal. The buffy coat fractions were removed and frozen at −20°C. DNA was extracted from a 100-μl aliquot of the buffy coat fraction or the whole blood. Cells were mixed with 0.5 ml of lysis buffer (10 mM Tris-HCl [pH 8.1], 10 mM EDTA, 50 mM NaCl, 2% SDS, 15 μl of proteinase K [10

TABLE 2. Sequences of oligonucleotide primers

Primer	Sequence (5' to 3')	Location within gene[a]
Pneumolysin[b]	AATAATGTCCCAGATAGAATGCAGTAT	424–450
	TGGAACAACTCAAGGTCAAGTTTGGTT	473–499
	AATGCACTGTTACATCAACGCTGGAAA	1006–1032
	GATACAACTCTGATTCCAATGTCGAAT	1047–1073
Autolysin[c]	AGAATGAAGCGGATTATCACTGGCGGA	107–133
	AACGGTTGCATCATGCAGGTAGGACCT	172–198
	AAAATCAATGGCACTTGGTACTACTTT	599–627
	TATATGCTTGCAGACCGCTGGAGGAAG	634–660

[a] Position numbers are from the start (ATG) codon.
[b] DNA sequence published by Walker et al. (30).
[c] DNA sequence published by Garcia et al. (9).

mg/ml] per sample) and were incubated at 60°C for 2 h. The DNA was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1; Sigma), precipitated at −70°C with 2 μg of oyster glycogen in 2 volumes of 100% ethanol containing 0.15 M sodium acetate, and pelleted by centrifugation at 8,832 × *g* for 10 min. The air-dried DNA pellets were then dissolved in 100 μl of TE buffer (10 mM Tris-HCl [pH 8.1], 1 mM EDTA). Ten-microliter samples were used for PCR analysis.

Oligonucleotide primers. There are two regions of the *S. pneumoniae* genome for which published sequences are available: the *lytA* gene, which codes for the major autolysin protein found in the cell wall of all pneumococci (9), and the pneumolysin gene, which codes for the pneumococcal hemolysin protein (30). From these sequences we constructed two sets of nested primers. The autolysin primers defined DNA target sequences of 445 and 553 bp in length, and the pneumolysin primers defined DNA target sequences of 559 and 649 bp in length. These sequences are described in Table 2. To verify the presence of amplifiable bacterial DNA in patient samples, we used broad-range primers in a separate single-step amplification protocol consisting of 25 cycles (6).

Amplification and detection. The PCR was performed in a 100-μl volume containing 1× PCR buffer II (Perkin-Elmer Cetus, Norwalk, Conn.), 0.2 mM (each) dATP, dCTP, dGTP, and dTTP, 6.5 mM $MgCl_2$, 1 μM (each) primer, 2.5 U of *Taq* polymerase (Perkin-Elmer Cetus), and 10-μl samples containing purified *S. pneumoniae* DNA, DNA from other bacteria, or DNA from blood specimens. Each tube was overlaid with 100 μl of mineral oil to prevent evaporation. After the mixtures were preheated at 90°C for 3 min, the amplifications were carried out in a programmable thermal cycler (Perkin-Elmer Cetus) in a two-step cycle, as follows: 2 min at 90°C to premelt the DNA, 30 s at 90°C for denaturation, 30 s at 70°C for annealing, and 30 s at 70°C for elongation. The elongation step was extended for 4 s for each subsequent cycle. The samples were amplified through 30 cycles by using the outer pair of primers for either the autolysin or pneumolysin genes. Ten microliters of the amplified mixture was then transferred to a fresh tube containing the reaction mixture with the inner pair of primers. The second amplification was allowed to proceed through 25 cycles. One positive and one negative control were processed at the end of each reaction series. Positive controls consisted of TE that contained 10 ng of pneumococcal DNA per ml. Negative controls consisted of TE only or blood samples from uninfected volunteers. Samples were prepared, and PCR amplification was carried out in two different rooms; positive-displacement pipettes were used throughout for dispensing all liquids to avoid contamination of samples.

After amplification, 25 μl of each sample containing amplified DNA was added to 12.5 μl of loading buffer containing 15% Ficoll (Sigma), 10 mM Tris-acetate (pH 8.2), 25 mM EDTA, and 0.36 mM Orange G (Sigma). Ten microliters of the amplified products was separated by electrophoresis in 4% agarose (3:1 NuSieve-Agarose; FMC Bioproducts, Rockland, Maine) gels in TAE buffer (Tris-acetate [pH 8.2], EDTA). The gels were stained with ethidium bromide (0.5 μg/ml), and the bands were identified by fluorescence with transilluminated UV light.

RESULTS

Optimization of reaction conditions. We examined the following parameters to optimize the pneumococcal amplification assay: (i) the concentration of *Taq* polymerase, (ii) the concentration of Mg^{2+}, (iii) the annealing temperature, and (iv) the number of cycles (11). In general, we obtained the best PCR amplification results with a primer-template annealing temperature of 70°C, a *Taq* polymerase concentration of 2.5 U, an Mg^{2+} concentration of 6.5 mM, and 30 cycles followed by 25 cycles of amplification.

Sensitivity of the nested primer amplification. To determine the lower limit of detection of pneumococcal DNA, 10-fold serial dilutions of a purified pneumococcal DNA stock solution with a known concentration of 1 μg/ml were analyzed by PCR with both the autolysin and pneumolysin nested primers in separate reactions. After PCR amplification, DNA was fractionated by agarose gel electrophoresis and was visualized under UV light by ethidium bromide fluorescence. Approximately 10 fg, or 4.3 genome equivalents of pneumococcal DNA, was the minimal amount of DNA needed to detect both the 559-bp pneumolysin fragment and the 445-bp autolysin fragment (Fig. 1A and B). To estimate the sensitivity of the PCR assay in terms of pneumococci which could be detected, whole blood and buffy coats were spiked with whole *S. pneumoniae* bacteria and were run as described above. The sensitivity in 50-μl volumes was found to be approximately 200 organisms for whole-blood samples and 20 organisms for buffy coat samples.

Specificities of the nested primers. To determine the specificities of the pneumolysin and autolysin primers, we tested the reactivities of DNAs from a variety of nonpneumococcal bacteria and fungi (Table 1) in the PCR assay. No PCR amplification products with either primer pair could be seen in the agarose gels. An additional primer pair, which has broad specificity for the conserved 16S rRNA sequences that are present in eubacteria (6), was used as an amplification control. This primer pair amplifies a 215-bp fragment. The broadly specific primers gave rise to a 215-bp DNA fragment in all samples tested. To demonstrate that the sequences amplified by the pneumolysin and autolysin primers are conserved within the various pneumococcal serotypes, DNAs from 20 serotypes of *S. pneumoniae* were purified and analyzed by PCR. The expected products were obtained from all pneumococcal serotypes tested when both the autolysin and pneumolysin primers were used (Fig. 2A to D).

PCR analysis of clinical samples. DNA was extracted from eight whole-blood and eight buffy coat fractions. The extracted DNA was subsequently tested by PCR with both the pneumolysin and the autolysin primer sets. Samples were examined in duplicate; only if both duplicate examinations

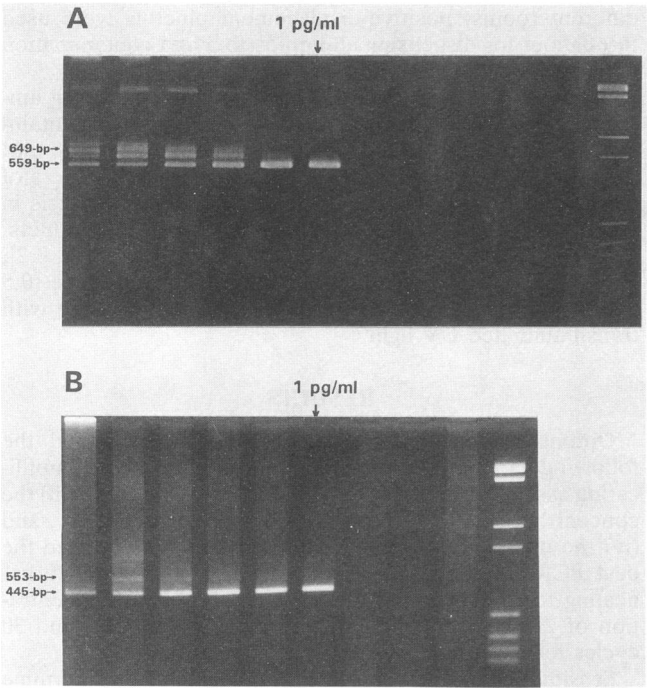

FIG. 1. Agarose gel electrophoresis of PCR-amplified products from 10-fold dilutions of purified *S. pneumoniae* DNAs. (A) Nested PCR with pneumolysin primers giving both the 649- and the 559-bp products; lane 12 (rightmost lane), molecular size markers. (B) Nested PCR with autolysin primers giving both the 553-bp and the 445-bp products; lane 10 (rightmost lane), molecular size markers.

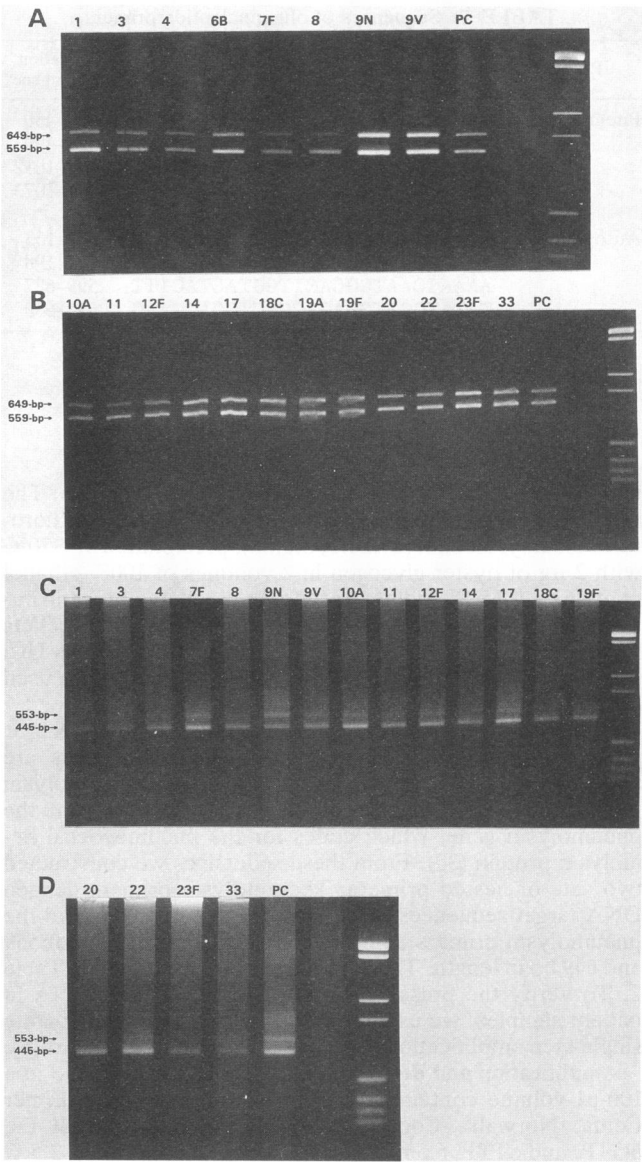

FIG. 2. Agarose gel electrophoresis of PCR-amplified products from pneumococcal isolates representing common invasive serotypes. (A and B) Nested PCR with pneumolysin primers giving both the 649- and the 559-bp products; gel A, lane 11, and gel B, lane 15 (unnumbered), molecular size markers. (C and D) Nested PCR with autolysin primers giving both the 553- and the 445-bp products; gel C, lane 15, and gel D, lane 7 (unnumbered), molecular size markers. PC, positive control (1 ng of pneumococcal DNA per ml).

were positive was the specimen reported as positive. Of eight buffy coat samples from patients with blood culture-confirmed pneumococcal bacteremia, six were positive with pneumolysin primers (sensitivity, 75%) and five were positive with autolysin primers (sensitivity, 63.5%) (Table 3). Of eight buffy coat samples from patients with blood culture-confirmed nonpneumococcal bacteremia, seven were negative by PCR with both primer sets (specificity, 87.5%). The one positive buffy coat sample was from a patient with blood culture-confirmed enterococcal bacteremia. For that patient, detectable pneumococcal capsular polysaccharide (serotype 3) was found in serum samples tested by counterimmunoelectrophoresis.

The diagnostic sensitivity decreased when DNA extracted from whole blood rather than from buffy coat samples was used. Of eight samples from patients with blood culture-confirmed pneumococcal bacteremia, only three were positive with both the pneumolysin and the autolysin primers (sensitivity, 37.5%). Of six whole-blood samples from patients with bacteremia of a nonpneumococcal cause, tested with both the pneumolysin and autolysin primers, none produced the expected products (specificity, 100%).

Buffy coat samples from five other patients who had radiographically confirmed pneumonia but whose blood cultures were negative for *S. pneumoniae* and who were positive for pneumococcal capsular polysaccharide antigen by counterimmunoelectrophoresis (serotypes 1, 6, and 19) were tested by PCR. Of the buffy coat samples from these five patients, pneumococcal DNA was detectable by PCR when both primer sets were used for three of the patients.

DISCUSSION

In the present study we evaluated the capability of a nested primer PCR to detect *S. pneumoniae* DNA in the blood of patients with invasive pneumococcal disease. Detection of pneumococcal DNA in the blood of patients with pneumonia and bacteremia by PCR was compared with detection in blood culture. While blood culture remains the diagnostic "gold standard" by which other techniques are measured, its sensitivity may be limited because patients may not be bacteremic at the time that the blood is drawn for culture (13, 33). In turn, this may limit the number of patients with pneumonia that show positive results for *S. pneumo-*

TABLE 3. PCR analysis of clinical samples from patients with pneumococcal and nonpneumococcal bacteremia

Blood culture result	No. of clinical samples	Buffy coat				Whole blood			
		PCR (pneumolysin)		PCR (autolysin)		PCR (pneumolysin)		PCR (autolysin)	
		No. (%) positive	No. (%) negative	No. (%) positive	No. (%) negative	No. (%) positive	No. (%) negative	No. (%) positive	No. (%) negative
S. pneumoniae	8	6 (75)	2 (25)	5 (63)	3 (37.5)	3 (37.5)	5 (62.5)	3 (37.5)	5 (62.5)
Other bacterial species[a]	8	1 (12.5)	7 (87.5)	1 (12.5)	7 (87.5)	0 (0)	6 (100)	0 (0)	6 (100)
Total	16	7	9	6	10	3	11	3	11

[a] *Haemophilus influenzae*, *Bacteroides fragilis*, *Morganella* sp., *Escherichia coli*, *Klebsiella* sp., beta-hemolytic streptococcus, group A streptococcus, *Proteus mirabalis*, *Candida albicans*, *Enterococcus* sp., *Pseudomonas* sp., gram-positive species, gram-negative species, viridans group streptococcus.

niae on blood culture. An added complication is that many patients are pretreated with antibiotics before their blood is cultured.

To ensure maximum sensitivity and specificity, a nested primer amplification strategy was used to reduce the possibility of nontarget amplification and to dilute out inhibitors that might initially be present in the sample. The two primer sets used in the present study were found to have similar specificities. Using this nested primer amplification protocol, we were able to detect 10 fg of purified pneumococcal DNA, which represents 4.3 genome copies. When whole blood or buffy coats were spiked with whole *S. pneumoniae*, the sensitivity was 200 organisms for whole blood and 20 organisms for buffy coat preparations. The increase in sensitivity when buffy coats were tested may result from a decrease in heme, which has been reported as a *Taq* polymerase inhibitor (11), and an increase in the concentration of target DNA. The decrease in sensitivity seen in these mock clinical samples when compared with the sensitivity obtained with purified pneumococcal DNA is not uncommon, because it has been reported previously that some sensitivity may be lost in the transition to clinical samples that require extraction and that may contain high levels of background human DNA (2).

The DNAs in 16 clinical samples (whole-blood or buffy coat preparations) were extracted with phenol to remove proteins and potential thermostable inhibitors of DNA polymerase activity; this was followed by precipitation with ethanol to concentrate amplifiable DNA (27). By this procedure, the sensitivity of PCR for the detection of pneumococcal DNA in whole blood of patients with culture-proven pneumococcal disease was only 35% with either autolysin or pneumolysin primers. This low sensitivity may be attributed to a relatively low concentration of target DNA (number of organisms per milliliter) or to the incomplete removal of DNA polymerase inhibitors. Also, samples tested by PCR were not always collected on the day of blood culture but were collected within 72 h of the day that blood was culture positive. This fact is important, because patients may have been treated with antibiotics soon after admission and after the initial blood sample for blood culture was taken. Such treatment may contribute to a reduced level of pneumococcal DNA in the blood of patients with invasive disease. In addition, although clinical samples were collected and stored at 4°C for up to 7 days prior to freezing and processing for PCR, the pneumococcal DNA originally present in culture-positive samples may have degraded. While improper storage of samples may lead to degradation of DNA and a false-negative result, we found that incubating whole *S. pneumoniae* organisms or purified pneumococcal DNA in whole blood for 12 days at 4°C failed to affect the amplification of pneumococcal DNA.

Clearance of viable and nonviable pneumococci from the blood depends on a complex interaction of specific and nonspecific factors, together with phagocytic cells (1). In vitro studies have shown that the addition of pneumococci to whole blood results in rapid phagocytosis. Consequently, phagocytes should provide a mechanism for collecting and concentrating bacterial pathogens. In the present study we tested DNA extracted from buffy coats collected from whole blood of patients with bacteremia. Of eight buffy coat fractions from patients with pneumococcal bacteremia, six produced the expected DNA products when the pneumolysin primers were used, while five of the eight produced the expected DNA products when the autolysin primers were used (sensitivities, 75 and 63%, respectively). We believe that the reason that pneumococcal DNA was not detected in buffy coat preparations from patients with culture-proven pneumococcal bacteremia was because it was present at levels below the sensitivity of the amplification technique, most likely because patients received antibiotics before the blood specimen tested by PCR was collected.

When blood specimens from 14 patients with culture-proven nonpneumococcal bacteremia were subjected to PCR analysis with both primer sets, one specimen yielded the autolysin and pneumolysin gene products. This specimen was from a patient with blood culture-confirmed enterococcal bacteremia. This finding was intriguing but could not be explained by the lack of specificity of the reaction. When DNAs from two strains of enterococci were used as templates in the PCR assay, no amplification products were detected by agarose gel electrophoresis. A more probable explanation is that this patient was coinfected with *S. pneumoniae*. Pneumococcal capsular polysaccharide (serotype 3) was detected in serum by counterimmunoelectrophoresis. The PCR also detected *S. pneumoniae* DNA in three buffy coat preparations from five patients whose blood cultures were negative yet whose urine had detectable pneumococcal capsular polysaccharide. This result supports the usefulness of PCR for detecting pneumococcal DNA in the absence of culturable organisms.

While PCR offers the possibility for improved detection of *S. pneumoniae* in the blood of patients with invasive pneumococcal disease, further work is needed to increase its utility. The clinical potential of this method has been limited by the complexities of the sample preparation and detection methods. Phenol extractions make specimen preparation time-consuming and cumbersome. A number of alternative methods for sample processing have been reported (7, 10, 20, 32). However, noted limitations include the use of very small amounts of whole blood (20), degrees of inhibition on DNA amplification, and a decrease in amplification efficiency of the processed samples over time (32). Most recently, investigators have described (4) a method that is

based on selective lysis and washing of erythrocytes with a washing solution and then DNA extraction with a heat-detergent treatment on the separated peripheral blood lymphocytes.

The initial detection methods that have been reported (15, 17, 26) involved gel electrophoresis combined with either ethidium bromide or ^{32}P-labeled probes to visualize amplified products. These methods are not entirely appropriate for screening large numbers of samples, and the inherent disadvantages with using radioisotopes include cost and the instability of the labeled probes. More recently, investigators (3, 14, 16, 19, 21, 28) have reported on methods that use nonradioactive techniques, such as time-resolved fluorescence, chemiluminescence, fluorescence, or color as substitutes for radioactivity. These methods have been found to have sensitivities similar to those of methods that use radioisotopes and can be easily designed for testing large numbers of samples.

The results reported here are encouraging for the potential use of PCR technology in the rapid and definitive identification of *S. pneumoniae* in blood specimens. A carefully controlled prospective study to assess this approach is now necessary.

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