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Real-Time Identification of *Pseudomonas aeruginosa* Direct From Clinical Samples Using a Rapid Extraction Method and Polymerase Chain Reaction (PCR)

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Pseudomonas aeruginosa has emerged as one of the most problematic Gram-negative nosocomial pathogens. Bacteremia caused by *P. aeruginosa* is clinically indistinguishable from other Gram-negative infections although the mortality rate is higher. This microorganism is also inherently resistant to common antibiotics. Standard bacterial identification and susceptibility testing is normally a 48-hour process and difficulty sometimes exists in rapidly and accurately identifying antimicrobial resistance. The Polymerase Chain Reaction (PCR) is a rapid and simple process for the amplification of target DNA sequences. However, many sample preparation methods are unsuitable for the clinical laboratory because they are not cost effective, take too long to perform, or do not provide a good template for PCR. Our goal was to provide same-day results to facilitate rapid diagnosis. In this report, we have utilized our rapid DNA extraction method to generate bacterial DNA direct from clinical samples for PCR. The lower detection level for *P. aeruginosa* was estimated to be 10 CFU/ml. In addition, we wanted to compare the results of a new rapid-cycle DNA thermocycler that uses continuous fluorescence monitoring with the results of standard thermocycling. We tested 40 clinical isolates of *P. aeruginosa* and 18 non-*P. aeruginosa* isolates received in a blinded fashion. Coded data revealed that there was 100% correlation in both the rapid-cycle DNA thermocycling and standard thermocycling when compared to standard clinical laboratory results. In addition, total results turn-around time was less than 1 hour. Specific identification of *P. aeruginosa* was determined using intragenic primer sets for bacterial 16S rRNA and *Pseudomonas* outer-membrane lipoprotein gene sequences. The total cost of our extraction method and PCR was $2.22 per sample. The accuracy and rapidness of this DNA-extraction method, with its PCR-based identification system, make it an ideal candidate for use in the clinical laboratory. J. Clin. Lab. Anal. 15:131–137, 2001.

Key words: polymerase chain reaction (PCR); bacteria; DNA extraction; real-time fluorescence; clinical samples; rapid PCR; *Pseudomonas*

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen that in recent years has emerged as one of the most important nosocomial pathogens and a leading cause of morbidity and mortality among patients compromised by surgical wounds, burns, trauma or cancer (1). This organism is inherently resistant to common antibiotics and even survives in antiseptics (2). Bacteremia due to *P. aeruginosa* is particularly life threatening and is associated with a high crude mortality rate ranging from 25–50% (3,4). Studies have shown that when administered early, appropriate antimicrobial therapy is associated with a lower attributable mortality rate, suggesting that this is one of the most important factors contributing to a favorable outcome (5). In the clinical laboratory, standard bacterial identification and susceptibility testing frequently requires as long as 48 hours to report and there may be difficulty in rapidly and accurately identifying antibiotic resistance. Therefore, it is crucial for clinical laboratories to have accurate and simple methods for the identification and confirmation of *P. aeruginosa* which are more rapid than standard culture.

The use of PCR for the detection of *P. aeruginosa* has been previously described (6–12) utilizing many different techniques to generate template DNA from various sources for PCR. These techniques may require additional steps for cell lysis or removal of potential inhibitors from some clinical specimens. In the clinical laboratory, these steps can add to the cost per test, increase sample processing time, and eliminate the formation of a general extraction procedure that can be easily and rapidly applied to identify a variety of microor-

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ganisms in a variety of clinical samples (blood, sputum, or urine). Also, some PCR methods require a pure sample from a subculture, thus delaying turn-around time (TAT) for results and necessitating the use of special media.

We have previously described a simple and rapid extraction method of bacteria DNA direct from clinical samples as well as a simple PCR procedure for the direct identification of Methicillin-Resistant Staphylococci (13). The entire procedure can be performed with a results TAT within 4 hours following the detection of positive blood-culture bottles or urine samples. The purpose of this study was to determine whether direct identification of *P. aeruginosa*, utilizing a new rapid-cycle DNA thermocycler (14), which uses continuous fluorescence monitoring utilizing a double-stranded-DNA-specific dye for real-time results, would agree with standard thermocycling results. We were able to detect 10 CFU/ml of *P. aeruginosa* and, using the rapid-cycle continuous fluorescent monitoring, we were able to decrease the TAT from 4 hours to less than 1. The total direct-supply cost (including PCR amplification of the target) was as little as $2.22 per sample and a cost-per-sample analysis was done to show that PCR detection optimized for use in the clinical laboratory could be cost effective when compared to standard methods.

MATERIALS AND METHODS

Bacterial Strains

To develop the rapid DNA-extraction method and test the limiting dilution, two *Pseudomonas* isolates (*P. aeruginosa* 31B-6843 clinical isolate and *P. stutzeri*, ATCC 17588) were used. In order to assess the specificity of the PCR for *P. aeruginosa*, we used a representative list of pseudomonads, Gram-negative and Gram-positive bacteria (Table 1). A total of 40 clinical isolates of *P. aeruginosa* were studied; 35 were obtained from Kaiser Permanente Reference Laboratories (Berkeley, CA). Additionally, five clinical isolates that tested positive for *P. aeruginosa* were obtained from the clinical laboratory at David Grant USAF Medical Center. A total of eight non-*P. aeruginosa* (pseudomonads) clinical isolates and two ATCC Gram-negative isolates were obtained from Armed Force Institute of Pathology (Washington, DC). Additionally, a total of eight clinical and ATCC isolates were obtained from the clinical laboratory at David Grant USAF Medical Center. Each clinical isolate was from a positive blood-culture bottle and *Pseudomonas* species were identified by Gram stain, and tested by MicroScan (Dade Behring, Deerfield, IL). Upon receipt of the blood-culture bottle by our institution, a subculture of each sample was performed on sheep blood agar plates and incubated at 37°C overnight. A 1×10^4 colony-forming unit/milliliter (CFU/ml) inoculum of each sample was mixed with 5 ml of anticoagulated blood and then inoculated into a new blood-culture bottle that was incubated in a BACTEC 9240 (Becton-Dickenson, Sparks, MD) 14–18 hours. Blood samples that did not contain bacteria and/or contained alternative Gram-negative bacterial strains were also inoculated into blood-culture bottles and incubated as well for negative controls. All samples inoculated with bacteria were positive after 14–18 hours in the BACTEC 9240. The next day, a 0.2-ml aliquot of the positive and negative blood-culture bottles were used for DNA extraction and further amplification using thermocycling conditions.

Rapid Extraction Method

The rapid extraction method, Bead Beating Plus CHELEX (BB+C), has been previously described (13). To test the method with *Pseudomonas*, each bacterial isolate was taken from an overnight subculture and resuspended into 1 ml of 1X phosphate buffered saline (PBS). One ml containing 1×10^9 CFU/ml bacterial cells was centrifuged at 7500*g* for 3 minutes. This was the starting point for the extraction proce-

TABLE 1. Rapid cycle DNA thermocycler results of bacterial species tested based on melting curve analysis (T_m)

	Standard PCR results		Rapid cycle PCR results	
Bacterial species (no. of isolates tested)[a]	16S rRNA 233bp band	*opr*L 504bp band	16S rRNA T_m (avg)	*opr*L T_m (avg)
Pseudomonas aeruginosa (40)	+	+	87.13	92.58
Pseudomonas putida	+	–	87.77	87.38
Pseudomonas stutzeri	+	–	87.35	89.02
Burkholderia cepacia (3)	+	–	87.18	89.19
Burkholderia cocovenenan	+	–	87.61	no peak
Xanthomonas sp.	+	–	87.29	no peak
Ochrobactrum anthropi	+	–	88.23	88.94
Ralstonia picketti	+	–	86.75	no peak
Salmonella enteritidis (3)	+	–	86.64	87.79
Klebsiella pneumoniae	+	–	85.77	no peak
Proteus vulgaris	+	–	85.62	no peak
Escherichia coli	+	–	88.51	87.23
Neisserria gonorrhea	+	–	86.82	87.77
Staphylococcus aureus (2)	+	–	84.49	no peak

[a]No number indicates that only one isolate was tested.

dure. The bacterial cells were mixed with 0.5 ml EDTA-anticoagulated blood, then centrifuged for 3 minutes at 10,000 rpm, washed with 1 ml 4% glacial acetic acid, then with 1 ml 1X PBS, then 500 µl 10mM Tris-EDTA (TE) buffer (pH 8.4). After the addition of the TE buffer, 1 g of 0.1-mm glass beads (Biospec Products, Inc., Bartlesville, OK) and ~0.25gm CHELEX-100 (BioRad, Hercules, CA) were added to the sample mixture. The samples were mixed and processed in the bead beater (Biospec Products, Inc.) at three-quarters speed for 5 minutes, then boiled for 5 minutes. The samples were then centrifuged for 5 minutes at 10,000*g*, and the supernatant was then moved to a clean 1.7-ml Eppendorf tube. All DNA samples were measured for concentration using DNA/RNA calculator (Pharmacia Biotech, Piscataway, NJ).

To test the clinical isolates, a 0.2-ml aliquot was taken from the positive blood-culture bottle and washed with 1 ml 4% acetic acid and continued as described above.

Lower Limiting Dilution Experiment

To determine the lower limits of detection of the target sequences, extracted DNA was diluted to 1 ng by serial dilutions and PCR was performed. To determine the lower limit of detection of CFU/ml, dilutions of *P. aeruginosa* 31B-6843 clinical isolate from 1×10^{10} to 1×10^{0} CFU/ml was extracted by BB+C method and PCR performed.

Direct Amplification of *Pseudomonas* From Extracted DNA With PCR (Standard Thermocycling)

The extracted DNA samples were used to PCR amplify different target sites in the genome to include the *P. aeruginosa opr*L gene and bacterial 16S rRNA gene (Table 2). Life Technologies-GibcoBRL (Gaithersburg, MD) synthesized the primers for PCR. One µl of the extracted DNA (50-ng minimum) was added to the Ready-To-Go PCR Beads (Pharmacia Biotech). When brought to final volume of 25 µl, the thermocycling mix contains 10 mM Tris-HCL (pH 9.0), 50 mM KCL, 1.5 mM $MgCl_2$, 200 µM of each dNTP and ~1.5 units Taq DNA polymerase along with 0.5 µM of each primer set. The thermocycling conditions were as follows: 94°C for 5 minutes for 1 cycle, then 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 1 minute for 35 cycles on Progene Thermocycler (Princeton, NJ). The reaction was then incubated for an additional 10 minutes at 72°C and was maintained at 4°C for up to 48 hours. After thermocycling, 5 µl was removed and subjected to agarose gel electrophoresis to determine quantity, quality, purity, and appropriate size of products. The resultant amplicons were resolved by agarose gel electrophoresis (1.5% agarose) at 120 volts for 30 minutes along with molecular weight size markers (Life Technologies-Gibco BRL). The gel was stained with ethidium bromide and the amplicons were visualized using UV light. All PCR testing was performed by dedicated personnel in a physical location distinct from the rest of the laboratory. Contaminant primer controls were included with the substitutions of deionized water for template DNA. Positive and negative controls were included with each run.

TABLE 2. Primer sets

DNA target	Primer set	Size	Ref.
*opr*L gene	(+)5′-ATG GAA ATG CTG AAA TTC GGC-3′	504bp	(6)
	(–)5′-CTT CTT CAG CTC GAC GCG ACG-3′		
16S rRNA	(+)5′-GAG GAA GGT GGG GAT GAC GT-3′	233 bp	(19)
	(–)5′-AGG CCC GGG AAC GTA TTC AC-3′		

Direct Amplification of *Pseudomonas* From Extracted DNA With PCR (Rapid-Cycle Thermocycling)

To develop the rapid-cycle thermocycling condition, two *Pseudomonas* isolates (*P. aeruginosa* 31B-6843 clinical isolate and *P. stutzeri*, ATCC 17588) were used. All 58 clinical isolates were coded and submitted to the technician in a blinded fashion. The extracted DNA samples were used to amplify target sites in the genome to include the *P. aeruginosa* gene and bacterial 16S rRNA gene using the rapid-cycle thermocycler (Table 2). Life Technologies-GibcoBRL synthesized the primers for PCR. One µl of the extracted DNA (50-ng minimum) was added to rapid cycler reaction cuvettes (Idaho Technology Inc., Salt Lake City, UT). When brought to a final volume of 10 µl, the rapid thermocycling mix contains 4 µl deionized water, 1 µl dNTP, 1 µl 30 mM Mg^{2+}, 1 µl Taq polymerase (0.5U/µl), 1 µl SYBR green double-stranded-DNA-specific dye (Molecular Probes, Eugene, OR), 1 µl targeted primer, and 1 µl template. PCR and fluorescent melting-curve analysis were performed on a LC32-Lightcycler (Idaho Technology Inc.). The samples were thermally cycled 25 times with temperature segments. The first segment was 94°C for less than a second at 20°C/sec for denaturation. A second segment of 60°C for less than a second at 20°C/sec allowed for primer annealing and a third temperature segment of 72°C for 5 sec at 20°C/sec allowed for extension. After amplification, the temperature was raised to 94°C for 5 sec, lowered to 65°C for 20 sec at 20°C/sec, and held for 20 sec. Melting-curve profiles were obtained by raising the temperature to 94°C at 0.2°C/sec while collecting fluorescent data constantly. Genotyping the samples by melting-curve temperature (T_m) was accomplished by converting the SYBR melting curves to –dF/dT derivative peaks and fitting the peak to Gaussian curves (Lightcycler software, Roche Molecular Biochemicals). All PCR testing was performed by dedicated personnel in a physical location distinct from the rest of the laboratory. Contaminant primer controls were included with the substitutions of deionized water for template DNA. Positive and negative controls were included with each run.

Result Turn-Around Time Measurement and Cost per Test Determination

Result TAT was measured from the time the positive blood-culture bottle was removed from the BACTEC 9240 for extraction of the DNA to the resolution of the PCR amplicons by agarose gel electrophoresis and the final observance of results for regular thermocycling or the T_m for rapid thermocycling. Cost per test was calculated by determining the one-time use of each individual item required to perform the test, including the supplies and media.

RESULTS

Rapid Extraction Method

P. aeruginosa 31B-6843 clinical isolate and *P. stutzeri* ATCC 17588 were used to test the BB+C DNA extraction method to generate sufficient and quality DNA for PCR. Median concentration of isolated DNA ranged from 24.0–1259.0 ng/µl. A consistent result with PCR was obtained from all 58 isolates extracted for standard thermocycling and for rapid cycle thermocycling (data not shown).

The BB+C method was tested for lower limits of detection of the target sequences. As little as 5 ng of DNA were required to amplify the target sequences using DNA generated from the BB+C method. The lower limits of CFU/ml were also determined. Dilutions of *P. aeruginosa* 31B-6843 clinical isolate from 1×10^{10}CFU/ml to 1×10^{0} CFU/ml were extracted, the DNA was measured, and standard thermocycling was performed. The *P. aeruginosa opr*L gene and bacterial 16S rRNA genes were easily amplified from DNA with the BB+C extraction method by standard PCR at the 1×10^{1} CFU/ml dilution.

Amplification of Bacterial Target Sites

The extraction method was then tested to see if it could generate DNA direct from the clinical samples for use in standard and rapid thermocycling. *P. aeruginosa* 31B-6843 clinical isolate and *P. stutzeri*, ATCC 17588 are shown in Figure 1. *P. aeruginosa opr*L gene (lane 4) and bacterial 16S rRNA genes (lanes 3 and 5) were easily amplified from DNA with the BB+C extraction method. Both primer sets were tested for contamination and no amplicons were detected after electrophoresis (lanes 7 and 8). As a negative control, *P. stutzeri*, ATCC 17588 was also tested. Only the bacterial 16S rRNA gene target sequence (lane 5) could be amplified by PCR.

Next we tested for the presence of the *opr*L and 16SrRNA genes in each of the 58 isolates. Only the expected amplified DNA products were produced from the *P. aeruginosa opr*L (504 bp) and bacterial 16S rRNA (233 bp) primer sets in every *P. aeruginosa* sample (n = 40). An amplified DNA product (233 bp) was seen only with 16S rRNA primer sets in every non-*P. aeruginosa* isolate (n = 18) (Table 1).

The BB+C extraction method was tested direct from clinical samples for identification of the bacterial target sites by the rapid-cycle DNA thermcycler with melting-curve analysis. DNA from *P. aeruginosa* 31B-6843 clinical isolate and *P. stutzeri*, ATCC 17588 was extracted and the melting curves generated are shown in Figure 2. Using the *opr*L primer set, a melting-curve temperature (T_m) was obtained at 92°C for only *P. aeruginosa* 31B-6843 clinical isolate (grey line) and nothing for *P. stutzeri*, ATCC 17588. The T_m for the 16S rRNA primer set was observed to be 87°C for both isolates (black and dotted line). Rapid-cycle thermocycling was then tested to see if we were able identify *P. aeruginosa* from non-*P. aeruginosa* isolates (Table 1). Forty clinical isolates of *P. aeruginosa* and 18 other isolates were received in a blinded fashion. Coded data revealed that there was 100% correlation for the rapid-cycle DNA thermocycling when comparing T_m results to standard clinical identification (Table 1). Multiple experiments allowed the compilation of a range for a significant T_m for each primer set used. The range for the *P. aeruginosa opr*L gene primer set was 90.00–93.50 (n = 66, mean = 91.82, SD = 1.79) while the 16S rRNA gene primer set was 85.60–88.00 (n = 72, mean = 87.14, SD = 0.98).

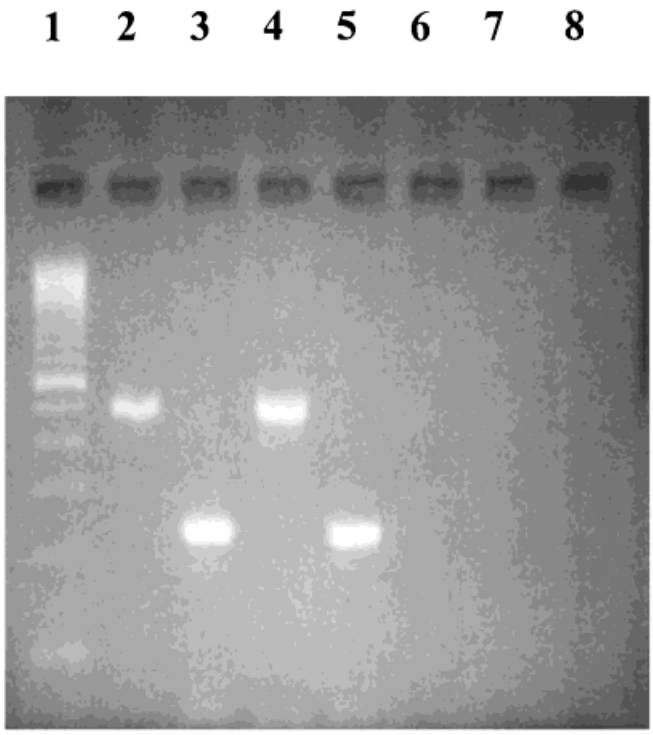

Fig. 1. Agarose gel electrophoresis of bacterial genomic DNA extracted from clinical isolates by BB+C method and amplified with *P. aeruginosa* primer sets using standard thermocycling. Lane 1, 100-bp DNA ladder (100–15,000 bp, and 2072-bp fragment) (Life Technologies, Gaithersburg, MD); lane 2, DNA PCR control from Ready-To-Go PCR Beads (Pharmacia Biotech, Piscataway, NJ) (500 bp); lanes 3 and 4, from a *P. aeruginosa* 31B-6843 clinical isolate; lanes 5 and 6, from *P. stutzeri* ATCC 17588; lanes 7 and 8, no DNA template (negative control) with each primer set; lanes 3 and 5, PCR-generated amplicons with bacterial 16S rRNA gene primer set (233 bp); lane 4, PCR amplicon generated with *opr*L gene primer set (504 bp).

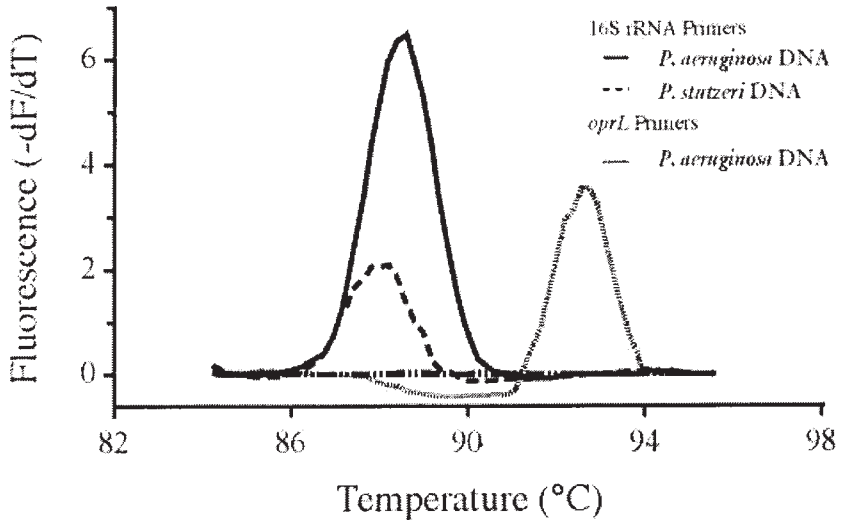

Fig. 2. Detection of *P. aeruginosa opr*L and bacterial 16S rRNA genes with melting-curve analysis; *P. aeruginosa* 31B-6843 clinical isolate vs. *P. stutzeri* ATCC 17588. The grey line represents the melting curve obtained with *opr*L gene primer set with extracted DNA from *P. aeruginosa* 31B-6843 clinical isolate (T_m = 92°C) while there was no T_m observed with extracted DNA from *P. stutzeri* ATCC 17588. The black line represents the melting curve obtained with the bacterial 16S rRNA gene primer set with extracted DNA from *P. aeruginosa* 31B-6843 clinical isolate (T_m = 88°C) and the dotted line corresponds to the melting curve obtained with DNA extracted from *P. stutzeri* ATCC 17588 (T_m = 87°C). The sequences of the primer sets utilized are listed in Table 2.

DISCUSSION

We have developed a procedure for rapid extraction of microorganism DNA directly from select clinical samples for molecular testing in our laboratory (13). Our mechanical lysis procedure generated DNA from the bacterial agent directly from the clinical sample within 20 minutes of sample submission. Significant progress has been made in the development of commercial extraction kits that can be used for rapid nucleic-acid extraction from microbial cultures for PCR. However, they require multiple steps (between 5 and 40) and extended times (15–150 minutes). They may require a pure culture and may be cost prohibitive for large numbers of samples (15). The most important component of an extraction method is its ability to obtain quality DNA for PCR. *P. aeruginosa* DNA was easily amplified by standard PCR and the target sites selected were readily amplified using specifically designed primer set and the results were available in less than 4 hours (Fig. 1).

We envision the use of PCR as a rapid and affordable confirmatory test that can easily and rapidly be applied to identify microorganisms directly from different clinical samples (blood, sputum, or urine). We used the blood-culture bottle as a screening tool since not every sample sent to the lab is positive. The minimum bacterial concentration in the blood-culture bottle, whose DNA could be extracted and detected by PCR with the *P. aeruginosa opr*L and bacterial 16S rRNA gene primer set, was determined to be 10 CFU/ml. This level of sensitivity for our BB+C method is lower than with our previous work with Methicillin-resistant staphylococci and in the range of other published extraction methods (6). Previously, we determined that a 1×10^4 CFU/ml concentration of bacteria requires a 10.1 hour incubation to test positive in the BACTEC 9240 (13). In our experience, once the blood-culture bottle becomes positive (bacterial density $1.25–4.0 \times 10^9$ CFU/ml), we can detect *P. aeruginosa* with our system. However, in our clinical microbiology lab, the blood-culture bottles are monitored continuously, but the positives are not worked up until the next morning. When we tested our positive blood-culture bottles the next day, all had greater than 1×10^7 CFU/ml. Therefore, there will be a sufficient concentration of bacteria to extract and detect by our method.

The cost of the rapid extraction and PCR-based method is affordable and set-up is readily applicable to the clinical lab. Standard identification methods, which include VITEK cards, media, inoculating loops, and reagents, can cost over $7 per sample for confirmation. If it is determined that a positive blood-culture bottle clinical isolate needs to be confirmed using the *Pseudomonas* primer set (bacterial 16S rRNA gene and *opr*L gene), the cost could be cheaper than standard identification. Cost per test for the Ready-to-Go tubes is $1.50/tube plus $0.10 for each set of primers, a total of $1.60/PCR for each primer set. If a panel were to be used consisting of two primer sets then the total for two different primer sets for identification would come to $3.20. Adding the cost of the Bead Beating Plus CHELEX-100 extraction method ($0.42/extraction) brings the total cost per test to $3.62. The cost per test for the new rapid-cycle technology is $0.90/PCR for each primer set. If our *P. aeruginosa* primer set were to be used then the cost would be double, or $1.80, and with the cost of the extraction, the total cost per test comes to $2.22. Techni-

cian time can also be calculated to include exact sample-processing time. One sample takes approximately 30 minutes for DNA extraction and PCR set-up. Therefore, the reported method was determined to be both time and cost effective when compared to standard clinical procedures. We found that results were ready in less than 1 hour even when we set up multiple samples.

The presence of the *opr*L gene, as detected by our PCR procedures, had a 100% agreement with clinical findings of *P. aeruginosa*, with 40 isolates tested using both standard and rapid-cycle PCR (Table 1). For rapid-cycle PCR, we have determined a range for the primer sets utilized that will specifically identify *P. aeruginosa*. We also tested 18 non-*P. aeruginosa* isolates to include other fluorescent pseudomonads and none reacted positively for the *opr*L gene in standard PCR or was observed within the specific T_m range for *P. aeruginosa*. Interestingly, using these primer sets, a T_m was generated for several of the non-*P. aeruginosa* isolates. The T_m was not in the specific range, and in addition, the *opr*L gene primer sets did not generate an amplicon with standard thermocycling. In the future and for other target sites, the double-stranded-DNA dye may not be a reliable screening tool. We are currently working on specific fluorescent probes with these target sites to eliminate the chance for any nonspecific binding. However, this is the first published report of the development of an observed range of T_m using a double-stranded-DNA dye for the identification *P. aeruginosa*.

The levels of sensitivity and specificity we achieved in our study are in agreement with other studies using PCR detection of *P. aeruginosa* direct from clinical samples (6,16). Although the classical clinical microbiology techniques currently being employed are satisfactory in most situations, a more rapid test may be useful in specific situations. This includes diagnosis for critically ill patients like burn victims or patients at risk for nosocomial infections, yet drug-susceptibility testing remains a problem even for conventional testing. Real-time PCR that uses continuous fluorescence monitoring holds great promise as a rapid diagnostic tool but may be cost prohibitive being that it is 10 times more expensive than regular thermocyclers. However, the sensitivity coupled with the speed of our bacterial DNA extraction procedure can assist the health care providers in making a rapid diagnosis and a prudent selection of chemotherapeutic agents to augment standard microbiological methods. This may be of primary value in clinical laboratories where standard microbiological testing capabilities are limited but agent identification is critical such as remote, deployed military medical facilities. In these places, routine samples are shipped to regional or stateside reference laboratories for testing which can increase result turnaround time. Recently, we have evaluated a field PCR laboratory set up in both a deployable medical system (DEPMEDS) at an Army Reserve Training Facility in Dublin, CA, and at an Air Force Field Hospital (17,18). Rigorous validation of these PCR technologies is currently underway at the Air Force and Department of Defense level. Therefore, the use of real-time PCR promises to provide clinical laboratory technicians with a versatile diagnostic tool with which to enhance microbiological capabilities in the deployed environment and will, along with other clinical information, improve patient management.

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