Original Article

Evaluation of broad-range 16S rRNA PCR for the diagnosis of bloodstream infections: two years of experience

Reem M Hassan[1], Mervat G El Enany[1], Hussien H Rizk[2]

[1] *Department of Clinical pathology, Faculty of Medicine, Cairo University, Cairo, Egypt*
[2] *Department of Cardiology, Faculty of Medicine, Cairo University, Cairo, Egypt*

Abstract

Introduction: Diagnosis of bloodstream infections using bacteriological cultures suffers from low sensitivity and reporting delay. Advanced molecular techniques introduced in many laboratories provide rapid results and may show improvements in patient outcomes. This study aimed to evaluate the usefulness of a molecular technique, broad-range 16S rRNA PCR followed by sequencing for the diagnosis of bloodstream infections, compared to blood culture in different patient groups.

Methodology: Conventional PCR was performed, using broad-range 16S rRNA primers, on blood cultures collected from different patients with suspected bloodstream infections; results were compared with those of blood culture.

Results: Though blood culture is regarded as the gold standard, PCR evaluation showed sensitivity of 86.25%, specificity of 91.25%, positive predictive value of 76.67%, negative predictive value of 95.22%, and accuracy of 88.8%.

Conclusions: Molecular assays seem not to be sufficient to replace microbial cultures in the diagnosis of bloodstream infections, but they can offer a rapid, good negative test to rule out infection due to their high negative predictive value.

Key words: blood stream infection; blood cultures; 16S rRNA; PCR.

J Infect Dev Ctries 2014; 8(10):1252-1258. doi:10.3855/jidc.4867

(Received 17 February 2014 – Accepted 19 June 2014)

Introduction

Blood cultures are considered the gold standard in the diagnosis of bloodstream infections (BSIs) [1]. Blood cultures have shown low sensitivity in the detection of bacterial agents in cases of low-grade bacteraemia, in cases where adequate sample volume for inoculation in blood culture bottles cannot be obtained, and in cases where antibiotics are used before blood sampling [2]. Also, microbial culture results may take at least 24 to 72 hours to become available. Automated systems for continuous blood culture monitoring improved blood cultures greatly. Also, results can raise an alarm signal whenever a positive culture is detected. Further subcultures are still required to perform specific biochemical assays needed for pathogen identification [3].

New developments in the diagnosis of BSIs include the use of new blood biomarkers [4], revised clinical criteria, and new molecular pathogen detection methods [5].

Molecular pathogen detection methods are based on two main principles – hybridization or amplification. Hybridization-based methods such as fluorescence *in situ* hybridization (FISH) can be applied to positive blood cultures using oligonucleotide probes that target consensus bacterial genes (typically rRNA genes) [6]. A recent development of the classic oligonucleotide probes is the peptide nucleic acid (PNA) probes. They are neutrally charged synthetic oligomers that mimic the DNA or the RNA structure, giving them a stronger hybridization capability [7] and a lower susceptibility to inhibition by impurities. PNA-FISH probes can be clinically applied to a wider range of clinical samples than can amplification methods [8].

Amplification methods (*e.g.*, polymerase chain reaction [PCR]) have been used to amplify specific target regions in the microbial genome. Broad-range PCR targets the 16S rRNA gene, a consensus gene that is present in all bacteria and consists of two regions – conserved and variable [9]. The conserved regions are targeted by universal primers for detection of the presence of a microorganism; the variable regions are targeted by genus or species-specific primers. Using universal primers, the amplified target regions are subjected to sequencing or microarray/probe

hybridization for identification of the microorganism genus and species [10].

PCR amplification of conserved regions of the bacterial genome, especially the 16S rRNA gene followed by sequence analysis, is a well-established technique for the identification of bacterial pathogens [11]. It has been applied to many types of samples for the diagnosis of different infections including blood stream infections, neonatal sepsis, and infective endocarditis [12,13].

This study was performed to evaluate the performance of 16S rRNA PCR followed by sequencing in the diagnosis of bloodstream infections (BSIs) and to compare its results to those of blood culture, the gold standard used in the diagnosis of BSIs.

Methodology

Study design

This prospective study included 280 patients of all age groups with clinically suspected bloodstream infections (BSIs) referred to Cairo University teaching hospital (Kasr alainy) between 2010 and 2012, to three different departments – cardiology, cardiothoracic surgery intensive care unit (ICU), and neonatal ICU. This included patients diagnosed with neonatal sepsis, infective endocarditis, central line-related bacteraemia, and bacteraemia secondary to other infections.

Written consent was obtained from the patients at admission for all the procedures performed, which were part of the routine investigations for patients with suspected BSIs established in the hospital. The Clinical and Chemical Pathology department's (Kasr alainy) Ethical Committee approval was obtained in August 2009 for the study to be done on patients admitted to these departments between 2010 and 2012.

Laboratory methods

A total of 288 blood samples were collected from 280 patients (8 patients developed a second attack of suspected BSI during their hospital stay) as part of the routine work-up based on hospital guidelines of cases with suspected BSIs. Simultaneous blood culture and broad-range 16S rRNA PCR followed by sequencing was performed on inoculated blood culture bottles.

Blood cultures

At least two blood culture samples from each patient were obtained as soon as possible after enrolment. Blood culture vials were supplied and stored according to the manufacturer's instructions. Blood samples were collected using sterile techniques to reduce contamination. A 10 cc syringe was used to draw 8-10 mL of blood from the patient twice; the needle was aseptically inoculated into each of the BACTEC Plus aerobic/F and BACTEC Plus anaerobic/F blood culture vials (Becton, Dickinson Dubai, UAE) at bedside after swabbing the septum with alcohol (not iodine). In neonates, 1-3 mL of blood was accepted for each bottle. The volume collected was monitored by the gradation marks on the vial label, and was transported to the laboratory as soon as possible. Inoculated vials were placed in the BACTEC fluorescent 9240 instrument (BACTEC Becton Dickinson, Dubai, UAE) for incubation and monitoring for 5 days unless the clinical diagnosis was infective endocarditis, in which case incubation was extended to 21 days. The instrument automatically tested the vials every 10 minutes, and positive vials were determined. Positive bottles were then sub-cultured and colonies were identified according to standard Gram staining and biochemical reactions [14].

Molecular techniques

For detection of broad-range bacterial 16S rRNA from inoculated blood culture bottles, samples were done in duplicate – from aerobic and anaerobic bottles for each patient in the same setting. Bacterial DNA was extracted from inoculated blood culture bottles, using the wash/alkali/heat lysis method previously described by Millar *et al.* [15]. Briefly, 5 mL was drawn from each bottle after a 24-hour incubation period in the BACTEC fluorescent 9240 instrument. Samples were centrifuged at 3000 rpm and the supernatant was removed to concentrate cells. A cell pellet containing any microbial DNA was washed three times with sterile saline solution, mixed by inversion, and subsequently centrifuged. The cell pellet was re-suspended in Tris-EDTA (20 mM Tris-HCl, pH 8, 2 mM EDTA, 1.2% Triton X-100) and heated at 100°C for 1 hour. The sample was frozen and thawed twice and subsequently centrifuged in a micro-centrifuge at 12,000 rpm for 30 seconds to remove the cell debris. The supernatant containing the extracted DNA was transferred to a sterile PCR quality tube and stored at -20°C prior to PCR [15].

Eubacterial broad-range 16S rRNA primer set 536f 5'CAGCAGCCGCGGTAATAC and RP2 5'eACGGCACCTTGTTACGACTT (AccuOligo, Bioneer, Daejeon, Korea) was used for DNA amplification and cycling conditions, as previously described [16,17]. Briefly, PCR reagents were added to each labeled tube as per PCR mix: Dream Taq PCR

Master Mix (2X) (Fermentas Vilnius, Lithuania) 25 µL, 0.2mM of each primer, template DNA 10 µL (approximately 500 ng), 5 µL 1X PCR buffer, 1U Dream Taq DNA Polymerase (Fermentas) completed to 50 µL with nuclease-free water. The reaction mixtures were vortexed briefly. Amplification reactions were carried out in an air-cooled Biometra UNO Thermoblock cycler (Biomedizinische GmbH, Göttingen Germany). Amplification started with an initial incubation at 95°C for 15 minutes, followed by 35 cycles of heating at 95°C for 1 minute, annealing at 62°C for 30 seconds, and extension at 72°C for 90 seconds. Amplification ended with an extension step at 72°C for 10 minutes. A positive control was included in each run using *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) in addition to a negative control, containing all PCR reagents except the DNA. The PCR products were purified using the GeneJET PCR Purification Kit (Fermentas), as defined by the supplier, and 4 µL of purified PCR product was sequenced in a 20 µL final volume containing sequencing buffer, 3.2 pmole of forward (536F) primer, 3 µL of BigDye Terminator V3.1 mix (Applied Biosystems, Foster City, California, USA), 3 µL of BigDye 5X dilution buffer, and 8 µL of deionized water. Sequencing reactions were purified using Centri-Sep columns (Princeton separations), and the purified products were sequenced on an ABI PRISM 310 genetic analyzer (Applied Biosystems). Sequences obtained were analyzed with Autoassembler software and compared with those available in the GenBank database for 16S ribosomal RNA sequences (bacteria and archaea) using MegaBLAST (basic local alignment search tool) optimized for highly similar sequences. The default search settings in www.ncbi.nlm.nih.gov were used, though environmental sample sequences were excluded [16,17].

Interpretation of the results

Identification to the species level was defined as sequence similarity of ≥ 99% with that of the GenBank prototype strain sequence; identification to the genus level was defined as sequence similarity of 97%–98.9% with that of the GenBank prototype strain sequence. A failure to identify was defined as sequence similarity of < 97% with sequences deposited in GenBank at the time of the analysis [18].

Statistical methods

Statistical analysis was performed using SPSS statistical software version 15 (SPSS Inc., Chicago, IL, USA). Agreement between different diagnostic techniques was tested using kappa statistic. A p value of < 0.05 was considered statistically significant.

Results

In this study, 280 patients were subjected to blood culture, and broad-range 16S rRNA PCR was performed on a total of 288 samples.

Blood culture was positive in 69 (24%) patients and negative in 219 (76%) patients with suspected sepsis.

Broad-range 16S rRNA PCR was positive in 79 (27.4%) blood culture samples.

Broad-range PCR and blood culture results showed an agreement of 68.7% in negative cases and of 20.1% in positive cases, collectively an agreement of 88.8% between the two methods (Table 1).

Though blood culture is regarded as the gold standard, PCR for 16S rRNA evaluation showed a sensitivity of 86.25% (95% confidence interval [CI]:76.72% to 92.92%), specificity of 91.25% (95% CI: 86.93% to 94.50%), positive predictive value of 76.67% (95% CI: 66.57% to 84.94%), negative predictive value of 95.22% (95% CI: 91.60% to 97.59%), and accuracy of 88.8%.

Microorganisms identified by blood culture were consistent with those identified by broad-range PCR followed by sequencing in 50% (46/92) of cases (Table 2).

The inconsistent results between blood culture and PCR could be divided into three categories. In the first category, PCR was positive in 21 cases with negative blood culture results and could identify an organism by sequencing in 19 of them (2 cases with inadequate bands). In the second category, PCR failed to diagnose 15 blood culture-positive cases, 4 of them due to double or inadequate bands which rendered them not suitable for sequence identification. In the third category, PCR showed a difference in the identification of microorganisms isolated from blood culture in 10 cases. Details are shown in Table 3.

Technique evaluation

The turnaround times and costs of blood cultures versus PCR were compared. Blood cultures were found to cost more money than PCR followed by sequencing, in both negative and positive cases. Negative cases required repetition of sampling, while positive cases needed further identification of the isolated microorganism using different culture media and biochemical tests (Table 4).

Table 1. The relation between blood PCR 16S primer and blood culture

			Blood culture		Total	
			Negative	Positive		
Blood PCR 16S primer	Negative	Count	198	11	209	**P value 0.000**
		% within Blood culture	90.4%	16.0%	72.6%	
		% of total	68.7%	3.8%	72.6%	
	Positive	Count	21	58	79	
		% within Blood culture	9.6%	84.0%	27.4%	
		% of total	7.3%	20.1%	27.4%	
Total		Count	219	69	288	
		% of total	76.0%	24.0%	100.0%%	

Table 2. Consistent results between organisms identified by blood culture compared to organisms identified by blood PCR sequencing

Blood culture organism	Blood PCR sequencing organism
Staphylococcus aureus (15)*	*Staphylococcus aureus* (15)*
Enterococcus spp. (4)*	*Enterococcus faecium* (3)*, *dispar*/*casseliflavus* (1)*
Streptococcus viridans (4)*	*Streptococcus mitis* (1)*, *oralis* (2*), *mutans* (1)*
Pseudomonas aeruginosa (2)*	*Pseudomonas aeruginosa* (2)*
CoNS (7)*	*Staphylococcus lugdunensis* (3)*, *haemolyticus* (1)*carnosus (1)*, warneri (2)*carnosus
Acinetobacter spp. (2)*	*Acinetobacter baumannii* (2)*
Klebsiella spp. (8)*	*Klebsiella pneumoniae* (8)*
E. coli (2)*	*E. coli* (2)*
Enterobacter spp. (1)*	*Enterobacter cloacae* (1)*
Corynebacterium spp.(1)*	*Corynebacterium jeikium* (1)*

*Number of cases identified

Table 3. Inconsistent results between organisms identified by blood culture compared to organisms identified by blood PCR sequencing

Blood culture organism	Blood PCR sequencing organism
Staphylococcus aureus (3)*	NR**
Enterobacter spp. and *Pseudomonas* spp.	NR**
Enterococcus spp. (4)*	NR (2)*, *Achromobacter denitrificans* (1)*, *Streptococcus anginosus* (1)*
CoNS (6)*	NR (5)*, *Salinicoccus albus* (1)*
CoNS and *Streptococcus viridans*	Double band
Streptococcus viridans (6)*	Inadequate band (3)*, *Acinetobacter baumannii* (1)*, *Brevibacillus brevi* (1)*, *Staphylococcus hominis* (1)*
Brucella spp.	*Staphylococcus aureus*/*saprophyticus*
Peptostreptococcus	*Streptococcus mutans*
Propionibacterium acnes	*Bacillus safensis*
Klebsiella spp.	*Brucella* spp.
NR** (21)*	*Geobacillus* (1)*, *Bacillus firmus* (1)*, *Staphylococcus aureus* (1)*, *Bordetella holmesi* (1)*, *Achromobacter denitrificans* (1)*, *Achromobacter xylosoxidans* (1)*, *Staphylococcus hemolyticus* (1)*, inadequate band (2)*, *Enterobacter cloacae* (1)*, *Proteus mirabilis* (1)*, *Staphylococcus carnosus* (1)*, *Haemophilus influenzae* (1)*, *Klebsiella pneumoniae* (3*), *Streptococcus pyogenes* (1)*, *Acinetobacter baumannii* (3)*, *Pseudomonas aeruginosa* (1)*

*Number of cases identified **NR: negative result

Table 4. Comparison between blood culture and PCR

Test	Labor	Turnaround time	Cost/ test/USD
Blood culture (BACTEC system) negative cases	Easy	5 days	15-50
Blood culture (BACTEC system) positive cases	Moderate	3-4 days	25-100
PCR	Moderate	4 hours	16
Sequencing	Intensive	6 hours	40

Discussion

Diagnosis of bloodstream infections is based on isolation of a causative agent in blood samples. Clinical suspicion of bloodstream infection is sufficient to begin administration of appropriate antibiotics. The best antibiotic is determined by trial and error, and broad-spectrum antibiotics that cover the most likely etiologic agents are usually prescribed. One major limitation to a specific regimen is the unavailability of a rapid and reliable diagnostic test used for the identification of the etiologic agents [19].

Blood culture is a highly specific, commonly used technique for the diagnosis of bloodstream infections, although its sensitivity remains low [19].

Antigen detection and serological testing are highly specific but have many variations in sensitivity, and the organism must be targeted according to clinical suspicion of each case [19].

Molecular methods have many advantages, as they are rapid and they require the analysis of a smaller sample volume than culture does. The disadvantage of broad-range PCR is that after the amplification of a specific sequence, a sequencing or probe identification step is required [20].

This in-house evaluation of conventional 16S rRNA PCR assay against blood culture, regarded as the gold standard, showed a sensitivity of 86.25%, specificity of 91.25%, positive predictive value of 76.67%, negative predictive value of 95.22%, and accuracy of 88.8%. These values are similar to those found in other studies using conventional or automated PCR techniques [20,21].

A multicenter study performed on 342 blood samples from 187 patients using a new commercial real-time multiplex PCR test (SepsiTest, Molzym, Bremen, Germany) found a diagnostic sensitivity and specificity of 87.0% and 85.8%, respectively [20]. In another study, PCR showed sensitivity of 88%, specificity of 96.3%, positive and negative predictive values of 74.3% and 98.5%, respectively, which are close to the results found in the present study [21].

Contrary to the present findings, another study using a commercial real-time multiplex PCR, Light Cycler SeptiFast Test (Roche Diagnostics, Mannheim, Germany) showed a higher specificity (95%) and a modest sensitivity (61%) [22]. This difference in specificity may be because their PCR technique could detect additional positive cases that were missed by blood culture but undetected in other body site infections (*e.g.*, urinary tract infection), as in cases of transient bacteremia of very low levels due to primary infections in other sites. The low sensitivity may be due to the presence of some organisms isolated by blood culture that were not on the list or panel of organisms detected by SeptiFast PCR [22].

In the present study, positive PCR results were found in 27.4% of cases, which is in the positivity range (from 3% to 29%) of many other different studies using molecular assays [23].

As for the identification of bacteria using PCR followed by sequencing technique, the length of the amplified PCR product controlled the efficiency of the sequence obtained. In the present study, the PCR product could not be identified by sequencing because of short PCR bands that led to the lack of identification of any related sequences in the database in 6.3% (5/79) of cases and because of a double band in 1.3% (1/79) of cases. In the present study, 92.4% (73/79) of positive PCR isolates were identified to the species level.

The present data is comparable to results of another study that evaluated the usefulness of 16S rRNA PCR sequencing for bacterial identification done over a period of 30 months on 683 bacterial isolates. The identification of microorganisms was limited to only the genus level (15.8%) and the sequence remained unidentifiable by 16S rRNA sequence analysis in only 1% of cases [11].

Less favorable results were reported by another study performed in Brazil, where 41% of the samples from patients were characterized to the genus level. This may have been due to the use of the broadly conserved bacterial 23S rRNA primers, although the same extraction method was used (wash/alkali/heat-lysis). The findings suggested a high degree of agreement between the results of blood culture and PCR, reaching 88.8%, with consistency in identified organism in 50% of cases [24].

In the present study, it does not appear that this PCR assay could replace blood culture for the identification of bloodstream infections in patients, as 15 samples could not be identified. Four of these were positive, but short PCR bands or double bands hindered organism identification using sequencing, and those patients were continued on empiric broad-range antibiotic treatment. Eleven cases were positive by blood culture but negative by 16S rRNA PCR, which missed a significant number of infections that would be critical to recognize.

Most of the missed cases were Gram-positive cocci (10/11); five were coagulase-negative staphylococci, three were *Staphylococcus aureus*, two were *Enterococcus* spp., and only one was Gram-negative bacilli. Positive blood cultures yielding

Gram-positive organisms were previously reported with negative PCR results and could be explained by a common technical factor – difficulties in breaking the cell walls of Gram-positive organisms during sample preparation – which resulted in the failure of the DNA extraction process [24].

PCR was positive in 21 blood culture-negative samples; in 13 cases, a common BSI causing organisms that may have been missed due to prior antibiotic intake or low level bacteraemia was identified. In 3 cases, unusual bacteria (*Achromobacter dentrificans*, *Achromobacter xylosoxidans*, and *Bordetella holmesi*), which are rare causes of central venous line-related bacteraemia in immunocompromised patients and patients on hemodialysis, were found [25].

Millar *et al.* stated that the major practical problem associated with the use of broad-range ribosomal RNA PCR is contamination of the assay by exogenous bacterial DNA. This occurred in two of our cases – the environmental contaminants *Geobacillus* and *Bacillus firmus* were identified. Millar *et al.* also recommended sticking strictly to the general precautions of PCR protocols to avoid contamination and also recommended adding special precautions for broad-range PCR such as UV irradiation of all PCR reagents (except primers) and PCR-dedicated pipettes and tubes before amplification [26]. These recommendations were followed in the present study.

Another method suggested to decrease the possibility of contamination was the treatment of all PCR reagents before amplification with the restriction endonuclease AluI, which destroys all contaminating DNA targets but leaves the primer set intact [24].

The low cost of the PCR method performed in the present study, including the rapid manual extraction of bacterial DNA from blood, broad-range primers, and PCR master mix, makes this method affordable and easily applicable to the clinical microbiology laboratory. This can result in early administration of appropriate antibiotics to patients with suspected bacteraemia, thus reducing mortality and morbidity, or in the reduction of empirical use of antibiotics in patients with no proven infection, lowering their exposure to the hazards of drug toxicity and high costs. However, DNA amplification techniques provide positive/negative results with nonviable microorganisms available for antibacterial susceptibilities, which still have to be determined by conventional methods using bacterial cultures.

In summary, PCR is a promising low-cost method that can be used in the rapid identification of bloodstream infections and to rule out infection. PCR must be interpreted cautiously and hand-in-hand with blood culture, offering the possibility of identifying more positive cases that may be missed by conventional culture methods.

References

1. Rodriguez-Creixems M, Alcala L, Munoz P, Cercenado E, Vicente T, Bouza E (2008) Bloodstream infections: evolution and trends in the microbiology workload, incidence, and etiology, 1985–2006. Medicine (Baltimore) 87 Suppl 4: 234-249.
2. Isaacman DJ, Karasic RB, Reynolds EA, Kost SI (1996) Effect of number of blood cultures and volume of blood on detection of bacteremia in children. J Pediatr 128 Suppl 2: 190-195.
3. Gaibani P, Rossini G, Ambretti S, Gelsomino F, Pierro AM, Varani S, Paolucci M, Landini MP, Sambri V (2009) Blood culture systems: rapid detection--how and why? Int J Antimicrob Agents 34 Suppl 4: S13-S15.
4. Pierrakos C, Vincent JL (2010) Sepsis biomarkers: a review. Crit Care 14: R15.
5. Murray PR, Masur H (2012) Current approaches to the diagnosis of bacterial and fungal bloodstream infections in the intensive care unit. Crit Care Med 40 Suppl 12: 3277-3282.
6. Kempf VA, Trebesius K, Autenrieth IB (2000) Fluorescent in situ hybridization allows rapid identification of microorganisms in blood cultures. J Clin Microbiol 38: 830-838.
7. Oliveira K, Procop GW, Wilson D, Coull J, Stender H (2002) Rapid identification of Staphylococcus aureus directly from blood cultures by fluorescence in situ hybridization with peptide nucleic acid probes. J Clin Microbiol 40: 247-251.
8. Forrest GN, Roghmann MC, Toombs LS, Johnson JK, Weekes E, Lincalis DP, Venezia RA (2008) Peptide nucleic acid fluorescent insitu hybridization for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. Antimicrob Agents Chemother 52: 3558-3563.
9. Woese CR (1987) Bacterial evolution. Microbiol Rev 51 Suppl 2: 221-271.
10. Relman DA (1999) The search for unrecognized pathogens. Science 284 Suppl 5418: 1308-1310.
11. Mignard S, Flandrois JP (2006) 16S rRNA sequencing in routine bacterial identification: a 30-month experiment. J Microbiol Methods 67: 574-581.
12. Klouche M, Schröder U (2008) Rapid methods for diagnosis of bloodstream infections. Clin Chem Lab Med 46: 888-908.
13. Madico GE, Rice PA (2008) 16S-Ribosomal DNA to diagnose culture-negative endocarditis. Curr Infect Dis Rep 10: 280-286.
14. Murray PR (2007) Manual of Clinical Microbiology, 9th edition, Washington D.C: American Society for Microbiology ASM Press,.
15. Millar BC, Jiru XU, Moore JE, Earle JAP (2000) A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material. J Microbiol Methods 42: 139-147.

16. Grijalva M, Horváth R, Dendis M, Cerný J, Benedík J (2003) Molecular diagnosis of culture negative infective endocarditis: clinical validation in a group of surgically treated patients. Heart 89: 263-268.
17. Bousbia S, Papazian L, Auffray JP, Fenollar F, Martin C, Li W, Chiche L, La Scola B, Raoult D (2010) Tropheryma whipplei in patients with pneumonia. Emerg Infect Dis 16: 258-263.
18. Simmon KE, Croft AC, Petti CA (2006) Application of SmartGene IDNS Software to Partial 16S rRNA Gene Sequences for a Diverse Group of Bacteria in a Clinical Laboratory. J Clin Microbiol 44: 4400-4406.
19. Kollef MH, Sherman G, Ward S, Fraser VJ (1999) Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. Chest 115: 462-474.
20. Wellinghausen N, Kochem AJ, DisquéC, Mühl H, Gebert S, Winter J, Matten J, Sakka SG (2009) Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. J Clin Microbiol 47: 2759-2765.
21. Ruppenthal RD, Pereira F de S, Cantarelli VV, Schrank IS (2005) Application OF Broad-Range Bacterial PCR Amplification and Direct Sequencing on the Diagnosis OF Neonatal Sepsis. Braz J Microbiol 36: 29-35.
22. Tsalik EL, Jones D, Nicholson B, Waring L, Liesenfeld O, Park LP, Glickman SW, Caram LB, Langley RJ, van Velkinburgh JC, Cairns CB, Rivers EP, Otero RM, Kingsmore SF, Lalani T, Fowler VG, Woodset CW (2010) Multiplex PCR to diagnose bloodstream infections in patients admitted from the emergency department with sepsis. J Clin Microbiol 48: 26-33.
23. Pammi M, Flores A, Leeflang M, Versalovic J (2011) Molecular assays in the diagnosis of neonatal sepsis: a systematic review and meta-analysis. Pediatrics 128: e973-e985.
24. Rothman RE, Majmudar MD, Kelen GD, Madico G, Gaydos CA, Walker T, Quinn TC (2002) Detection of bacteremia in emergency department patients at risk for infective endocarditis using universal 16S rRNA primers in a decontaminated polymerase chain reaction assay. J Infect Dis 186: 1677-1681.
25. Ahmed MS, Nistal C, Jayan R, Kuduvalli M, Anijeet HK (2009) Achromobacter xylosoxidans, an emerging pathogen in catheter-related infection in dialysis population causing prosthetic valve endocarditis: a case report and review of literature. Clin Nephrol 71 Suppl 3: 350-354.
26. Millar BC, Xu J, Moore JE (2002) Risk Assessment Models and Contamination Management: Implications for Broad-Range Ribosomal DNA PCR as a Diagnostic Tool in Medical Bacteriology. J Clin Microbiol 40 Suppl 5: 1575-1580.

Corresponding author

Dr Reem Mostafa Mohamed Hassan, MD
Department of Clinical Pathology
Faculty of Medicine, Cairo University
Cairo, 11562, Egypt
Phone: +2 01141399472
Email: reem.mostafa@kasralainy.edu.eg

Conflict of interests: No conflict of interests is declared.