International Journal of Infectious Diseases 57 (2017) 144–149

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Utility of 16S rRNA PCR performed on clinical specimens in patient management

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ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form 7 February 2017
Accepted 8 February 2017
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
16S
16S rRNA
Utility
Bacterial infections
Cost analysis

SUMMARY

Background: Broad-range 16S rRNA PCR can be used for the detection and identification of bacteria from clinical specimens in patients for whom there is a high suspicion of infection and cultures are negative. The aims of this study were (1) to compare 16S rRNA PCR results with microbiological culture results, (2) to assess the utility of 16S rRNA PCR with regard to antimicrobial therapy, and (3) to compare the yield of 16S rRNA PCR for different types of clinical specimen and to perform a cost analysis of the test.
Methods: A retrospective study was performed on different clinical specimens which had 16S performed over 3 years (2012–2015). Standard microbiological cultures were performed on appropriate media, as per the laboratory protocol. Patient clinical and microbiological data were obtained from the electronic medical records and laboratory information system, respectively. 16S rRNA PCR was performed in a reference laboratory using a validated method for amplification and sequencing. The outcomes assessed were the performance of 16S rRNA PCR, change of antimicrobials (rationalization, cessation, or addition), and duration of therapy. Concordance of 16S rRNA PCR with bacterial cultures was also determined for tissue specimens.
Results: Thirty-two patients were included in the study, for whom an equal number of specimens ($n = 32$) were sent for 16S rRNA PCR. 16S rRNA PCR could identify an organism in 10 of 32 cases (31.2%), of which seven were culture-positive and three were culture-negative. The sensitivity was 58% (confidence interval (CI) 28.59–83.5%) and specificity was 85% (CI 61.13–96%), with a positive predictive value of 70% (CI 35.3–91.9%) and negative predictive value of 77.2% (CI 54.17–91.3%). Antimicrobial therapy was rationalized after 16S rRNA PCR results in five patients (15.6%) and was ceased in four based on negative results (12.5%). Overall the 16S rRNA PCR result had an impact on antimicrobial therapy in 28% of patients (9/32). The highest concordance of 16S rRNA PCR with bacterial culture was found for heart valve tissue (80%), followed by joint fluid/tissue (50%).
Conclusions: Despite the low diagnostic yield, results of 16S rRNA PCR can still have a significant impact on patient management due to rationalization or cessation of the antimicrobial therapy. The yield of 16S rRNA PCR was highest for heart valves.
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Introduction

Bacterial infections are a leading cause of inpatient hospital mortality and are a significant financial burden to the hospital system.[1,2] The accurate identification of bacterial isolates is important for the clinical microbiology laboratory, as it is crucial for making a diagnosis and choosing the correct antimicrobial. It also aids in determining the duration of therapy and taking appropriate infection control precautions. The traditional microbiology workflow for bacterial identification consists of Gram stain and isolation of the organism on culture media, followed by phenotypic identification. Often even with appropriate and prompt incubation on routine and selective culture media, growth of the organism does not take place. As a clinical consequence, this leads to the use of broad-spectrum antimicrobial therapy, which is often inappropriate and leads to side effect.[3]

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http://dx.doi.org/10.1016/j.ijid.2017.02.006
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While automated identification systems like Vitek 2 (bioMérieux, Marcy l'Etoile, France) and Phoenix (BD Diagnostics, San Jose, CA, USA) utilize biochemical reactions, other technologies like matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) utilize mass spectrometry for organism identification. However all of these systems need viable growth of the bacteria from the clinical specimen.

In the absence of growth on culture media, the laboratory often has to rely on other genomic tests for organism detection directly from the clinical specimen. Technologies like broad-range PCR and next-generation sequencing are available, although their use is limited by their cost and availability only in tertiary referral laboratories. Broad-range 16S rRNA PCR is becoming increasingly available and is generally quick and easy to perform in a laboratory with relevant expertise in molecular microbiology and an understanding of bioinformatics.[4]

16S rRNA gene polymerase is present in all bacteria as a variable and conserved region.[5,6] With the use of broad-range primers, the conserved sequences within the 16S rRNA gene are recognized, and amplification of the variable regions leads to the provision of unique signatures that are utilized for identification of the bacteria to the species level.[7–9] 16S rRNA gene sequencing has been used for the study of bacterial taxonomy and phylogeny.[10] Data from previous studies suggest that 16S rRNA provides genus identification in up to 90% of cases, but can only identify to the species level in 65–91%.[11–13] 16S rRNA has gained an important place in clinical microbiology, as it provides an attractive alternative for the detection and identification of bacterial pathogens in clinical specimens from patients for whom there is a high suspicion of infection but bacterial cultures are negative.[14–16]

16S rRNA PCR can also detect non-viable bacterial DNA after the initiation of antibiotics from sterile sites, which gives it an advantage over culture methods.[16] The aims of this study were (1) to compare 16S rRNA PCR results with microbiological culture results, (2) to assess the utility of 16S rRNA PCR for antimicrobial management, and (3) to determine the yield of 16S rRNA PCR for different tissues and to assess the cost benefit of performing such a test and its impact on overall hospital and ancillary costs.

Methods

This was a retrospective analysis of 32 patients for whom 16S rRNA PCR was performed on clinical specimens from sterile sites ($n = 32$). The specimens were analysed during the period January 2012 to May 2015 in a large diagnostic microbiology laboratory that serves six acute care hospitals within its geographical area. This study was approved by the Sydney South West Local Health District Ethics Committee (HREC reference LNR/15/LPOOL/10.3).

Patient identification and microbiological methods

Thirty-two patients who had an equal number of clinical specimens ($n = 32$) sent to the laboratory were identified in the laboratory database. 16S rRNA PCR was performed on these clinical specimens. Standardized clinical details including primary diagnosis, primary site of infection, and empirical antibiotic therapy were obtained from the electronic medical records. Details of the specimen, initial microscopy, Gram stains, blood culture, and tissue culture were obtained from the microbiology database. Changes in the choice of antibiotic agent, duration of therapy, and decision to cease antibiotic therapy after 16S rRNA PCR results was evaluated by two infectious diseases physicians. 16S rRNA PCR testing is not currently a routine test in the laboratory workflow and is undertaken upon the request of the infectious diseases physician involved in the clinical case. Standard bacterial cultures were performed as per the current laboratory protocols. For blood cultures, the BACTEC system (Becton Dickinson Diagnostic Instrument System, North Ryde, Australia) was utilized; for tissue/fluid specimens, standard, selective, and enrichment media in combination were used, as per laboratory protocols. The calculation of sensitivity and specificity was done using bacterial culture (blood and/or tissue/fluid) as the gold standard for comparison. For the purpose of this study, the clinical impact was defined by (1) the cessation of therapy based on the result, (2) a change of category of antibiotic after the result, and (3) a change in duration of therapy after the result. This information was recorded in a Microsoft Excel spreadsheet.

Methodology of 16S rRNA PCR

16S rRNA PCR was performed in the State reference laboratory (Institute of Clinical Pathology and Medical Research, Westmead Hospital) using a previously validated and published method. [7] Specimens including cardiac valve tissue, joint aspirate or tissue biopsy, pleural fluid, brain biopsy, and bone biopsy were sent to the reference laboratory. For tissue samples, 16S rRNA paraffin sections were extracted manually by spin column purification using a GenElute Genomic DNA Miniprep Kit (Sigma Corp. St. Louis, MO, USA) as per the recommended protocol. For the cerebrospinal fluid (CSF) specimen, extraction was performed on an automated NucliSENS EasyMag instrument (bioMérieux, Marcy l'Etoile, France).

The PCR itself was performed by a single amplification targeting the short U1 and U3 regions in two separate reactions. The amplicon product was visualized by agarose gel electrophoresis and then sent for sequencing with each reaction's respective forward primer. The amount of DNA sequenced and the primer concentration were optimized. The amplification was done with GoTaq Flexi DNA Polymerase (Promega Corporation, Madison, WI, USA). Sequences were copied to sequence analysis software (Bio Manager) and analysed with an appropriate database using a BLAST search.[17–19]

Cost analysis

The cost analysis was done based on cost estimates from the Australian Refined Diagnosis Related Groups (AR-DRG). AR-DRG is an Australian admitted patient classification system that provides a clinically meaningful way of relating the number and type of patients treated in a hospital to the resources required by the hospital. An Independent Hospital Pricing Authority (IHPA) has been established in Australia, which utilizes information from the AR-DRG to evaluate the cost of medical treatment in public hospitals. The current cost of hospitalization and hospital-in-the-home (HITH) healthcare costs were modelled from this system (2015–2016 version).

Results

Of the 32 patients included in this study, eight (25%) had a clinical diagnosis of infective endocarditis and 18 (59.3%) had bone and joint infections (of which five were prosthetic joint infections, eight were native joint septic arthritis, and five were vertebral osteomyelitis/discitis/epidural abscess); one patient had both infective endocarditis and septic arthritis of the elbow. Two patients (6%) had central nervous system (CNS) infections (of which one had a confirmed brain abscess on imaging and one had a clinical diagnosis of meningitis), two had endovascular graft infections, and two had pleural empyema.

16S rRNA PCR could identify an organism in 10 cases (31.2%), of which seven were culture-positive and three were culture-negative. All three patients with 16S rRNA PCR-positive and

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