BRAZILIAN JOURNAL OF MICROBIOLOGY xxx (2018) xxx–xxx

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Medical Microbiology

A broad-range PCR technique for the diagnosis of infective endocarditis

Ines Boujelben[a], *Radhouane Gdoura*[b,*], *Adnane Hammami*[a]

[a] *Hospital Sfax, Department of Microbiology and Research Laboratory "Microorganismes et Pathologie Humaine" Habib Bourguiba, Sfax, Tunisia*

[b] *Université de Sfax, Faculté des Sciences de Sfax, Laboratoire de recherche Toxicologie Microbiologie Environnementale et Santé LR11ES06, Sfax, Tunisia*

ARTICLE INFO

Article history:
Received 19 March 2016
Accepted 23 March 2017
Available online xxx

Associate Editor: Afonso Barth

Keywords:
Broad-range PCR
Infective endocarditis
Conventional methods
Negative culture
Phenotypic investigations

ABSTRACT

Infective endocarditis (IE) remains a severe and potentially fatal disease demanding sophisticated diagnostic strategies for detection of the causative microorganisms. The aim of the present study was to develop a broad-range 16S ribosomal RNA gene polymerase chain reaction in the routine diagnostic of IE for the early diagnosis of fatal disease. A broad-range PCR technique was selected and evaluated in terms of its efficiency in the diagnosis of endocarditis using 19 heart valves from patients undergoing cardiovascular surgeries at the Habib Bourguiba Hospital of Sfax, Tunisia, on the grounds of suspected IE. The results demonstrated the efficiency of this technique particularly in cases involving a limited number of bacteria since it helped to increase detection sensitivity. The technique proved to be efficient, particularly, in the bacteriological diagnosis of IE in contexts involving negative results from conventional culture methods and other contexts involving bacterial species that were not amenable to identification by phenotypic investigations. Indeed, the sequencing of the partial 16S ribosomal RNA gene revealed the presence of *Bartonella henselae*, *Enterobacter sp.*, and *Streptococcus pyogenes* in three heart valves with the negative culture. It should be noted that the results obtained from the polymerase chain reaction-sequencing identification applied to the heart valve and the strain isolated from the same tissue were not consistent with the ones found by the conventional microbiological methods in the case of IE caused by *Gemella morbillorum*. In fact, the results from the molecular identification revealed the presence of *Lactobacillus jensenii*. Overall, the results have revealed that the proposed method is sensitive, reliable and might open promising opportunities for the early diagnosis of IE.

* *Corresponding author.*
E-mail: gdoura.radhouane@gmail.com (R. Gdoura).

https://doi.org/10.1016/j.bjm.2017.03.019

Please cite this article in press as: Boujelben I, et al. A broad-range PCR technique for the diagnosis of infective endocarditis. *Braz J Microbiol.* (2018), https://doi.org/10.1016/j.bjm.2017.03.019

Introduction

Conventional bacteriological diagnostic methods consist in the isolation and identification of the causative agent of an infection from a pathological product. This identification is based on the study of the phenotypic traits (morphological, cultural and biochemical) of the isolated bacteria. The microbiological diagnosis of infective endocarditis (IE) is mainly based on blood culture, excised cardiac valve tissue, or infected emboli. The conventional approach for the microbiological diagnosis of IE is successful in 92–95% of cases wherein a microorganism is present. Streptococci and Enterococci account for 45–60% of the cases of IE. Staphylococci represent the second most frequent group associated with IE. Various fungi or bacteria, such as *Enterobacteriaceae*, cause the remaining cases of IE. In fact, some bacteria are more specifically associated with IE, including those from the HACEK group (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella* and *Kingella*).[1]

Although conventional cultures are negative in 5–8% of IE cases, IE is a challenging disease to diagnose. This is particularly due to the wide variation in the criteria used to define it even after surgery. Cultures and microscopy may not reveal the causative organism in the infected valve. In such cases, slow-growing or non-culturable microbes may be the etiologic agents, and the patient may have received antimicrobial treatment at the time the specimen was obtained.[2] In addition, phenotypic identification techniques are not applicable for bacteria expressing little discriminating phenotypic characteristics. In recent years, researchers have increasingly become interested in the search for viable strategies and techniques to overcome the inadequacies and limitations associated with the conventional microbiological methods of IE diagnosis.

The literature indicates that culture-independent molecular techniques that involve the use of broad-range PCR to detect and amplify bacterial DNA and sequencing of the genes coding for 16S rRNA may open promising opportunities for establishing the etiology of the infection. In fact, the use of these techniques has revolutionized the detection and analysis of infectious diseases and the ways to combat them.[2,3] Furthermore, the literature indicates that the nested-PCR approach offers a rapid, efficient and reliable tool for the detection of bacterial infections from clinical specimens.[4,5] Some studies have, however, reported that the frequent incidence of false-positive results is a major problem hampering the application of nested PCRs in large-scale clinical settings.[4,6] Accordingly, the literature highlights the importance of the amplification of part of the 16S rRNA gene and direct sequencing of the amplicon to identify microbes in valve tissues. The determined sequence is often compared to the reference sequences, a procedure that often allows for the identification of the causative agent. The results of conventional bacteriological or/and histopathological diagnostic methods for IE have often been compared to those obtained by PCR.[1,2] The broad-range PCR appeared a relatively easy and reliable method that gives accurate results when applied to surgically removed heart valves of patients with IE and may be used as an adjunct for cases where cultural methods fail.[2]

The amplification of the gene that encodes the 16S rRNA of eubacterium-specific sequences from heart valve tissues seem to offer a new promising tool for the etiological diagnosis of IE. This method has allowed for the detection of fastidious organisms, such as *Tropheryma whipplei*, *Coxiella burnetii* and *Bartonella* spp, which require special culture conditions.[1,2] The broad-range PCR technique has also proved efficient for confirming the presence of several microorganisms responsible for IE, including *Granulicatella elegans*, Streptococci, Staphylococci, *Enterobacter*, *Borrelia burgdorferi*, *Candida albicans*, and *Aspergillus* species.[7,8] Lactobacilli have been identified in some clinical reports as causal agents of IE, meningitis and several other health diseases.[9]

Considering the growing global concerns over the prevalence of IE and the promising potential that molecular techniques might open for the identification and analysis of this serious infection, the present study aimed to investigate the feasibility and potential of a broad-range PCR technique, based on bacterial 16S rRNA gene, in the diagnosis of IE.

Materials and methods

Bacterial strains and specimens

The reference strains used as control organisms in the PCR performed in this study were *Escherichia coli* ATCC 25 922, *Staphylococcus aureus* ATCC 43 300, *Streptococcus pneumoniae* ATCC 49 619, *Haemophilus influenzae* ATCC 1399, *Salmonella Typhimurium* WHO 32, *Klebsiella pneumoniae* WHO 1, *Enterococcus faecalis* ATCC 159, *Citrobacter freundii* WHO 9, *Pseudomonas aeruginosa* WHO 8, and *Serratia marcescens* WHO 10. Some other strains that posed identification problems were also isolated from the heart valves of the patients, namely *Acinetobacter baumannii*, *Micrococcus lylae*, and *Gemella morbillorum*. The isolates of each of the above bacteria were obtained from the laboratory of clinical microbiology in the Habib Bourguiba hospital of Sfax, Tunisia.

Suspensions of *E. coli* ATCC 25 922, *S. aureus* ATCC 43 300, and *S. pneumoniae* ATCC 49 619 containing (10^6, 10^5, 10^4, 10^3, 10^2 and 10 CFU/mL) were also prepared by routine culture and dilution counts.[10]

The study used a total of 19 heart valves from patients who underwent heart valve replacement surgeries (Prosthetic valve) at the cardiovascular service of the Habib Bourguiba hospital of Sfax, Tunisia, on the grounds of suspected IE.

DNA extraction

The DNA of the bacterial strains of each specimen valve tissue was extracted by the Cetyltrimethylammonium Bromide–Phenol-Chloroform/Isoamyl Alcohol method ((CTAB)–Phenol-Chloroform/Isoamyl Alcohol). Prior to (CTAB)–Phenol-Chloroform/Isoamyl Alcohol extraction, 500 μL of lysis buffer (200 mM NaCl, 20 mM Tris HCl, pH 8, 50 mM EDTA, pH 8, and 1% SDS) and 25 μL of Proteinase K (10 mg/mL) (Sigma) were added to approximately 10 mg of valve tissue. The mixture was then vigorously agitated and incubated at 65 °C for 30 min or until the complete dissociation of valve tissue fragments. The enzymatic reaction

Please cite this article in press as: Boujelben I, et al. A broad-range PCR technique for the diagnosis of infective endocarditis. *Braz J Microbiol.* (2018), https://doi.org/10.1016/j.bjm.2017.03.019