



# Molecular Diagnosis of Bacterial Infective Endocarditis in Tabriz, Azerbaijan

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## ABSTRACT

**Introduction:** The aim of this study was to analyze a PCR based approach for detection of infective endocarditis in Azerbaijan.

**Methods:** Ten aortic valves, 8 mitral valves and 2 tricuspid valves, were analyzed for the presence of bacterial infective endocarditis using Gram staining, culture and PCR methods.

**Results:** Of the 20 valves, 5 and 4 cases were positive by Gram staining and culture assay, respectively. Bacterial DNA was positive in 12 of the 20 valves (60%) by broad-spectrum PCR. Direct sequencing for species identification was possible in 10 cases.

**Conclusion:** PCR and direct molecular identification of the etiological agents responsible for infective endocarditis may enable specific treatment to begin at an earlier phase of the disease.

## Introduction

Infective endocarditis (IE) is an important disease because of its high morbidity and mortality if untreated or not treated aggressively. Incidence of IE is between 1.7 to 6.2 cases per 100000 in the general population. Since, recurrent endocarditis is an ominous complication; the identification of causative agents could affect operative outcomes.<sup>1</sup> The diagnosis of IE remains challenging and nowadays, diagnosis of IE is based on the Duke criteria, principally positive blood culture and abnormal echocardiography.<sup>2</sup> The critical diagnostic finding is bacteremia, but blood cultures remain negative up to 30% of suspected IE patients despite the use of appropriate laboratory techniques.<sup>3</sup> Sterile blood culture may be caused by slow growing or non-cultivable microbes, or previous antibiotic therapy.<sup>4</sup> Recent advances in molecular assays, provides a significant improvement in detection of IE.<sup>5</sup> In this research, we applied a PCR based approach and direct sequencing for detection of IE.

## Materials and Methods

Blood cultures (Darvash, Iran) were incubated at 37 °C for at least 7 days. In case of growth, solutions were subcultured on Columbia agar (Merck, German), and on Brucella agar (Merck, German), both supplemented with 5% sheep blood, and incubation was done at 37 °C for 72h. Culture negative endocarditis was present when no microorganisms could be identified in serial blood cultures or valvular tissue cultures. The heart valves were

processed aseptically under a laminar flow unit. Then, portions of the valve tissue were ground with a mortar and pestle and cultured on mediums, and were incubated for 2 to 3 days. In addition, a piece of valve tissue was inoculated into Brain Heart Infusion and Thioglycolate mediums and incubated for 10 days. Finally, isolated colonies stained with Gram, and were identified according to standard methods.<sup>6</sup>

DNA was extracted from the tissues valves as previously described.<sup>4</sup> For each specimen, we used universal bacterial primers targeting conserved sequences at 16SrRNA bacterial gene, as described previously.<sup>4,7</sup> Finally, the amplicons were sent for direct sequencing.

## Results

From 2009 to 2012, 20 patients with IE (14 males and 6 females; mean age 56 years) were studied. Ten patients had the aortic valve IE, 8 and 2 patients suffered from IE of the mitral valves and tricuspid valves, respectively. The causes of operation were progressive infection despite drug treatment, infectious embolism, or increasing heart failure. The infected valves were native in 14 (70%) and prosthetic in 6 (30%) of patients. Twenty cardiac valves were analyzed for the presence of IE by Gram staining, culture and PCR methods.

Before surgery, the blood culture for 2 of 20 patients was positive. For 3 patients, the single isolation of coagulase negative staphylococci was contamination. The isolated microorganisms were *Staphylococcus aureus*

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and *Pseudomonas aeruginosa*. Culture and molecular results were analyzed with respect to the patients' clinical background and the Duke Criteria.

Of the 20 valves, 5 were positive by microscopy and Gram staining. The valve cultures were positive in 4 cases. Therefore, culture-positive endocarditis was present in 20%; whereas, 80% of the patients had culture-negative endocarditis.

Bacterial DNA was detected in 12 of the 20 valves (60%) by broad-spectrum PCR (Figure 1). Direct sequencing for species identification was possible in 10 cases. The remaining 2 cases were impossible to identify the microbe by sequencing. The results of microscopy, culture, and PCR are presented in Table 1.

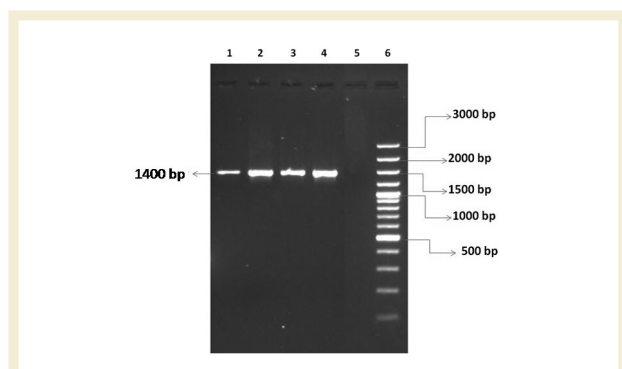
## Discussion

IE is a disease with high morbidity and mortality. Adequate antimicrobial management depends on the microbial diagnosis of the causative pathogen.<sup>8</sup> Most cases of IE are caused by staphylococci and streptococci species. The frequency of culture negative IE varies among different previous studies, ranging from 2.5-31%.<sup>1,7</sup>

In this study, a significant percentage (80%) remained culture-negative, because of previous antibiotic treatment

or the presence of microorganisms with fastidious growth. In such cases, blood culture systems require longer incubation periods (> 6 days). Also in culture negative IE, all tissue excised during cardiac surgery should be examined.<sup>5</sup> The theory of culture negative IE is likely to be more difficult than those where the microorganism is known.<sup>9-11</sup> The value of serology has been proven for some IEs as Bartonella, Legionella, Chlamydia and Coxiella.<sup>5,11</sup> In cases of surgical therapy of IE in the negative blood cultures, microbiological assessment of excised heart valves is the only way to identify the causative microorganism. Paradoxically, the improved antibiotic treatment of IE during the past decades may have increased the diagnostic failure rate because the microorganisms present on the excised valve are nonviable. This has limited our knowledge of the current spectrum of organisms causing IE.<sup>8</sup>

IE usually is detected by culture and serology. Recently diagnosis relies more on the PCR-based methods thanks to their accuracy, usefulness, and likely widespread availability for identification.<sup>1,12,13</sup> When traditional methods are negative, PCR assay may establish the etiology of culture negative IE.<sup>7,11,14-17</sup> We used a broad-spectrum PCR to amplify bacterial ribosomal sequence, followed by direct sequencing to detect and differentiate the causative agents of IE. In the previous studies, molecular assays such as PCR and subsequent direct sequencing were useful in diagnosing the cause of IE.<sup>1,3,7,11,14,18</sup> Goldenberger *et al.*, first reported the use of PCR followed by sequencing, in 18 excised heart valves.<sup>4</sup> Newly, it was recommended that molecular diagnosis of IE should be included in the Duke's classification system.<sup>1,11,19,20</sup> In this research, sensitivity of culture and Gram staining methods in comparison to PCR were 33.3% and 41.6%, respectively. In this study, molecular assay showed to be more sensitive and specific than conventional blood and tissue culturing techniques for the detection of bacteria. The main advantages of this technique are that it is effective irrespective of prior antibiotic therapy, and approximately all bacteria can be detected in a single reaction with the use of broad-spectrum primers. Also, both live and dead bacteria may result in positive PCR assays.<sup>19</sup> When we considered bacterial species separately, all patients with endocarditis due to bacteria were positive by PCR. This may reflect greater amounts of DNA in infected valves or the fact that DNA of bacteria remains for longer periods in tissues. The cost of PCR-based methods are not cheap. When bearing in mind the costs of experimental therapy, numerous expensive antibiotics and multiple investigations done in the workup, these costs are modest. Moreover, availability of the medications and diagnostic modalities should be considered based on the geographical patterns.<sup>18-20</sup> Such knowledge and understanding of the conditions would contribute to better diagnosis, treatment and management of the diseases.



**Figure 1.** Gel electrophoresis of PCR products (1400 bp). Lane 1, 2 and 3: Samples that were 16s rRNA positive, Lane 4: Positive control, Lane 5: Negative control, Lane 6: DNA ladder 1000-bp

**Table 1.** Identified bacteria in this study

Bacteria (N)	Microscopy (5)	Culture (4)	Molecular assay (10)
<i>Streptococcus</i> spp.	1	1	3
<i>Staphylococcus epidermidis</i>	1	0	2
<i>Staphylococcus aureus</i>	1	1	1
<i>Enterococcus faecalis</i>	1	1	2
<i>Propionibacterium acnes</i>	0	0	1
<i>Pseudomonas aeruginosa</i>	1	1	1

## Conclusion

In conclusion, the most commonly isolated microorganism was *Streptococcus* spp. and, PCR have the potential to detect the agents causing blood culture negative IE. Thus, molecular assays provide useful clinical and epidemiological information with significantly improved specificity and sensitivity.

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**Ethical issues:** This study was reviewed and confirmed by the ethics committee of Tabriz University of Medical Sciences.

**Conflict of interests:** The authors declare no conflicts of interest.

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