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Review Article

The diagnosis of microorganism involved in infective endocarditis (IE) by polymerase chain reaction (PCR) and real-time PCR: A systematic review

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Abstract Broad-range bacterial rDNA polymerase chain reaction (PCR) followed by sequencing may be identified as the etiology of infective endocarditis (IE) from surgically removed valve tissue; therefore, we reviewed the value of molecular testing in identifying organisms' DNA in the studies conducted until 2016. We searched Google Scholar, Scopus, ScienceDirect, Cochrane, PubMed, and Medline electronic databases without any time limitations up to December 2016 for English studies reporting microorganisms involved in infective endocarditis microbiology using PCR and real-time PCR. Most studies were prospective. Eleven out of 12 studies used valve tissue samples and blood cultures while only 1 study used whole blood. Also, 10 studies used the molecular method of PCR while 2 studies used real-time PCR. Most studies used 16S rDNA gene as the target gene. The bacteria were identified as the most

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common microorganisms involved in infective endocarditis. *Streptococcus* spp. and *Staphylococcus* spp. were, by far, the most predominant bacteria detected. In all studies, PCR and real-time PCR identified more pathogens than blood and tissue cultures; moreover, the sensitivity and specificity of PCR and real-time PCR were more than cultures in most of the studies. The highest sensitivity and specificity were 96% and 100%, respectively. The gram positive bacteria were the most frequent cause of infective endocarditis. The molecular methods enjoy a greater sensitivity compared to the conventional blood culture methods; yet, they are applicable only to the valve tissue of the patients undergoing cardiac valve surgery.

Introduction

Infective endocarditis (IE) is a serious infection of the endocardium because of several bacteria and fungi that usually involve the valves and neighbor structures [1]. Firstly, IE was described by Osler in 1885 in patients with a history of the valvular disease [2]. It occurs in 1.7—6.2 in 100,000 in the general population with high morbidity and mortality rates in misdiagnosed and untreated patients [1]. The symptoms of IE are not specific; however, fever, vegetation in echocardiography, and positive blood culture present in the most of the cases [3]. In clinical practices, the traditional and modified Duke criteria are the most-frequently used criteria for diagnosis of IE in suspected patients [4—7]. Although remarkable improvements have been achieved in the diagnosis and treatment of IE, however, the mortality rate is about 20% in hospitalized patients [8,9], and about 50% of the cases with IE develop at least one complication [10]. In line with these findings, the epidemiological practices indicated that in recent years, the incidence of the IE has increased especially in the elders and in hospitalized patients [11—13]. Several risks factors are related to the IE such as bacterial resistance [14—16], old age [17], intracardiac devices [18], prosthetic valves [19], and hemodialysis [20]. For pathogen identification, the blood culture is an accepted gold standard; however, the blood culture may be negative in 2—30% of the patients with suspected IE [21] because of prior antibiotic therapy, small amounts of microorganisms, slow growing pathogens, and some other factors [22,23]. Moreover, the valvular cultures are not reliable due to antibiotic therapy and contamination [3,24]. Therefore, the scientists introduced the culture-independent universal 16S rRNA gene polymerase chain reaction (PCR) and sequencing to the diagnosis of IE and to the identification of new pathogens in IE [25—30]. In harmony, the studies indicated that culture-negative endocarditis (CNE) patients are almost complicated patients [31], and in these patients, polymerase chain reaction (PCR) is a more helpful technique. Furthermore, they suggested that due to the large number of microorganisms obtained from the infected heart valves (HVs), PCR evaluation is usually a very effective and accurate diagnostic technique [32—35]. Additionally, in the real-time PCR, the scientists can monitor PCR cycle by cycle and can perform post-PCR evaluation in the same PCR tube, resulting in lower contamination risks. Also, the real-time PCR is faster, simpler, and more sensitive than the traditional PCR [36]. In this systematic review, we analyzed the literature evaluating pathogens involved in infective endocarditis using PCR and real-time PCR.

Materials and methods

Data sources and searches

In this systematic review, in order to investigate the molecular methods of PCR and real-time PCR to diagnose causative microorganisms involved in the etiology of infective endocarditis, we searched Google Scholar, Scopus, ScienceDirect, Cochrane, PubMed, and Medline electronic databases without any time limitations up to December 2016 with language restrictions (only English language) for studies reporting microorganisms involved in infective endocarditis microbiology using PCR and real-time PCR. All the potentially relevant papers were reviewed independently by two investigators using the standard key words. Any conflict between the first two researchers was resolved by the third researcher. The key words included: "infective endocarditis, PCR, and real-time PCR" which were selected by OR & AND operators in combination. Then, all the reviewed studies that observed the inclusion criteria were evaluated. The Preferred Reporting Items for Systematic Reviews (PRISMA) guideline was followed for this review, which is a standard guideline for systematic reviews [37].

Study selection and data collection

All original prospective, retrospective, and cross-sectional, observational studies were included; however, the case reports, the editorials, and the lack of access to the full texts of articles were not included. The criteria for enrolment were: a clear definition of the time of the study, infective endocarditis, and the kind of the microorganisms. To avoid bias, we included the studies on general population with IE and studies on patients with some specific diseases such as HIV, immunodeficiency, and drug abusers were excluded. From each study, the data such as first author's last name, year of publication, countries, time of the study, the types of studies, the traditional PCR and real-time PCR, and the kind of microorganisms were

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extracted and recorded. All the searched articles were imported to EndNote, the repetitious cases were omitted, the titles and abstracts were studied, and the irrelevant articles were excluded.

Results

In this systematic review, we evaluated 12 studies without any time limitations up to December 2016. In so doing, first 80 probably-relevant articles were found. Of these, 15 repetitious articles were omitted, i.e., articles with the same author, title, and journal. Then, the abstracts of the 65 remaining articles were delved into and 30 irrelevant articles were excluded. Next, the full text of the remaining 35 articles were contemplated on leading again to the exclusion of 23 more articles due to irrelevance and low quality. Ultimately, 12 articles qualified for inclusion in the systematic review (Fig. 1).

Most of these studies have been conducted in Europe, but two have been conducted in Canada and Egypt. Six studies were prospective, 2 studies were retrospective, and 2 studies were observational cross-sectional, while the design of 2 other studies was not mentioned or clear. Eleven out of 12 studies used valve tissue samples and blood cultures while only 1 study used whole blood. Also, 10 studies used the molecular method of PCR and 2 studies used real-time PCR (Table 1). The microorganisms involved in infective endocarditis by PCR and real-time PCR in the all of the studies are presented in Table 1. On the basis of this table, gram positive microorganisms were the most frequent cause of infective endocarditis. The target genes used in these 12 studies were 16S rDNA in 6 studies, 16S rRNA in 2 studies, and 23S rDNA in 1 study for bacteria and 28S rDNA for fungi. In another study, 16S rDNA, 18S rDNA, and 28S rDNA were used for bacteria and fungi, respectively. Another study used 16S rDNA for bacteria and 18S rDNA and 28S rDNA for fungi and finally, another study used universal rRNA.

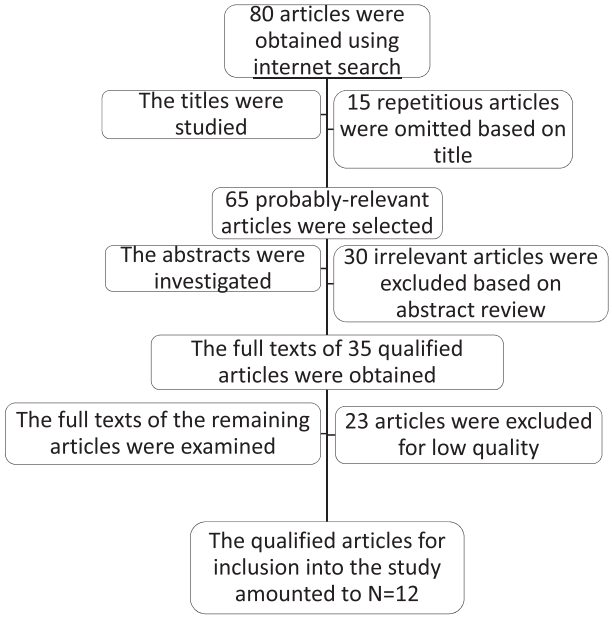

Figure 1. Flow chart of investigation and final selection processes of articles for inclusion into the systematic review.

In all of these studies, PCR and real-time PCR identified more pathogens than blood and tissue cultures. Moreover, the sensitivity and specificity of PCR and real-time PCR were more than cultures in the most of the studies. The highest sensitivity and specificity were 96% and 100%, respectively (Table 2).

Discussion

The use of molecular methods for the diagnosis of infective endocarditis does not have a long history. Even many studies introduce these methods as innovative modalities of diagnosing infective endocarditis [26,29]. Goldenberg et al. (1997) reported for the first time the results of their study on infective endocarditis using PCR. They concluded that the molecular method is of high sensitivity and reliability, and is a fast method of diagnosis of microbial infective endocarditis [38]. Most of the studies investigated in this systematic review suggested that the wide-range bacterial DNA PCR is useful in many cases including the following: 1) when a small amount of bacteria is the cause of infective endocarditis, 2) when blood culture is positive for just one case, 3) when replacement of uninfected valve leads to histological diagnosis of infective endocarditis, and 4) when the patient has previously used antibiotics [26,38,39]. Even Millar et al. and also Tak et al. have recommended that molecular diagnostic methods be added to Duke's criteria as a gold standard [1,29]. In this systematic review, 12 studies were analyzed. In 11 of these studies, valve sample culture and blood culture were used to perform the molecular tests. The blood sample culture is important since it can determine the causative organism that induces the disease and this facilitates the appropriate prescription and administration of the required antibiotic resulting in more effective treatment [5,6,27,40,41]. However, in 2.5%—31% of cases of infective endocarditis, blood cultures are negative [3,5,27,42]. Many studies demonstrate that the negative result of blood culture may be due to previous antibiotic therapy, small amount of microorganisms per volume, presence of slow-growing or hard-growing microorganisms such as *Tropheryma whipplei*, *Coxiella burnetii*, *Legionella* species, *Mycoplasma* species, *Haemophilus* species, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella* species, and *Chlamydia* specie and also *HACEK* group [6,27,35,40,42]. Of course, it should be kept in mind that culturing method, number of blood cultures, and meticulous care at the time of blood sampling or phlebotomy may affect the results of blood culture tests [43]. The analysis of the results of 12 studies indicated that the sensitivity of molecular methods is higher than that of the conventional blood culturing methods so that the highest sensitivity belonged to Marín's study with 96% sensitivity [36] while the PCR results in some cases have been even negative. Marín and Miller attribute the negative results of PCR test to the presence of low amounts of microorganisms in the sample or to the high probability of sampling error [36,41]. Kotilainen et al. stated in their study that negative PCR result does not mean that the patients have responded

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Table 1 The characteristics of the studies and identified microorganisms.

Author/Year	Type of studies	Country	Type of specimens	Technique (Target gen)	Microorganism detected (n values)
Marín [36] 2007	Prospective	Spain	HV	Real-time PCR (16S rRNA)	*Streptococcus* spp. *(6)* *Staphylococcus* spp. *(2)* *Corynebacterium glutamicum (1)* *Enterococcus faecalis (1)* *Tropheryma whipplei (1)* *Bartonella quintana (1)* *Abiotrophia defective (1)* *Peptostreptococcus micros (1)* *Candida albicans (1)*
Vollmer [35] 2010	Retrospective	Germany	HV	Real-time PCR (targeting bacterial 23S rDNA, fungal 28S rDNA, and mycoplasma tuf gene)	*Streptococcus* spp. *(1)* *Staphylococcus* spp. *(1)* *Enterococcus* spp. *(1)* *Abiotrophia* spp. *(1)* *Actinobacillus actinomycetemcomitans (1)* *Bartonella* spp. *(2)* *Candida* spp. *(1)* *Citrobacter koseri (1)* *Enterobacter aerogenes (1)* *Gemella sanguinis (1)* *Haemophilus parainfluenzae* *Proteus mirabilis (1)* *Serratia marcescens (1)* *Tropheryma whipplei (1)*
Kuhn [44] 2011	Prospective	Germany	HV & WB	Broad-range PCR (universal rRNA)	*Streptococcus* spp. *(4)* *Staphylococcus* spp. *(1)* *Enterococcus faecalis (1)* *Candida* spp. *(2)* *Haemophilus parainfluenzae (1)*
Harris [40] 2014	Prospective	UK, Ireland	HV	Broad-range PCR (16S rDNA)	*Streptococcus* spp. *(12)* *Staphylococcus* spp. *(2)* *Bartonella* spp. *(3)* *Coxiella burnetii (1)* *Haemophilus* spp. *(2)* *Lactococcus lactis (1)* *Legionella pneumophila (1)* *Neisseria gonorrhoeae (1)* *Prevotella denticola (1)* *Propionibacterium acnes (1)* *Terrahaemophilus aromaticivorans (1)* *Tropheryma whipplei (1)*
Miller [41] 2016	Prospective	Canada	HV	Broad-range PCR (16S rDNA)	*Streptococcus* spp. *(3)* *Staphylococcus* spp. *(2)* *Enterococcus faecalis (1)* *Haemophilus parainfluenzae (1)* *Gram-negative bacilli (1)* *Propionibacterium acnes (1)* *Cardiobacterium hominis (1)* *Delftia tsuruhatensis (1)* *Acinetobacter junii (1)*
Vondracek [45] 2009	Prospective	Sweden	HV	Broad-range PCR (16S rDNA)	*Streptococcus* spp. *(6)* *Staphylococcus* spp. *(3)* *Enterococcus* spp. *(2)* *Gemella species (1)* *Abiotrophia* spp. *(1)*

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Table 1 (*continued*)

Author/Year	Type of studies	Country	Type of specimens	Technique (Target gen)	Microorganism detected (n values)
Voldstedlund [46] 2005	Retrospective	Denmark	HV	Broad-range PCR (16S rDNA)	*Streptococcus* spp. *(12)* *Staphylococcus* spp. *(3)* *Enterococcus faecalis (1)* *Tropheryma whipplei (1)* *Gemella morbillorum (1)*
Bosshard [25] 2001		Switzerland	HV	Broad-range PCR (16S rDNA)	*Streptococcus* spp. *(7)* *Staphylococcus* spp. *(1)* Unclassified oral streptococci *(1)* *Enterococcus faecalis (1)* *Haemophilus parainfluenzae/paraphrophilus (1)*
Breitkopf [39] 2004	Cross-sectional, observational	Germany	HV	Broad-range PCR (targeting bacterial 16S rDNA, fungal 18S & 28S rDNA)	*Streptococcus* spp. *(5)* *Staphylococcus* spp. *(2)* *Bartonella quintana (1)* *Enterococcus faecalis (1)* *Nocardia paucivorans (1)* *Propionibacterium acnes (1)*
Guern [47] 2014	Prospective	France	HV	Broad-range PCR (16S rDNA)	*Streptococcus* spp. *(10)* *Staphylococcus* spp. *(3)* *Granulicatella* spp. *(2)* *Enterococcus faecalis (1)* *Serratia* spp. *(1)* *Cryptosporidium hominis (1)* *Propionibacterium avidum (1)* *Bartonella quintana (1)* *Serratia marcescens (1)*
El-Kholy [48] 2010	Cross sectional	Egypt	HV	Broad-range PCR (targeting bacterial 16S rRNA, fungal 18S & 28S rRNA)	*Streptococcus* spp. *(3)* *Staphylococcus* spp. *(4)* *Acinetobacter* spp. *(2)* *Brucella* spp. *(1)* *Aspergillus flavus (1)* *Bartonella quintana (1)* *Kytococcus schroeteri (1)* *Candida albicans (1)* *Pseudomonas stutzeri (1)* *Micrococcus (1)*
Greub [49] 2002		France	HV	Broad-range PCR (16S rRNA)	*Streptococcus* spp. *(2)* *Coxiella burnetii (1)* *Mycoplasma hominis (1)*

HV = heart valve.
WB = whole blood.

appropriately to antibiotics [50]. Various reasons for negative PCR results may include: 1) the long interval lapsing between antibiotic therapy and surgical operation, 2) the application of an improper valve segment for PCR; 3) or the effect of PCR inhibitors (though albeit extraction and amplification as controls led to the expected outcomes; moreover, the result of amplification of the beta-globin gene was positive), 4) Lack of designing of the proper primer, 5) Low quality of the extracted DNA, 6) Inappropriate concentrations of enzymatic buffers, 7) Lack of proper setting up of thermal cycles with respect to time and lack of proper selection of bonding temperature of the primer, 8) Low quality of electrophoresis gel, specially for low product genes [26]. In addition to sensitivity which is the most important difference between blood sample culture and molecular methods, it should also be noted that blood sample culture is still used as the gold standard for diagnosing and identifying blood pathogens while it takes a long time to achieve results. This is the case while many important microorganisms are hardly ever cultivable or cannot be cultured at all [51]. However, the molecular methods enjoy a very high speed of giving results. The high speed in giving results is mandatory for some acute infectious disorders such as infective endocarditis. Yet, the molecular methods are much more expensive than blood culture method and are not in widespread clinical use [52]. Moreover, despite the easy application, high speed, and great accuracy, the molecular methods suffer from many deficiencies like infection. The use of protected Oligonucleotide primers for various genes of microorganisms may

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Table 2 The validity of PCR and real-time PCR methods were investigated in the studies.

Author/Year	Validity test				n values
	Sensitivity	Specificity	PPV	NPV	
Marín [36] 2007	96%	95.3%	98.4%	88.5%	177
Vollmer [35] 2010	80.6%	100%	100%	71%	357
Kuhn [44] 2011	85%	—	—	—	30
Harris [40] 2014	67%	91%	96%	46%	169
Miller [41] 2016	92%	77.8%	92%	77.8%	68
Vondracek [45] 2009	81%	100%	—	—	57
Voldstedlund [46] 2005	72%	100%	—	—	74
Bosshard [25] 2001	82.6%	100%	100%	76.5%	63
Breitkopf [39] 2004	41.2%	100%	100%	34.8%	52
Guern [47] 2014	72.7%	94.7%	—	—	82
El-Kholy [48] 2010	88.3%	92%	79.1%	95.8%	156
Greub [49] 2002	61%	100%	100%	74%	245

PPV = positive predictive value.
NPV = negative predictive value.

be associated with many problems induced by the simultaneous reproduction of the alien DNA and target DNA. These complexities would lead to reduced efficiency of molecular methods in diagnostic services [53]. Another sample used in the reviewed studies was valve tissue. The valve tissue samples are not considered as appropriate as they may be contaminated during surgery and excision. This is because the isolated microorganism may not be the causative organism for infective endocarditis; rather, it may be transmitted during surgery [23,24,36]. Nonetheless, the valve tissue samples are considered as appropriate today for investigating cases of endocarditis with negative blood culture [30] because bacterial DNA may remain in the infected valve for several weeks despite antibiotic therapy [27,50,54]. The long survival of bacterial DNA in the valve may be attributed to: 1) a persistent infection, 2) the original infection not treated fully because of deep infiltration into valve, or because it could not be treated even by sufficient antibiotic therapy, so bacteria survived though they could not grow, and 3) PCR method detected DNA from the killed bacteria [32,35]. Marín et al. asserted in their study that the most important limitation of the use of valve tissue for molecular tests is that it could be used only for patients who need cardiac valve surgery while it could not be used for patients with infective endocarditis who do not require valve surgery [36]. In our review, only 1 out of 12 studies used whole blood [44]. Additionally, Vollmer et al. postulated in their study that their previous study with unpublished data investigated 23 whole blood samples of 33 patients with infective endocarditis using rDNA PCR assay. However, the PCR findings were not successful. They attributed their failure to low number of bacteria in whole blood samples (35). Nevertheless, it appears that the development of methods for identifying the etiological parameters of IE using blood samples of patients' not undergoing surgery who have negative blood cultures is still a challenging issue [45]. Yet, Fournier et al. believe that greatly sensitive PCR tests which specifically aim at bacteria can serve as worthwhile detection instruments for blood samples in the future [42]. The target genes used in these 12 studies were 16S rDNA in 6 studies, 16S rRNA in 2 studies, and 23S rDNA in 1 study for bacteria and 28S rDNA for fungi. In another study, 16S rDNA, 18S rDNA, and 28S rDNA were used for bacteria and fungi, respectively. Another study used 16S rDNA for bacteria and 18S rDNA and 28S rDNA for fungi and finally, another study used universal rRNA. The existence of extensive databases renders the application of rDNA as primary target as a highly favorable daily approach [50]. Vollmer et al. asserted in their study that in Real-time PCR, using 23S rDNA target was more beneficial than the common standard 16S rDNA for the detection of microorganisms in valve tissues; nevertheless, 16S rDNA remained more helpful for identifying the species level when proper [35]. It is mandatory to improve the etiological diagnosis of blood culture-negative endocarditis (BCNE). BCNE is rendered as a famous clinical procedure with clinical implications for treatment and prognosis. This stands as the main justification for the adoption of 16S rDNA test to analyze tissue from heart valves. Furthermore, 16S rDNA sequencing is helpful when it is difficult to interpret blood culture isolates [45]. The analysis of the results of 12 studies indicated that the causative pathogenic microorganisms inducing infective endocarditis were first bacteria, and second, fungi while gram positive bacteria were most frequent among the bacteria. Generally, fungal or mycoplasma endocarditis is rare [35]. Moreover, 45%—60% of cases of infective endocarditis were caused by *Streptococci* and *enterococci* [11,26,55,56], 17%—41% by *viridans streptococci* (such as *Streptococcus sanguinis*, *Streptococcus salivarius* and *Streptococcus mutans*) [11,26,55,57,58], 5%—15% by D group such as *Streptococcus bovis* [8,26,55], and 5%—10% by *Enterococcus faecalis* [11,26,55]. *Staphylococci* are considered as the second group of microorganisms causing infective endocarditis so that 15%—23% of cases are caused by *Staphylococcus aureus* while 3%—8% of cases are caused by coagulase-negative *Staphylococcus* [11,26,55,59]. Other causative organisms inducing infective endocarditis include various bacteria such as *Enterobacteriaceae* or fungi like *Candida* spp. Furthermore, some bacteria like HACEK (*Haemophilus parainfluenzae*, *Haemophilus aphrophilus*, and *Haemophilus paraphrophilus*, *Actinobacillus actinomyces comitans*, *C. hominis*, *Eikenella corrodens* and *Kingella kingae* account, on the average, for 4% of cases [11,26,55,57]. Based on the findings of the causative microorganisms of infective endocarditis mentioned in the studies above, it could be said that gram positive bacteria accounted for most cases of infective endocarditis. Nonetheless, various other microorganisms are involved in infective endocarditis, as well and the incidence of this disorder cannot be

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attributed to just one specific group of microorganisms [26]. The only reason for the dominant role of gram positive bacteria in the etiology of this disorder is their rapid growth compared to other microorganisms, specially the fastidious bacteria [60].

Conclusion

Regarding the 12 studies analyzed in this systemic review, it can be said that the molecular methods used in diagnosing the causative microorganisms involved in the etiology of infective endocarditis are only applicable to the valve sample of patients undergoing cardiac valve surgery. So, it could not be helpful in the prompt diagnosis of pathogenic microorganisms. It is recommended that some future studies be conducted on samples other than cardiac valve tissue to diagnose promptly the pathogenic microorganisms involved in the etiology of infective endocarditis and to obtain a therapeutic option other than surgical intervention.

Study limitations

This study excluded non-English articles and articles without full text.

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