بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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Role of Multiplex PCR in Early Diagnosis of Infective Endocarditis

Thesis

Submitted for Fulfillment of the Master Degree in Medical Microbiology and Immunology

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Omnia Abdelaziz

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List of Abbreviations

Abb.	Full term
18F FDG-PET/CT	18F-fluorodeoxyglucose positron emission tomographic computed tomographic
AE	Elusion buffer
AL	Tissue lysis buffer
AMA	American Heart Assosiation
AMB	Amphotericin B
ASD	Atrial septal defect
AV	Aortic valve
AW	Wash buffer
BCNE	Blood culture negative endocarditis
CDC	The Center for Disease Control and Prevention
CI	Confidence interval
CIED	Cardiac implantable electronic device
CoNS	Coagulase negative staphylococci
ECG	Electrocardiogram
EDTA	Ethylene diamine tetraacetic acid.
ESC	European Society of Cardiology
FDA	Food and drug administration
FE	Fungal endocarditis
GNB	Gram negative bacilli
HACEK	Haemophilus, Aggregatibacter, Cardiobacterium spp., Eikenella and Kingella spp
HAI	Hospital acquired infection
IE	Infective endocarditis
IVDU	Intravenous drug users

List of Abbreviations Cont...

Abb.	Full term
MDR	Multidrug resistant
MIC	Minimal inhibitory concentration
MRI	Magnetic resonance imaging
MRSA	Methicillin resistant *staphylococcus aureus*
MSCRAMMS	Microbial surface components recognizing adhesive matrix molecules
MSCT	Multislice computed tomography
MSSA	Methicillin sensitive *staphylococcus aureus*
MV	Mitral valve
NBTE	Non bacterial thrombotic endocarditis
NPV	Negative predictive value
NVE	Native valve endocarditis
PCR	Polymerase chain reaction
PPV	Positive predictive value
PVE	Prosthetic valve endocarditis
ROC	Receiver operating characteristic curve
SD	Standard deviation
TAE	tris acetate EDTA buffer
TEE	Transeosophageal echocardiography
TTE	Transthoracic echocardiography
TV	Tricuspid valve
VGS	Viridans group streptococci
VRE	Vancomycin resistant enterococci
VSD	Ventricular septal defect
VSE	Vancomycin susceptible enterococci
WHO	World health organization

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INTRODUCTION

Infective endocarditis (IE) is an infection of the endocardial surface of the heart, most commonly the valves, but also may occur on mural endocardium, on cardiac septal defects, on arteriovenous or arterioarterial shunts and on intravascular devices ***(Cuervo et al., 2021).***

Infective endocarditis (IE) encompasses native, prosthetic valves or any intracardiac devices within the heart. It is caused by the sowing of any of these structures by bacterial or, less frequently, fungal organisms ***(Shmueli et al., 2020).***

The clinical presentation of IE is highly changeable and may represent as an acute, subacute or chronic condition that mirrors the variable causative microorganisms, underlying cardiac conditions and pre-existing comorbidities ***(Rajani & Klein, 2020).***

Among cardiovascular disorders, infective endocarditis (IE) is considered an uncommon cardiovascular condition - its overall incidence is estimated at 3-10 episodes per 100 000 person-years. Nonetheless, this number is almost certainly underestimated as the diagnosis of endocarditis is difficult and some patients remain undiagnosed ***(de Sousa et al., 2021).***

The overall complication rate is high in Egypt (39.4%). The main complications are potentially life-threatening,

including heart failure, severe sepsis, and renal failure ***(Rizk et al., 2019).***

Despite therapeutic advances, the mortality rate remains high with 14–22% in-hospital mortality rates and up to 51% mortality at 10 years. It is also associated with significant morbidity including prolonged hospital stay, reduced quality of life and a high risk of re-infection ***(Williams et al., 2021).***

The number of cases of IE per annum is probably growing due to the increasing use of prosthetic valves and cardiac implantable electronic devices also due to an increase in the number of immunocompromised patients, and extended survival of patients with congenital heart disease ***(Cahill & Prendergast, 2016).***

It is noteworthy to state that neither the incidence nor the mortality of IE has declined in the previous 30 years. In addition, IE represents in different clinical presentation and its management varies according to physician experience which render IE a peculiar disease ***(Sadaka et al., 2013).***

Blood-culture is commonly known as the gold standard for the detection of microbial pathogens in the bloodstream. This methodology, though, possesses some intrinsic restrictions, for example it is difficult and can only identify microbes that grow under optimal cultural conditions. In contrast, PCR based techniques are sensitive to small amounts

of pathogen's DNA and are able to directly detect it in blood samples within 3–6hours, thus supporting the treatment subsequently ***(Trung et al., 2019).***

A predisposing condition, such as rheumatic heart disease, is nowadays less common except in low-income countries. Intravenous drug use have decreased globally, but a dramatic increase of this habit has continued to alter the epidemiology of endocarditis in North America and in some Eastern European countries. Other risk factors are being increasingly detected in high-income countries, such as degenerative valve disease, intracardiac devices, indwelling catheters and immunosuppressive conditions ***(Cuervo et al., 2021).***

Nonetheless most microorganisms linked with IE are diagnosed by blood cultures and occasionally with serology, diagnosis in the forthcoming decade will expected to rely further on molecular biology methods in attempt to perform detection and identification of bacteria. That is owing to their noticeable competence in the etiological diagnosis with accuracy, efficiency, and anticipated wide accessibility. This is significantly crucial and feasible in the case of culture-negative endocarditis caused by preceding antibiotic intake or incapability of fastidious microorganisms to cultivate ***(Brouqui & Raoult, 2006).***

PCR-based diagnostics have been effectively developed for a wide range of microbes. Due to its incredible sensitivity, specificity, and speed of amplification, PCR has been championed by infectious disease experts for identifying organisms that cannot be grown *in vitro*, or in instances where existing culture techniques are insensitive and/or need prolonged incubation times ***(Bajinka & Secka, 2017).***

Multiplex PCR is a novel methodology that permits simultaneous detection of several organisms through introduction of different primers to amplify DNA region coding for particular genes of each bacterial strain targeted ***(Rajapaksha et al., 2019).***

The multiplex PCR assay is sensitive, specific, rapid and less costly. This methodology could be used in clinical laboratories for rapid identification and induction of specific and effective treatment, decreasing patient mortality and morbidity. Additionally, it may help decreasing abuse of antimicrobials that are more expensive and toxic ***(Ali et al., 2018).***

There are several multiplex assays for the fast recognition of microorganisms in clinical samples within about 8h ***(Bloos et al., 2012; Tsalik et al., 2010)***. *Staphylococcus aureus* has overpassed streptococci as the most common causative pathogen throughout the world and particularly in the developed world given its common association with health-care contact and invasive procedures ***(Khalid et al., 2020).***