بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

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Nested Multiplex PCR Assay for Simultaneous Detection of Bacterial and Fungal Blood Stream Infections in Patients with Hematological Malignancies

Thesis

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Nesma Abdel Aziz Hamdi Hassanin

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List of Abbreviations

Abb.	Full term
ANC	*Absolute Neutrophil Count*
ASCO	*American Soceity of Clinical Oncology*
BC	*Blood Culture*
BSI	*Blood Stream Infection*
CDC	*Centers for Disease Control & Prevention*
CLABSI	*Central Line Associated Blood Stream Infection*
CRP	*C-Reactive Protein*
CoNS	*Coagulase-Negative Staphylococci*
DNA	*Deoxyribonucleic Acid*
ESBL	*Extended Spectrum Beta Lactamase*
FISH	*Fluorescence in Situ Hybridization*
GNB	*Gram-Negative Bacilli*
HCT	*Hematopoietic Cell Transplantation*
HM	*Hematological Malignancy*
ICU	*Intensive Care Unit*
IDSA	*Infectious Disease Society of America*
LCBI	*Laboratory Confirmed Blood Stream Infection*
MALDI-TOF MS	*Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry*
MBI	*Mucosal Barrier Injury*
MDR	*Multi-Drug Resistant*
MRSA	*Methicillin-Resistant Staphylococcus aureus*
MSSA	*Methicillin-Sensitive Staphylococcus aureus*

List of Abbreviations cont...

Abb.	Full term

NF**.....***Neutropenic Fever*
NHSN**.....***National Healthcare Safety Network*
PCR**.....***Polymerase Chain Reaction*
PMNLs**.....***Poly-morpho-nuclear Leucocytes*
rDNA**.....***Ribosomal Deoxyribonucleic Acid*
TTP**.....***Time to Positivity*
WBCs**.....***White Blood Cells*

Abstract

Introduction: Bloodstream Infections (BSIs) are a main cause of life-threatening complications among patients with cancer.

Methodology: This study aimed to identify microbial pathogens causing BSI in febrile neutropenic patients with hematologic malignancy and compare the results of conventional blood culture with a nested multiplex real time PCR assay done directly on whole blood samples. The nested multiplex PCR was based on *16S rDNA* and *18S rDNA* sequence-specific primers; hence, it allowed the identification of most species of bacteria and fungi.

Results: Forty adult patients with febrile neutropenia, admitted at Hematology ward of Ain Shams University Hospitals, were included in this study. Each patient was subjected to conventional blood culture and nested multiplex PCR. Blood culture was positive in 19 patients (47.5%). About 68.4% of the positive cultures were monomicrobial, while 31.6% were polymicrobial. A total number of 26 isolates were grown from positive cultures; *Staphylococcus aureus* was the most common (30.8%), followed by *Klebsiella pneumoniae* (19.2%). Regarding nested PCR, positive results were detected in 37/40 patients (92.5%) which was statistically significantly higher than that of blood culture. Eighteen samples that tested negative by culture were positive using the molecular approach. The agreement between the two approaches was 55%.

Conclusion: Nested multiplex real time PCR can be a promising tool in order to achieve rapid diagnosis in cancer patients clinically suspected of BSIs. Its utilization could affect the choice of antimicrobial treatment whether bacterial or fungal and, therefore avoid unnecessary use of antimicrobials.

Key words: Bloodstream Infections; Nested Multiplex PCR; Hematological Malignancy; Diagnosis.

INTRODUCTION

Bloodstream infections (BSIs) are life-threatening especially in patients with haematological malignancies (HMs). These patients are at increased risk of infections, not only because of the malignancy itself, but also because of neutropenia induced by intensive chemotherapy & its cytotoxic effect on the cells that line the gastrointestinal tract. Neutropenia in these patients is considered an important factor in assessment of infection risk and treatment choice. According to Infectious Diseases Society of America (IDSA) guidelines, the infection risk is described as high risk if neutropenia is prolonged; >7 days, and neutrophil count is $\leq 100/mm^3$ ***(Habip et al., 2014).***

Bacteria are incriminated in most cases of these infections; coagulase-negative staphylococci (CoNS), *Streptococcus viridans* and *Escherichia coli* have been found to be the most commonly isolated pathogens. Risk factors for bacteraemia in patients with haematological malignancies were found to be use of a central venous catheter and neutropenia for more than 6 days. Fungi play an important role as well. In fact, fungal BSIs are rapidly increasing over the last decades mostly due to increased cases of immune suppression. *Candida and Aspergillus spp.* are the most commonly isolated fungal pathogens and Candida is considered the 4th most common cause of BSI in patients with haematological malignancies ***(Chen et al., 2017).***

Mixed fungal/bacterial infections are also not uncommon; as combined Candida and bacterial bloodstream infections have been reported in as many as 23% of all episodes of candidaemia ***(Gosiewski et al., 2014b)***.

The early stages of BSI are associated with nonspecific clinical symptoms leading to difficult and delayed diagnosis. It's of utmost importance to rapidly diagnose such infections to swiftly initiate the proper antimicrobial therapy with the least side effects. The diagnostic "gold standard" nowadays for bloodstream infections is still blood culture (BC) techniques, performed either in the conventional specialized media or in automated culture systems. Blood culture is advantageously of low cost but hampered by the long period it takes for the results to properly appear, delaying the initiation of appropriate treatment. It's also unfortunately associated with low sensitivity as positive results are given in less than 50% of cases in spite of the clear signs of sepsis in patients. Moreover, subjecting the patients to empiric antimicrobial therapy before collection of blood samples for culture might inhibit the growth of microorganisms, especially if they are of fastidious nature ***(Habip et al., 2014).***

Molecular methods are a promising reliable alternative to BCs for the detection of these life-threatening infections. These techniques showed much higher sensitivity and less affection by the implementation of empirical antimicrobial therapy ***(Lamoth et al., 2010).***