

**Simultaneous Detection of MRSA,
Selected gram negative bacilli,
clinically relevant Candida species and
Aspergillus by PCR in Critically ill
Patients with Sepsis**

Thesis

*Submitted for Partial Fulfillment of Master
Degree in Pediatrics*

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2019

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العليم

صدق الله العظيم

سورة البقرة الآية: ٣٢



*First and foremost, I feel always indebted to **ALLAH**, the Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to **Prof. Mervat Gamal Eldin Mansour**, Professor of Pediatrics - Faculty of Medicine- Ain Shams University for her keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to **Dr. Iman Ahmed Ragab**, Assistant Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

*I am deeply thankful to **Dr. Iman Mohamed Amin Elkholy**, Professor of Microbiology, Ain Shams Specialized Hospital - Ain Shams University, for her great help, active participation and guidance.*

*I wish to introduce my deep respect and thanks to **Dr. Marwa Saad**, Assistant Professor of Microbiology, Faculty of Medicine, Ain Shams University, for her kindness, supervision and cooperation in this work.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Last but not least my sincere thanks and appreciation to all patients participated in this study.

List of Contents

Title	Page No.
List of Tables	i
List of Figures.....	iv
List of Abbreviations	viii
Introduction	i
Aim of the work	3
Review of literature	4
▪ Prevalence of sepsis	4
▪ Hospital acquired infection (HAI).....	7
▪ The most common organisms causing sepsis in icu patients	10
▪ Multidrug resistant (MDR) bacteria	14
▪ Fungal infection in PICU	17
▪ Diagnosis of sepsis	20
▪ Advantages of pcr technique in diagnosis of infection over the conventional blood culture	22
▪ Management of critically ill patients with sepsis in the pediatric ICU	26
▪ Outcome of sepsis.....	33
Patients and methods.....	34
Results	52
Discussion.....	79
Summary	91
References	95
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	Epidemiology of pediatric sepsis in multicenter studies in developed countries since 2003.....	6
Table (2):	Demographic data of the studied patients	52
Table (3):	Disease characteristics of the studied patients	52
Table (4):	Initial presenting system at PICU admission of the studied patients.....	53
Table (5):	Severity and mortality risk scores of the studied patients.....	54
Table (6):	Different risk factors of the studied patients	54
Table (7):	Laboratory results of the studied patients	56
Table (8):	Frequency of Antibiotic use in the studied patients.....	56
Table (9):	Frequency of antifungal use in the studied patients.....	58
Table (10):	Outcome of the studied patients.....	59
Table (11):	Comparison between the results of PCR and conventional blood culture.....	60
Table (12):	Comparison between survivors and deceased regarding laboratory results and risk scores	62
Table (13):	Comparison of blood culture results among survivors and deceased	63
Table (14):	Comparison of PCR results among survivors and deceased	64
Table (15):	Sensitivity results of positive blood culture for <i>Klebsiella pneumoniae</i> to different antibiotics.....	66

List of Tables

Table No.	Title	Page No.
Table (16):	Sensitivity results of positive blood culture for Acinetobacter to different antibiotics.....	67
Table (17):	Sensitivity results of positive blood culture for Candida albicans to different antifungals	68
Table (18):	Correlation between Sofa score and laboratory results.....	70
Table (19):	Comparison between patients with community acquired infection and nosocomial infection regarding mortality.....	71
Table (20):	Comparison between patients with community acquired infection and nosocomial infection regarding blood culture positivity.....	72
Table (21):	Comparison between patients with community acquired infection and nosocomial infection regarding PCR positivity.....	74
Table (22):	Comparison of the exposure to the risk factors among deceased and survivors.....	76
Table (23):	Diagnostic accuracy of PCR according to blood culture as the gold standard.....	78

List of Figures

Fig. No.	Title	Page No.
Figure (1):	The American College of Critical Care Medicine-Pediatric Advanced Life Support (ACCM-PALS) algorithm.	26
Figure (2):	Macroscopic and microscopic characteristics of <i>Candida albicans</i>	44
Figure (3):	<i>Candida albicans</i> by chromogenic media (blue)	44
Figure (4):	Antimicrobial sensitivity test of <i>Candida albicans</i>	45
Figure (5):	The results of Gel electrophoresis of PCR.....	50
Figure (6):	Initial presenting system at PICU admission of the studied patients	53
Figure (7):	Different risk factors of the studied patients	54
Figure (8):	Frequency of Antibiotic use in the studied patients.....	57
Figure (9):	Frequency of antifungal use in the studied patients.....	58
Figure (10):	Comparison between the results of PCR and conventional blood culture.....	61
Figure (11):	Comparison of blood culture results among survivors and deceased	63
Figure (12):	Comparison of PCR results among survivors and deceased	65
Figure (13):	Sensitivity results of positive blood culture for <i>Candida albicans</i> to different antifungals	69

List of Figures

Fig. No.	Title	Page No.
Figure (14):	Comparison between patients with community acquired infection and nosocomial infection regarding mortality	71
Figure (15):	Comparison between patients with community acquired infection and nosocomial infection regarding blood culture positivity	73
Figure (16):	Comparison between patients with community acquired infection and nosocomial infection regarding PCR positivity	75
Figure (17):	Comparison of the exposure to the risk factors among deceased and survivors	77

List of Abbreviations

Abb.	Meaning
BC	Blood culture
CL	Central line
CVC.....	Central venous catheter
CVP.....	Central venous pressure
ESBL.....	Extended spectrum β Lactamase
HA-BSI	Hospital acquired-blood stream infection
HAI	Hospital acquired infection
IAI.....	Invasive Aspergillus infection
ICI.....	Invasive Candidal infection
IFI	Invasive fungal infection
MDR.....	Multidrug resistant
PCR.....	Polymerase chain reaction
PICU	Pediatric intensive care unit
PIM2	Score Pediatric Index of Mortality
PRISM	Pediatric Risk of Mortality Score
SD	Standard Deviation
SOFA	Score Sequential Organ Failure Assessment Score

Abstract

Background: Pediatric severe sepsis remains a public health problem. Sepsis remains the most expensive condition in hospital stays. The delay in results of conventional blood cultures may results in inappropriate treatment with increased costs and delay in delivery of adequate treatment.

Objective: To determine the frequency of infection by selected organisms including *Candida* species, *Aspergillus* species, MRSA, *Acinetobacter baumannii*, *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* among the pediatric patients admitted to pediatric intensive care unit with sepsis by PCR, to compare PCR as detection methods with the conventional blood culture methods and study sensitivity patterns of detected organisms to antimicrobial therapy.

Methods: A cross-sectional observational study was carried out in Pediatric ICU, Children Hospital, Ain Shams University. It included 45 critically ill patients in pediatric age group (30days-18years) fulfilling the criteria of sepsis. Details of admission and risk factors were recorded. Blood samples were collected from each case for bacterial and fungal culture and sensitivity and PCR for selected organisms.

Results: Total number of positive PCR was 30 in 23 patients with polymicrobial infection in 6 patients (51.1%) compared to 10 for blood culture in 10 patients (22.2%). The frequency of MRSA was 16 (35.6%) of the studied patients by PCR compared to (0%) by blood culture; E.Coli 3 (6.6%) by PCR compared to (0%) by blood culture; *Candida albicans* 8 (17.8%) by PCR while only 7 (15.6%) were detected by culture and 2 patients (4.4%) with *Acinetobacter* and one (2.2%) with *Klebsiella* were detected by both blood culture and PCR .

Conclusion: There was a disagreement between PCR and conventional blood culture as regard the detection of both MRSA and E coli. PCR methods were able to rapidly detect a wider panel of microorganisms and to determine the presence of bacteria especially in those patients with prior antimicrobial treatment.

Keywords: pediatric sepsis, MRSA, *Candida albicans*, *E coli*, *Klebsiella*, *Acinetobacter*, PCR, PICU.

INTRODUCTION

Sepsis is a whole-body inflammatory response to an infection. Common signs and symptoms include fever, increased heart rate, increased breathing rate, and confusion (*Dellinger et al., 2013*).

The infection is most commonly by bacteria, but can also be by fungi, viruses, or parasites (*Jonathan, 2011*). Disease severity partly determines the outcome with the risk of death from sepsis being as high as 30%, severe sepsis as high as 50%, and septic shock as high as 80%. In the developed world about 0.2 to 3 per 1000 people get sepsis yearly or about a million cases per year in the United States (*Jawad et al., 2012*).

Sepsis was the most expensive condition treated in U.S. hospital stays in 2011, at an aggregate cost of \$20.3 billion for nearly 1.1 million hospitalizations (*Torio et al., 2013*).

Invasive fungal infections (IFIs) are being increasingly recognized as a major threat in critically ill adult and pediatric patients. They can range widely in severity and can be life threatening in some patients. *Candida* and *Aspergillus* species are the most common causes of IFIs, but other yeasts and filamentous fungi are emerging pathogens. *C. albicans*, the most significant pathogenic species, is seen in almost all of the 17% of patients treated in the intensive care unit (ICU) who develop IFIs, and is associated with significant morbidity and mortality (*Gullo, 2009*).

Blood culture (BC) is still considered the gold standard for diagnosis and identification of bloodstream pathogens by many. However, this conventional laboratory method lacks sensitivity, has a low pre-test probability in certain clinical settings, and is impaired by the delay in the time to result especially in fungal infection since there are negative outcomes in as high as 50% autopsy-confirmed cases of candidemia. In addition, cultures may only become positive late in the infection (*Kirn, 2013*).

The early detection and adequate treatment of bacterial infections have a great impact on the outcome of patients with systemic infection. In practice, most infections are treated empirically with broad-spectrum antibiotics because of the usual delay of 24 to 48 h for routine microbiological processing of the clinical samples (*Kumar et al., 2006*). PCR has been successfully used to detect bacterial DNA in clinical samples and has improved the rate of microbial detection (*Carroll et al., 2000*).

Quick and early detection allows the clinician to immediately prescribe better targeted antibiotic therapies. Real-time PCR is revolutionizing microbiological diagnostics because of the sensitivity of detection and specificity for determination of variants. In addition, there may be substantial time and cost savings over traditional culture methods for determining the quantity of a given pathogen in a clinical specimen. Real-time PCR most notably benefits patients when used to detect and identify bacteria (*Mackay, 2004*).

AIM OF THE WORK

- Determine the frequency of infection by selected organisms including *Candida* species, *Aspergillus* species, Methicillin resistant staph aureus (MRSA), *Acinetobacter Baumannii*, *Escherichia coli*, *Klebsiella Pneumoniae* and *Pseudomonas Aeruginosa* among the pediatric patients admitted to pediatric intensive care unit with sepsis by PCR
- To compare PCR as detection methods with the conventional culture methods.
- To study sensitivity patterns of detected organism to antimicrobial therapy.

REVIEW OF LITERATURE

PREVALENCE OF SEPSIS

Fever is a common problem in ICU patients as it complicates up to 70 percent of all intensive care unit (ICU) admissions. The presence of fever frequently results in the performance of diagnostic tests and procedures that significantly increase medical costs and expose the patient to unnecessary invasive diagnostic procedures and the inappropriate use of antibiotics. ICU patients frequently have multiple infectious and noninfectious causes of fever, necessitating a systematic and comprehensive diagnostic approach (*Paul, 2010*).

According to the National Center for Health Statistics and the Centers for Disease Control and Prevention, sepsis was the 10th leading cause of death overall in 2007 (*Xu et al., 2009*).

Pediatric severe sepsis remains a burdensome public health problem, with prevalence, morbidity, and mortality rates similar to those reported in critically ill adult populations (*Weiss et al., 2015*).

Estimates suggest that there are between 77 to 240 new cases of sepsis per 100,000 population each year (*Martin et al., 2003; Finfer et al., 2004*). The population is growing older, and patients are living longer, even in the face of diseases that were previously considered universally fatal. Hospitalized patients are becoming more dependent upon the use of invasive devices and

technology, all of which are associated with increased risk of infection. As such, the incidence of sepsis is expected to increase by 1.5% every year, resulting in an additional 1 million cases per year by 2020 (*Martin et al., 2003, Dombrovskiy et al., 2003*). The story in children is fairly similar. There are between 20,000 – 42,000 cases of severe sepsis every year in the United States alone, half of which occur in children with underlying diseases like cancer and congenital heart disease (*Odetola et al., 2007*). Again, as more children survive diseases that were previously fatal, the incidence of sepsis will likely increase further (*Simon et al., 2010*).

While the management of critically ill patients with sepsis is certainly better now compared to 20 years ago, sepsis-associated mortality remains unacceptably high (*Wheeler et al 2009*). Studies suggest that there are approximately 4,500 children who die every year from sepsis in the United States alone (*Watson et al., 2005*). The actual number of deaths associated with sepsis is likely to be much higher, as many patients usually die from sepsis during the course of an underlying disease, such as prematurity, congenital heart disease, or cancer. In many of these cases, deaths are frequently attributed to the underlying disease process, rather than to sepsis (*Watson et al., 2005*).

According to data from the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and the Bill and Melinda Gates Foundation, nearly 70% of the 8