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

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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 baumanni*, *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 poly-microbial 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 Baumanni, 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 10^{th} 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