Faculty of Science
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Epidemiology of fungal infections in neonates correlated with antifungal drug susceptibility testing

A Thesis

Submitted in partial fulfillment of the requirement for

M.Sc. Degree in Microbiology

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(2019)

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Acknowledgment

First and forever, thanks to **ALLAH**, who gives me everything in my life, and I supplicate to Allah to make my life in a perfect way.

With the deepest gratitude, I wish to thank every person who has come in to my life and inspired, touched and illuminated me through their presence. I would also like to acknowledge and express my gratitude to the following people for their magnificent support and contributions to this manuscript.

For the soul of ***Prof. Dr. Alzahraa Karam Eldin***, Prof. of Microbiology, Ain Shams University, who suggested the topic of this work and gave a lot of effort to accomplish it, a lot of support and kind help, continuous encouragement and valuable guidance

Grateful thanks to***Prof. Dr. Yousseria M. Hassan Shetaia,*** Professor of Microbiology (Mycology), Microbiology Department, Faculty of Science, Ain Shams University for her continuous help, valuable supervision and reviewing this work

Wish to express my sincere gratitude to***Prof.Dr. Iman Mohamed El-Kholy,*** consultant of microbiology at Ain shams university hospitals for her continuous help, constructive criticism, valuable supervision during the experimental work, continuous encouragement, valuable guidance as well as reviewing this work.

Grateful thanks are also extended to ***Dr. Eman Mokhtar Mahmoud***Manger of Abu El-Azzayem Hospitals, Laboratories for her continuous help, valuable supervision during the experimental work, and sincere help in preparing the manuscript. My heartfelt gratitude is expressed to her.

I would like also to express my deepest love to my husband, for his support, encouragement and continuous help.

I am also deeply grateful to my mother, my father, my sister, my brother and my uncles for their unconditional support, encouragement and patience in all; better and worse.

Moreover, I would like to acknowledge gratefully all members of Abu El-Azzayem Hospitals Laboratories for their cooperation and help which enabled this work to be accomplished.

Also, I offer my regards and blessing to all of those who supported me in any respect during the completion of work

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List of abbreviations

AFG	Anidulafungin
AMB	Amphotericin B
BHI	Brain heart infusion
BMT	Blood and marrow transplant
BSI	Blood stream infection
BW	Birth weight
CAS	Caspofungin
CNS	\Central nervous system
DMSO	Dimethyl sulfoxide
ELBW	Extremly low birth weight
EOS	Early onset sepsis
FLC	Fluconazole
FUO	fever of unknown origin
ICUs	Intensive care units
IFIs	invasive fungal infections
ITC	Itraconazole
KTC	Ketoconazole
LOS	Late onset sepsis
MFC	Minimum fungicidal concentration
MFG	Micafungin
MIC	Minimum inhibitory concentration
NAC	Non albicans *Candida*
NICUs	Neonatal intensive care units
POS	Posaconasole
PRRS	porcine respiratory and reproductive syndrome
SDA	Sabaroud dextrose agar
TPN	Total parental nutrition
TRB	Terbinafine
UTI	Urinary tract infection
VLBW	Very low birth weight
VRC	Voriconazole
YCB	Yeast carbon base
YNB	Yeast nitrogen base

Abstract

Background: Invasive fungal diseases (IFDs) are opportunistic infections associated with significant mortality in paediatric patients, especially in those with compromised immune system and neonates with very low birth weight (VLBW). The objectives of this study are to determine the prevalence, clinical features and fungi isolates of neonatal sepsis in three hospitals in Egypt. Methodology: The study is a cross sectional survey of 176 neonates with clinical sepsis admitted to the neonatal intensive care units (NICU) of the three hospitals over a period of one year (February 2015 to January 2016). A minimum of two blood samples (collected within 24 hours) from each neonate were cultured for bacteria in automated BacT/AlerT and conventional culture bottles, while Saboraud-Brain Heart Infusion broth was inoculated for fungi culture. Positive growths from the broth were sub-cultured on Sabouraud Dextrose Agar (SDA) plates for aerobic incubation at 25oC and 37oC for 2 weeks. Identification of fungi colonies on SDA was by conventional morphology and confirmation on chromogenic agar media. Phylogenetic analysis of representative fungi isolates was done by partial nucleotide sequencing of D1-D2 domain of the large subunit rRNA gene. Results: Of the 176 neonates, blood culture was positive for pathogens in 55 (31.3 %) samples and fungi were isolated in 26 (14.8 %); yeast (25) and mould (1). The commonly isolated yeasts were Candida albicans, Candida tropicalis, and Candida krusei representing 34.6%, 30.8% and 23.1%, respectively of the total fungi isolated. The phylogenetic analysis in comparison to Genbank data showed defined clades for Candida tropicalis, Candida parapsilosis, Candida albicans and Pichia kudriavzevii Conclusion: This current study highlights the changing pattern of neonatal infections in Egypt caused by Candida, with

increasing incidence of infections caused by non-albicans Candida species .

Key words: fungal infection, neonatal, risk factors, PCR, yeast

Introduction

Opportunistic infections are an increasing common problem in neonatal intensive care units (NICUs), (Latha *et al*., 2017) More than 40% of under-five deaths globally occur in the neonatal period, resulting in 3.1 million newborn deaths each year (UNICEF,2011). Almost 1 million of these deaths are attributed to infectious causes, Including neonatal sepsis, meningitis, and pneumonia (Black *et al.*, 2010).

Fungal infections in children appear to have increased over the past few decades, primarily because there has been an increase in children with primary or secondary immune deficiencies. Premature neonates are at high risk for opportunistic infections. The risk for invasive fungal infections is high in very low birth weight (VLBW) infants (<1500 g) and highest infection were recorded for infants born at the youngest gestational ages (Mokhtar *et al*., 2005).

Fungal infections are an important cause of mortality and morbidity. Although these infections have been well characterized in adults, the incidence and analysis of risk factors, diagnostic tools, treatments and outcomes have not been well described for large cohorts of pediatric or neonatal patients in the neonatal period, especially in preterm infants (Steinbach, 2010).

Candida species are one of the most common causes of blood stream infections among neonates (Shrivastava *et al*., 2015).Significance of *Candida* species, in neonatal intensive care units (NICU) is increasingly being recognized. It is the third most common cause of late onset sepsis in NICU patients and accounts for 9-13% of blood stream infections (BSI) in neonates (Juyal *et al.,* 2013).

Although *Candida albicans* has historically been the most frequently isolated species, non-albicans Candida (NAC) has been emerged as important opportunistic pathogen, notably *Candida tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata* (Goel *et al.,* 2009, Oberoi *et al.*, 2012).

There is growing evidence suggesting a role of increasing use of azole agents in this epidemiological shift. Several of these NAC species exhibit intrinsic resistance to traditional triazoles like fluconazole (FLC) and may also demonstrate cross resistance to newer triazoles. This makes it imperative to perform both speciation and antifungal susceptibility (AFG) of all the yeast isolates from blood or any other specimens (Juyal *et al.*, 2013).

Candida infections are a common cause of late-onset sepsis in the NICU and are associated with significant mortality and neurodevelopmental impairment. One of the most important reasons in managing candidal infection in NICU is the use of

prophylactic fluconazole in very low birth-weight infants to prevent invasive candidiasis (Benjamin *et al*., 2014).

The introduction of antifungal agents caused a shift from complete dominance of *Candida albicans* to non-albican species, which now constitute more than half of all cases of candidemia. Recognition of this change is clinically important, since the various species differ in susceptibility in the newer antifungal agents (Shrivastava *et al.*, 2015).

Aim of the work

The objectives of this study were as the following:

1. Determination the incidence and etiology of fungal infection among the neonates in the intensive care units in some hospitals.
2. Isolating the causative agent from different sites of infection in neonates and identification of these fungal isolates.
3. Evaluation the efficacy of antifungal drugs against the isolated fungal pathogens.