"Prevalence of Candidemia in High-risk Neonates of Neonatal Intensive Care Unit" -A one-year cross sectional study.

By

REG. NO. BIO120002

Dissertation

Submitted to the KAHER, Belgaum, Karnataka
In partial fulfillment of the requirements for the degree of

M. D. (DOCTOR OF MEDICINE)

IN

MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
J. N. MEDICAL COLLEGE,
BELGAUM – 590010. KARNATAKA. INDIA

JUNE/JULY -2023

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Dr. Manjula.A.Vagarali M.D.Ph.D
Prof & Head
Department of Microbiology,
J. N. Medical College,
Belgaum – 590010.

Date: 29th DEC 2021
Place: BELAGAVI

Dr. (Mrs) N.S. Mahanthshetti MD.
Principal
J. N. Medical College,
Belgaum – 590010.

Principal
Jawaharlal Nehru Medical College
BELAGAVI.

Date: 29th DEC 2021
Place: BELAGAVI

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Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831-2471350 0831-2470759 www.jnmc.edu principal@jnmc.edu

Ref No: MDC/PG/ Date: 25-11-2022

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Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BI0120002.
Postgraduate Student,
2020-21 Batch,
Department of Microbiology,
J. N. Medical College, Belagavi.

ETHICAL CLEARANCE LETTER

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 55 **Date: 25/01/2021**

To,

Dr. (Mrs) Anugula Amritha
PG student in Microbiology
J. N. Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"PREVALENCE OF CANDIDEMIA IN HIGH RISK NEONATES OF NEONATAL INTENSIVE CARE UNIT – A ONE YEAR CROSS SECTIONAL STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Smita Sonoli)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Harsha Hegde)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

LIST OF ABBREVATION

AMR	-	Antimicrobial resistance
BSI	-	Blood stream infections
CDC	-	Center for disease control
CLSI	-	The clinical and standard laboratory institute
CCL-2	-	CC Chemokine ligand-2
CFU	-	Colony forming unit
E-test	-	Epsilometer test
MIC	-	Minimum inhibitory concentration
GTT	-	Germ tube test
LBW	-	Low birth weight
VLBW	-	Very low birth weight
NAC	-	Non-albicans Candida
NICU	-	Neonatal intensive care unit
SDA	-	Sabouraud's dextrose agar
CMA	-	Corn meal agar
TPN	-	Total parental nutrition
IV	-	Intravenous injection
UAC/UVC	-	Umbilical artery catheterization/ Umbilical Venous catheterization.

FLC	-	Fluconazole
CAS	-	Caspofungin
5FC	-	5 Flucytosine
VRC	-	Voriconazole.
AP	-	Amphotericin-B
DC	-	Dendritic cell
PAMPS’s	-	Pathogen associated molecular pattern
PRR’s	-	Pattern recognition receptors.
AMA	-	Against medical advice
R	-	Resistance
S	-	Sensitive

ABSTRACT

Background: Fungal sepsis in neonates is on rise among blood stream infection, it is the third most common etiological agent for sepsis and the changing epidemiology to non-albicans *Candida* is grossly evident. Fungal isolates are showing azole resistance which were the most common prophylactic treatment used, now made treatment choices difficult for neonate ICU clinicians. Newer class of antifungal drugs especially Echinocandins (Caspofungin) came into real time practice in azole resistant *Candida* causing sepsis.

Objective:

1. To study the prevalence of Candidemia in high-risk neonates of NICU
2. To study Caspofungin (Echinocandin) antifungal susceptibility in Fluconazole resistant *Candida* isolates by E test / MIC test strip method.

Material and methods: The blood sample collected from all high-risk neonates admitted during the study period in NICU of Dr. Prabhakar kore charitable hospital, Belagavi from January 2021 to December 2021 received in the Department of Microbiology, J. N. Med. College, KAHER, Belagavi, were included in the study. All the samples were processed according to the standard operating procedures of Mycology, conventionally. Isolation and identification followed by speciation was done using Corn meal agar and Chrom agar. Antifungal susceptibility was determined by Disc diffusion method and E-strip method, as per the Standard Mycological techniques followed by antifungal susceptibility was done according to CLSI guidelines (M44 & M60)

Results: A total of 230 Blood samples collected from NICU from the high-risk neonates with suspected sepsis, who were admitted in the study period and were processed and were evaluated. Of which total of 63 were Fungal isolates (27.39%), *Candida albicans* were 25.4% (n=16) and NAC species (n=47), predominant being *C. glabrata* 46.03% (n=29). By disc diffusion method 53.97 (n= 34) isolates are resistant to Fluconazole and 76.19 (n=48) are resistant to Voriconazole. By E-strip method 52.38(n= 33) and 20.63 (n=13) were resistant to Fluconazole and Caspofungin respectively. 50 isolates of 63 (79.36%) were sensitive to Caspofungin. Mortality was seen in 10 neonates of total 586 admitted in NICU during study period.

Conclusion: Prophylactic usage of Fluconazole as an antifungal drug is now questionable. This study concludes that Fluconazole resistance is on rise. A newer class of drugs Echinocandins have come into rescue. Strict infection control strategies, appropriate preventive, and therapeutic measures such as prophylactic antifungal use and a restrictive policy of antibiotic use should be implemented by the health care workers and officials in NICU. Care should be taken for emerging multi drug resistant species like *C. auris*.

Keywords: Fungal sepsis, *Candida*, NAC species, critically ill high-risk neonates.

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INTRODUCTION

Fungi once considered as non-pathogenic or less virulent are now recognized as the common cause of morbidity and mortality especially in neonates who are at high risk. Over last few years mycotic infections have been progressively increased. Among these mycotic infections *Candida* species being the most common pathogen. And these *Candida* species are the source for many clinical manifestations ranging from mucocutaneous to life threatening disseminated infections mainly including blood stream infection notoriously known to cause fungal sepsis [1,2,19].

Fungal sepsis synonymously known as Candidemia is now showing its increasing prevalence in all age groups more considerably in high-risk neonates as many challenges are to be faced in this age group. Who are hospitalized with in the first week of birth approximately 10% sick neonates show fungal colonization, which increases tremendously with increasing weeks of hospital to 90% (i.e., second and third week of hospital stay [2,4].

The potential source of *Candida* infection to the neonate is via maternal vaginal flora through vertical transmission or cross transmission via hands of health care personnel. Incidence of invasive fungal sepsis due to *Candida* species in neonates is dramatically increasing these recent years and recorded as the third most common cause for blood stream infection in NICU accounting for 9-13%[2.14].

Recent advances pertaining to neonatal care has led to remarkable improvement with respective to increased survival of extremely low birth weight and lower gestational age preterm neonates [3]. Neonates such as Preterm, extremely/ very/ low birth weight, on total parenteral nutrition, on mechanical ventilator,

immunocompromised, on broad spectrum antibiotics, prolonged stay in hospital are at high-risk for *Candida* infections [1,2,3].

Not only *Candida albicans* but also there is a good percentage rise in Non albicans *Candida* species infection. This epidemiological shift of increased prevalence of Non albicans *Candida* infection in high-risk neonates has created a menace and it gives us warning sign for changing strategies in choosing empirical treatment protocols in above categorized high risk neonates. As a result of which identification and isolation of *Candida species* in blood stream of critically ill neonates should be mandatory. [2,17]

Accurate and rapid identification of the *Candida* species along with antifungal fungal susceptibility testing helps the clinician to decrease mortality and morbidity rate of NICU caused due to these invasive Candidiasis and choose appropriate antifungal treatment in future.

Azole group of antifungals are the most used drugs to treat *Candida* infection. Mainly Fluconazole is the most preferred treatment in Candidiasis [13,14]. However, increasing trends of azole resistance is observed in the recent past. Hence now it has become very crucial to study the resistance in order to improve azole group of antifungals or change the class of drugs to treat *Candida* infections. [23]

The gross evidence in increasing prevalence of Non albicans *Candida* species and their emerging resistance to Fluconazole and other azole group of drugs represents a major challenge for empirical, therapeutical, and prophylactic strategies. [24]

Newer class of drugs Echinocandins have become the savior for azole resistant *Candida* species. Hence to study the efficacy of Echinocandins in the treatment of invasive Candidiasis is the need of the hour. [32]

Thus, the study is undertaken to identify the isolate of *Candida* species accurately, correctly and efficiently in the blood stream of critically ill neonate of NICU using conventional techniques under the standard Mycological laboratory protocols. And to study their antifungal susceptibility pattern to achieve better clinical results and further aids in selecting appropriate antifungal therapy which in turn is beneficial for the patient to reduce the hospital stay and treatment cost as well.

Key challenges to the management of candidemia and invasive candidiasis include prevention, early recognition, and rapid initiation of appropriate systemic antifungal therapy [6].

Hence, this study helps the NICU health care worker in giving the better treatment and management of late onset sepsis caused by *Candida* species.

AIMS AND OBJECTIVES

- To study the prevalence of Candidemia in high-risk neonates of NICU
- To study Caspofungin (Echinocandin) antifungal susceptibility in Fluconazole resistant Candida isolates by E test / MIC test strip method.

REVIEW OF LITERATURE

Yeast like fungi namely the most prevalent *Candida* is the commonest cause for fungal diseases in human mucosa, skin, nails, blood and disseminated into multiple organs with a wide range of clinical spectrum such as acute or chronic, superficial, or deep [19,33]. And most importantly Candidiasis is seen as secondary infections in immunocompromised patients, cancer patients under treatments like chemotherapy, patients on corticosteroid therapy, pediatrics population such as neonates and geriatrics population usually tend to have very poor immune status [6,7].

A review of Candida History:

In fourth century, Candidiasis was described in pediatric patients by Rosen Von Rosenstein and Under Wood, and recorded the term "Thrush". Yeast like organisms causing thrush was first described by Lagenbeck and David Gurby presented in Academy of Science, Paris. Bennet first time isolated *Candida* from sputum of a Tuberculosis patient. Initially it was named as *Odium albicans* and *Monilia albicans*, hence the disease Moniliasis. The first Non-albicans *Candida* isolated from grapes and was named as *Cryptococcus glabratus* eventually Lodder and de Vries in 1938 renamed as Torulopsis glabratus. In 8th Botanical Congress, Paris genus Candida proposed by Berkhout was accepted. In Latin *Candida* means "glowing white" [5,33]

Taxonomy:

This notorious Fungus taxonomically placed under [19]

- Phylum: Fungi Imperfecti
- Order: *Moniliales*
- Family: *Cryptococcaceae*
- Genus: *Candida*

Out of 163 anamorphic species of *Candida* 20 of them are considered to be significantly pathogenic in humans causing various clinical manifestations and of which the following are well known opportunistic pathogens:

- *Candida albicans*
- *Candida glabrata*
- *Candida krusei*
- *Candida tropicalis*
- *Candida auris*
- *Candida parapsiliosis*
- *Candida kefyr*
- *Candida rugosa*
- *Candida lusitaniae*
- *Candida dubliniensis*
- *Candida guilliermondii*
- *Candia viswanathii*

*Candida s*pecies are normally populated as normal commensals or flora of humans such as in GI tract, female genital tract and skin. Usually, these commensals turn into pathogens when the person becomes immunocompromised. Blood stream infection due to *Candida* species is termed as Candidemia under the broad umbrella term invasive Candidiasis. Late onset sepsis due to fungal infections is most seen in high-risk neonates. Invasive Candidiasis is most caused due to *Candida albicans*, however Non-albicans *Candida* is now taking *over Candida albicans* as seen in these last decade. At least 15 distinct *Candida spp*. can cause human disease, but most invasive infections are caused by five pathogens: [(6)].

- *Candida albicans,*
- *Candida glabrata,*
- *Candida tropicalis,*
- *Candida parapsilosis*
- *Candida krusei*

Candida species colonization is regarded as a prerequisite for subsequent infection. And each *Candida* species possesses its own unique characteristics relative to invasive potential, virulence, and antifungal susceptibility. Overall, *Candida albicans* is the most common pathogen in most clinical settings, but Non-albicans *Candida* collectively could represent >50% of the bloodstream isolates in certain regions. The prevalence rate of *Candida species* various globally [(6,19)].

Classification of candida species: The CTG clade and beyond.

Since the origin of *Candida* and its related discoveries researchers have been facing difficulties in characterizing *Candida species* as they do not share a unique single evolutionary origin. The nomenclature "*Candida*" represents imperfect fungi due to unknown sexual cycle which isn't clearly defined. Many *Candida species* are categorized under CTG or CUG clade, in which CTG codes for amino acid serine. *Candida albicans, Candida tropicalis, Candida parapsiliosis* come under CTG clade. Whereas other two species that are major causes of infection i.e., *Candida glabrata* and *Candida krusei* lie outside CTG clade. Apart from these less infective species like *Candida kefyr* are non CTG clade *Candida* species [(5)].

Following the figure 1 and table 1 shows that *Candida albicans* and *Candida dubliniensis* share similar clade which is CTG clade, but whereas the other pathogenic Non-albicans *Candida* such as *Candida glabrata* share may characteristics with *Saccharomyces cerevisiae*. It shows major genome is shared by *Saccharomyces cerevisiae*. Which is also known as whole genome duplication.

Table 1: Showing the classification of *Candida* species basing on CTG or CUG

S.A. Turner and G. Butler

Table 1. *Candida* pathogenic species

Name[a]	Common teleomorphs/synonyms	Ploidy[b]	Mating[c]	Incidence[d] (%)
CTG clade species				
C. albicans		Diploid	P	65.3
C. dubliniensis		Diploid	P	0.1
C. tropicalis		Diploid	P	7.20
C. parapsilosis		Diploid	NO	6.00
C. orthopsilosis		Diploid	NO	0.50[e]
C. metapsilosis		Diploid	NO	<0.1[e]
C. famata	*Debaryomyces hansenii*	Haploid	Ho	0.30
C. lusitaniae	*Clavispora lusitaniae*	Haploid	Het	0.60
C. guilliermondii	*Meyerozyma guilliermondii; Pichia guilliermondii*	Haploid	Het	0.70
Other species				
C. krusei	*Issatchenkia orientalis; Pichia kudriavzevii*	Haploid	Het	2.40
C. glabrata		Haploid	NO	11.30
C. kefyr	*Kluyveromyces marxianus*	Haploid	Ho	0.50
C. norvegensis	*Pichia norvegensis*		Ho	0.10
C. inconspicua	*Pichia cactophila*		ND	0.20
C. lipolytica	*Yarrowia lipolytica*	Haploid	Het	0.05

[a]Species are listed in approximate order of phylogenetic relationship.
[b]Haploid indicates isolates can exist as stable haploids; diploids may also be formed.
[c]P, parasexual; NO, not observed; ND, not determined; Ho, homothallic; Het, heterothallic (data from Kurtzman et al. 2011).
[d]Average incidence 1997–2007 (Pfaller et al. 2010b), except for *C. metapsilosis* and *C. orthopsilosis*.
[e]Estimated from Canton et al. 2011; *C. orthopsilosis* isolates are ~8% and *C. metapsilosis* isolates are 1% of isolates identified as *C. parapsilosis*.

S. A. Turner and G. Butler

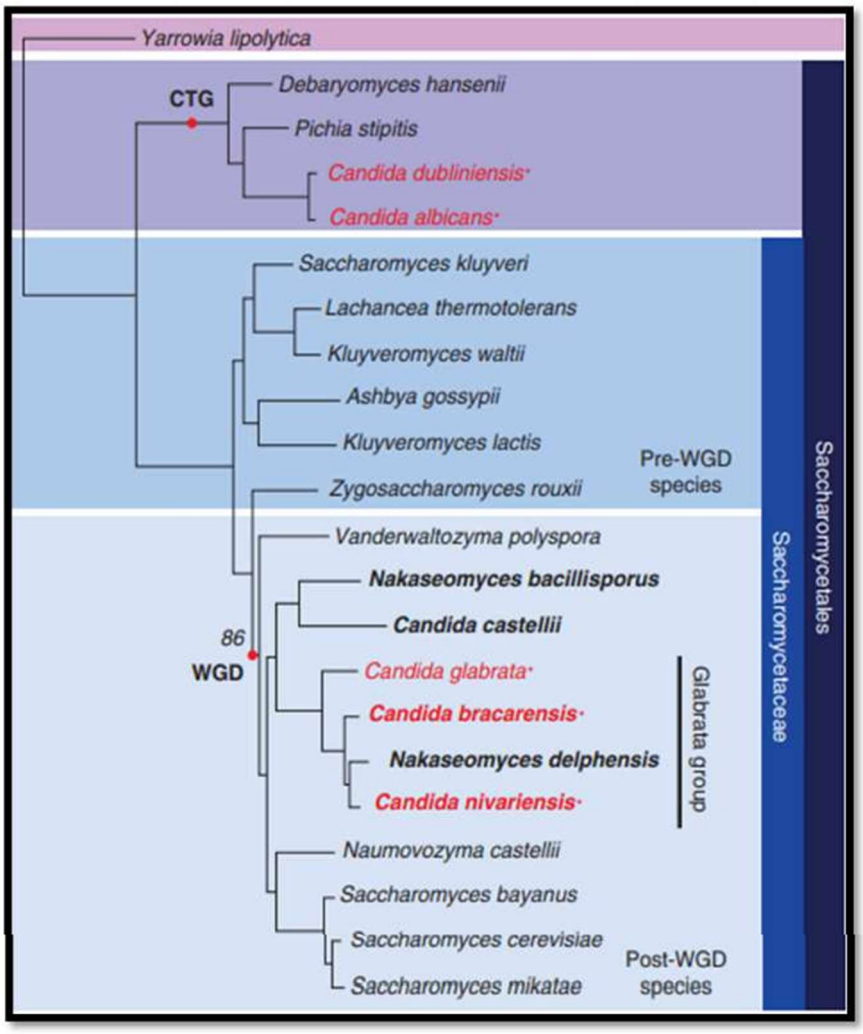


Figure 1: The phylogenetic tree is taken from Gabaldo´n et al. (2013) and was derived from a concatenated alignment of 603 one-to-one orthologs.

Pathogenic species are shown in red and are indicated with an asterisk. CTG, CTG clade; WGD, whole-genome duplication.

Epidemiology of Disease burden globally and with respective to health care settings:

There is evident geographical variability of prevalence of *Candida species.* The factors which impact the prevalence rate of *Candida species* infections are local epidemiology, age, background antifungal usage pattern in the hospital, clonal outbreaks, health care environment and individual risk factors. Infections caused due to *Candida* stands third in health acre associated blood stream infections next to *Staphylococcus aureus,* Coagulase negative *Staphylococcus, Enterococcus* species accounting to 18% of blood stream infections, among which 50% of episodes of candidemia are recorded in ICU's. The mortality rate due to candidemia as per data ranges between 10% to 47%. And now its one of the most leading causes of morbidity and mortality in neonates worldwide [(6)].

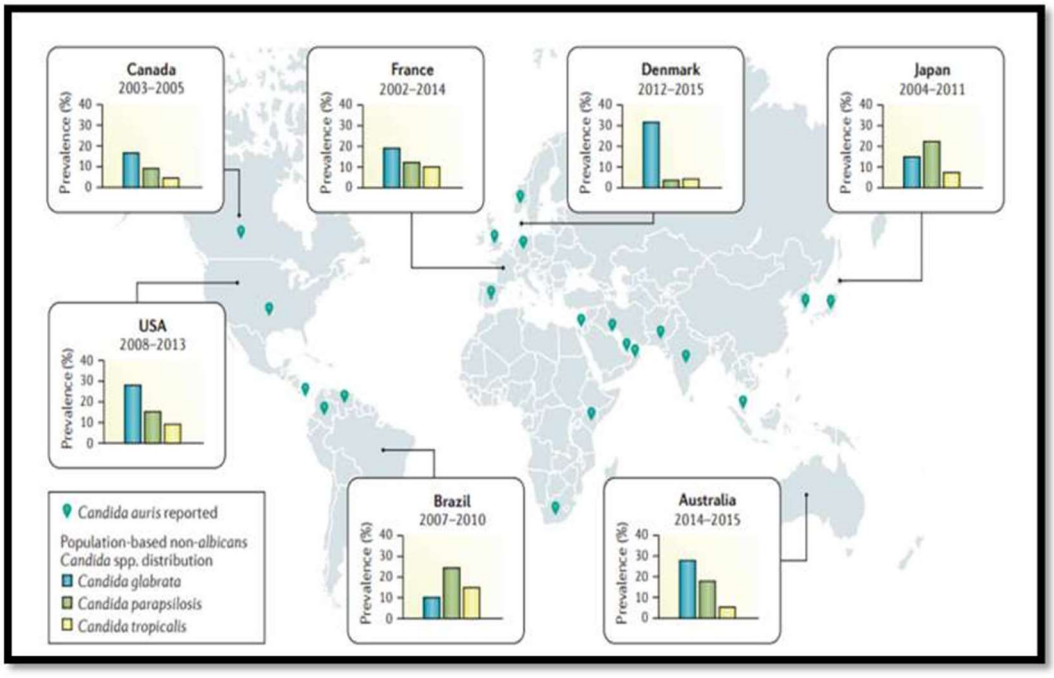
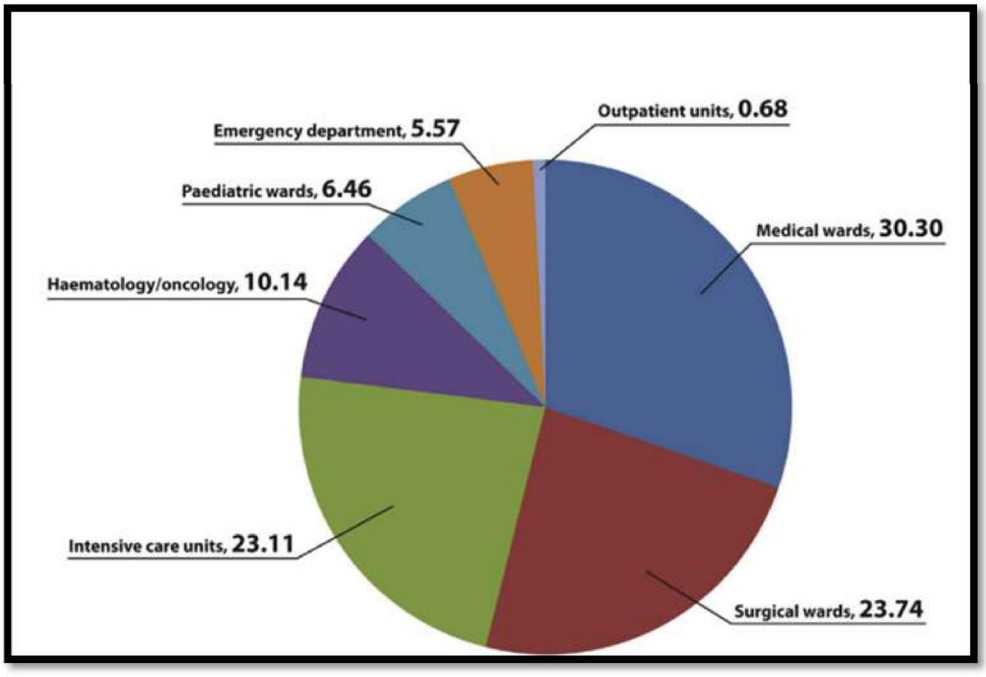


Figure 2: Geographical variations in the distribution of *Candida species.* Globally, *Candida albicans* is the most prevalent species associated with invasive candidiasis.

Globally *Candida albicans* were more prevalent and most common causes of invasive candidiasis, but in the Figure 2 the global burden represents that there are quite evident number of Non-albicans *Candida* all over. And now especially the newly evolved environmental Non-albicans *Candida* i.e., *Candida auris* is geographically distributed in Asia, parts of India and Pakistan, initially isolated from the ear sample of the patient residing in Japan hence also the name *Candida auris.*

Below graph:1 is the pie diagram showing various sources of *Candida* infection the health care settings, highest being Medical and Surgical wards followed by ICU's, especially in the immunocompromised patients admitted in the ICU, prolonged stay in the hospital, on interventions like Mechanical ventilator, Central line tips, long line tips, and patients on TPN [47].



Graph 1: The above pie chart shows the distribution of patients with Candidemia by hospital service (number denotes%)

Table 2: List of virulence factors of *Candida species*:

Sl.no	Virulence factor	Description / Function	Present / works	Names
1.	Adhesin	Major role in pathogenesis. Binding of *Candida* to epithelial and endothelial cells is controlled by adhesins	The cell wall protein mass constitutes to: Mannose residues N-glycosylation, O-glycosylation, and/or Glycosylphosphatidylinositol (GPI) anchoring GPI proteins that are covalently bound to β-1,6-glucan	Als family Hwp family Hyr family
2.	Enzymes	Plays a major role in invasion, tissue damage, immune system evasion, as well as its dissemination Aids host-pathogen interactions	The extracellular enzymes are: Superoxide dismutase's, Aspartyl proteases, Phospholipases, Esterase's, Phosphatases, Hemolysins	hydrolytic enzymes
3.	Toxins	Cytolytic peptide toxin secreted by the invasive form of the human pathogenic fungus.	Candida lysin is critical for mucosal and systemic infections and is a key driver of host cell activation, neutrophil recruitment and Type 17 immunity.	Candida lysin
4.	Compleme nt receptors	The ability to bind compliment-derived opsonin.	Facilitated by directly triggering the three dominant pathways, but also indirectly via the coagulation and fibrinolysis systems	
5.	Phenotypic switching	The ability to morphologically change from blastospore to true/pseudo hyphae	Helps to aid adapt to changing environment and evade host defense system. White-opaque switch.	
6.	Biofilm production	Survival in host environment, Antifungal resistance	They attach, adhere, proliferate, form microcolonies, production of extracellular matrix,	

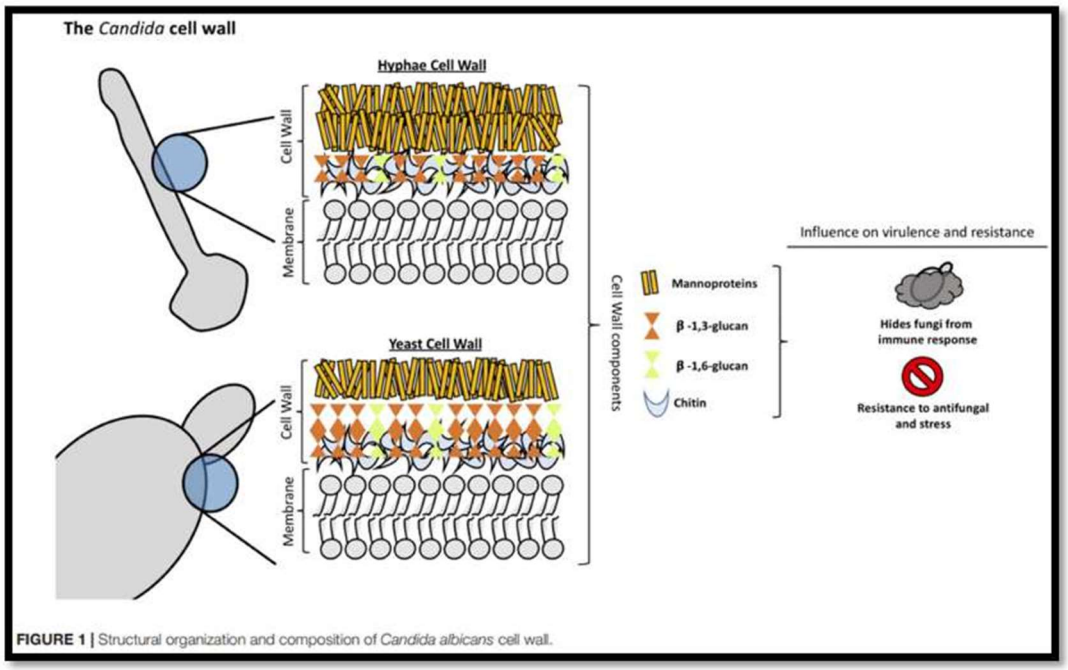


Figure 3: cell wall composition of *Candida* and its hyphae

Cell wall plays a vital role in maintaining the integrity of the cell and protects the intracellular components from harsh and toxic environment. Cell wall is the primary component in host-pathogen interactions. The cell wall has a great capability to change according to the environment aiding pathogen to survive. As in figure 3 shows the cellular composition of *Candida* species main constituents of outer cell wall layer are β-glucan-chitin skeleton which is responsible for strength and shape of the cell wall. Inner layer is formed by cross linkage of β-1,3- glucans and β-1,6-glucans connecting the outer cell wall to inner. There are other proteins present on the outer cell wall called as mannan proteins which are glycosylphosphatidylinositol proteins. Basing on their composition they are N-linked mannan and O-linked mannan. These mannan proteins and other cell wall components acts as virulence factors and have Antigenic properties, inducing host cellular mechanism hence used in laboratory diagnosis. And, the targets site for many Antifungal drugs. [8]

Pathogenesis:

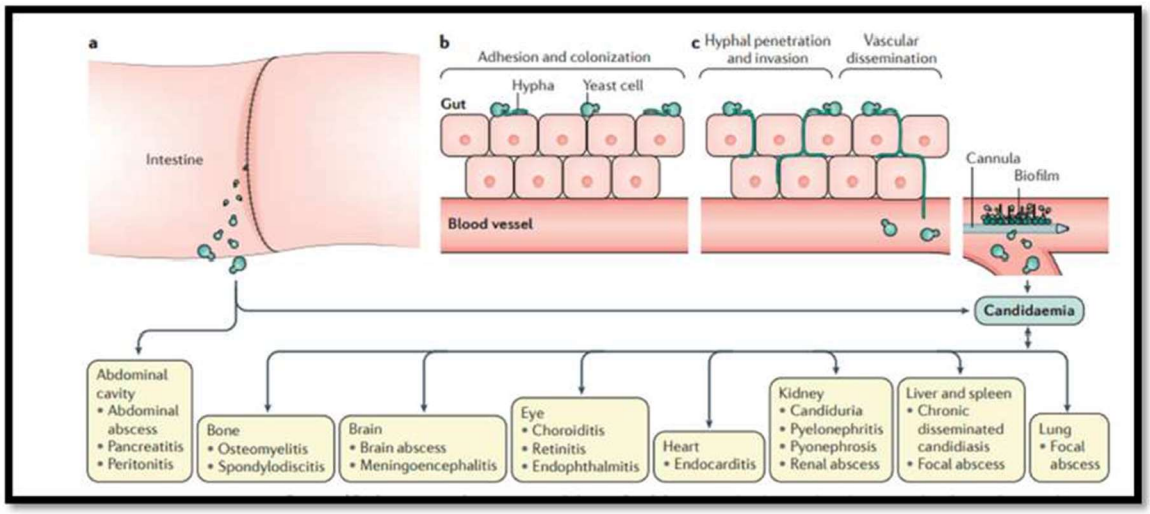


Figure 4: Pathogenesis of invasive Candidiasis

Figure 4 explains when there is a breach in the intestinal barriers occur, for example, after gastrointestinal surgery, *Candida spp.* can disseminate to the abdominal cavity directly and invade the bloodstream (Candidemia). Under normal conditions, the fungus behaves as a commensal organism without causing disease. Impairment of immune response, among other factors, can promote fungal overgrowth in the gut and candidemia, which can lead to deep-seated opportunistic infections in various organs (invasive candidiasis). Not only in surgeries but any kind of intervention like central line catheterization, mechanical ventilator etc., under such favourable condition there is a risk for high chances of *Candida* infection [(6)].

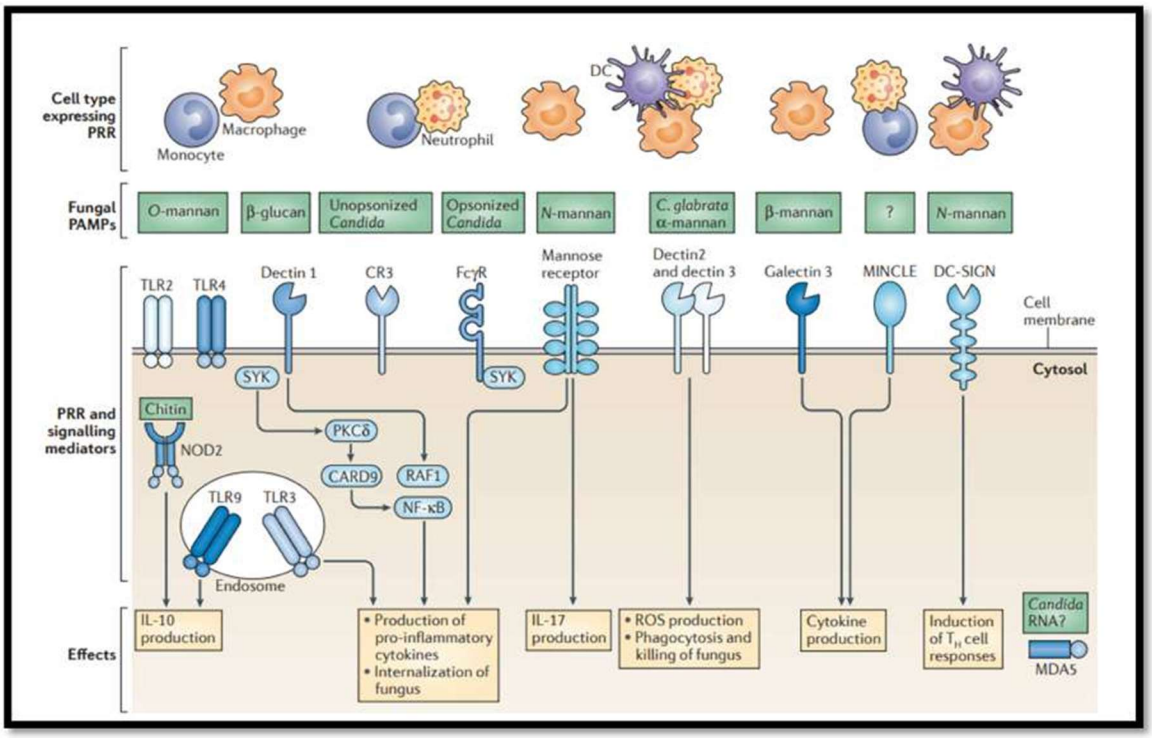


Figure 5: Recognition of *Candida species* by innate immune cells.

As described earlier mannans and glucans both acta as fungal PAMP's (pathogen associated molecular patterns) which are recognised by the cell of host like monocytes, macrophages, neutrophils, dendritic cells (DC) as these cells express pattern recognition receptors (PRR). These PRR's are toll like receptors (TLR-2, TLR-4) which recognises mannan proteins and TLR's 3 and 9 recognise fungal pathogen intracellularly present on nuclear membrane. After the interaction between these PRR'S and PAMP's there is a elicitation of signalling pathway using PRR's signalling mediators in the cytosol of the host cell and stimulates in production of chemokines like pro-inflammatory cytokines, interleukins (IL-10, IL-17) as shown in the above figure 5. (7)

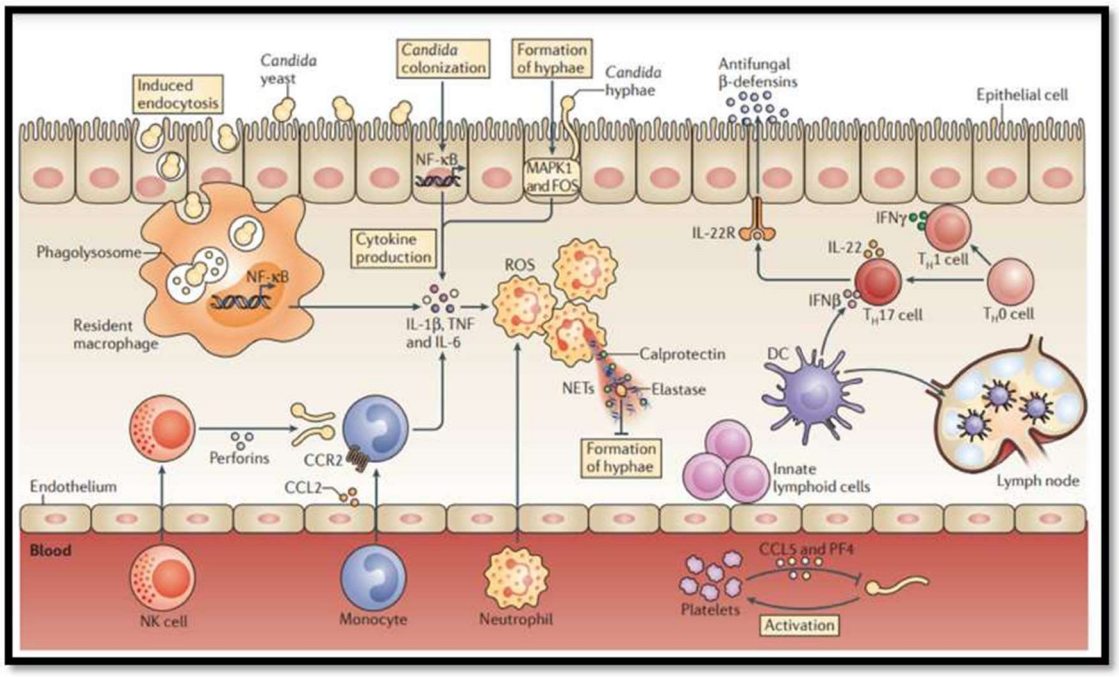


Figure 6: Clearance of *Candida species*- effector mechanism by host

Different types of host cell show different mechanisms for fighting against pathogen and their clearance. As in figure 6 clearly explains all possibilities by host cell to clear the fungal pathogen. Epithelial cells recognize the *Candida* hyphae and release cytokines via mitogen activated protein kinase 1 (MAPK1) and FOS dependent pathway. Epithelial cells can directly act as anti-*Candida* activity by producing β-defensins. Inflammatory monocytes via CC-chemokine ligand 2 (CCL-2) will further clear the *Candida.* Antigen presenting cells like Dendritic cell migrate to regional lymph nodes and start stimulation for production T helper cells 17 which in turn recruits and activates neutrophils and increased production of defensins which ids induced by IL-22. Innate and cell mediated immunity both help in clearance of *Candida* infection. [7]

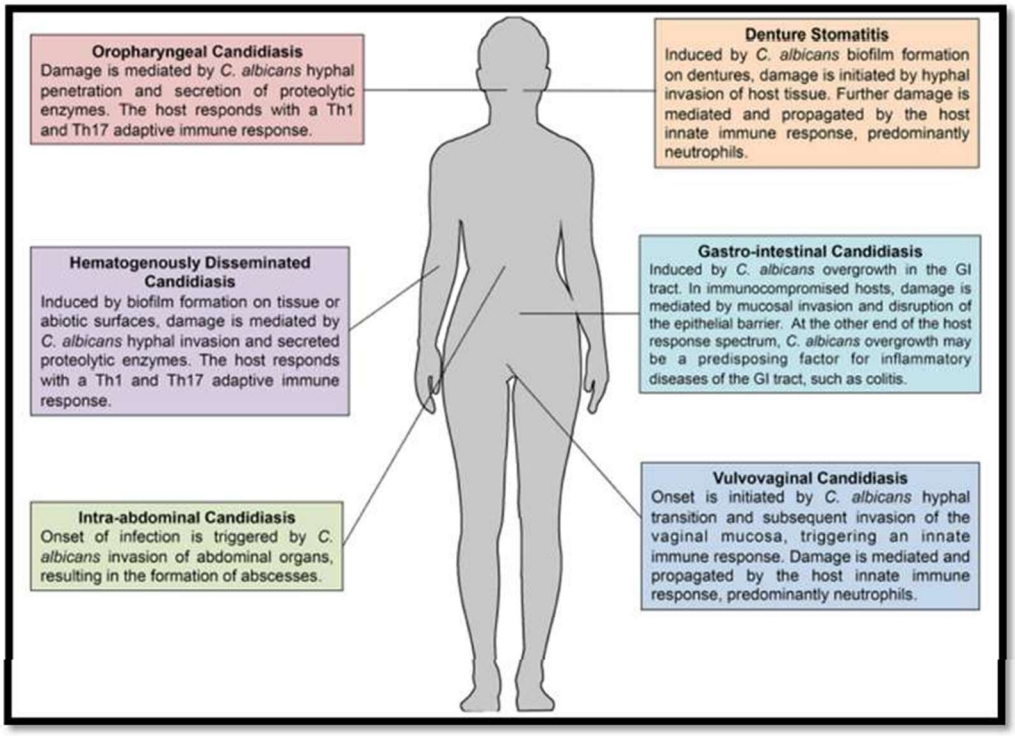


Figure 7: Diseases caused by *Candida* species- A overall view:

Table 3:The following table enlists the clinical manifestation of *Candida* infection: [19]

Mucocutaneous manifestation:	**Cutaneous manifestation:**
Oral: thrush, stomatitis, glossitis, cheilitis	Intertrigo
Alimentary: esophagitis, gastritis	Paronychia
Vulvovaginitis, balanitis, balanoposthis.	Onychomycosis
Chronic mucocutaneus candidiasis	Diaper dermatitis
Ocular candidiasis	Candida granuloma
Systemic manifestation:	**Allergic diseases:**
Candidemia	Eczema
Meningitis	Asthma
Disseminated	gastritis
Urinary tract infection	
Endophthalmitis	
endocarditis	

Table 4 Differential diagnosis: [19,33]

Oral thrush	• *Staphylococcus aureus* • *Corynebacterium diphtheriae* • Leukoplakia • Lichen planus • Tertiary syphilis
Vaginal candidiasis	• Trichomoniasis • Bacterial vaginosis • Herpes simplex infection
Systemic candidiasis	• Other mycoses • Tuberculosis • Neoplasia • Chronic bacterial infections

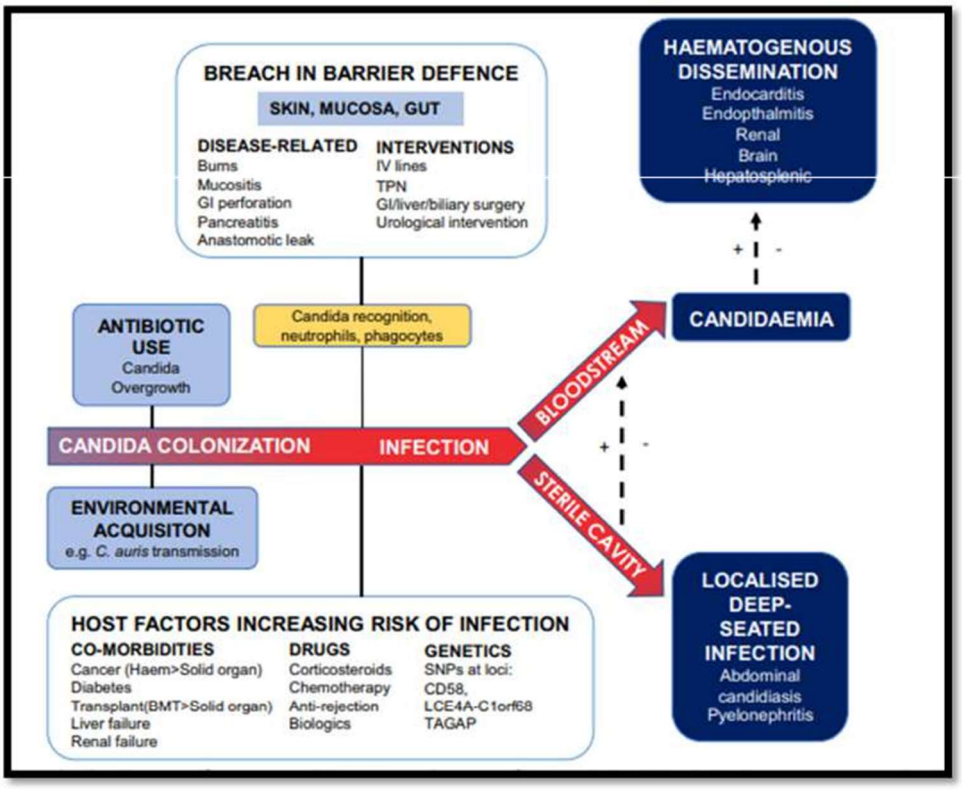


Figure 8: Key factors in the development of invasive candidiasis in critical care units.

Key factors include: [48]

- *Candida* colonization
- Broad-spectrum antibiotic
- Breach in barrier defences
- critical care interventions (e.g., skin; line insertion)
- Predisposing host risk factors:
- BMT bone marrow transplant,
- Gastrointestinal, Haematological, IV (intravenous), SNPs single-nucleotide polymorphisms,
- TPN- total parenteral nutrition

Fungal sepsis is common occurrence in high-risk neonates of NICU and is important cause of morbidity and mortality. Fungal colonization occurs in 10% of sick neonates during their first week of birth and in 90% in second and third week of hospitalization after birth which indicates the prolonged stay in hospital. Candidemia has become increasing major problem in neonates in ICU accounting for 9-13% blood stream infections. Mortality associated with Candidemia in high-risk neonates was as high as 20-34%. *Candida albicans* has been historically been the most isolated species, recently Non-albicans *Candida* have emerged as important cause for invasive Candidiasis. [1,8].

Nearly 50-57% of mucosal surfaces is habitat for *Candida* species in healthy individuals. When there is breach in these mucosal lining there are high chances for these commensal *Candida* species to enter blood stream and cause systemic infection. The capability of strong adhesion and evasion of this pathogen in one of the main causes for survival in host and cause pathogenesis. The other important virulence

factor is biofilm formation, resistant to commonly used antifungal group of drugs that is azoles like Fluconazole Voriconazole due to its efflux pump mechanisms and mutations in the genes coding for them [6]

CDC declared global emergence of *Candida auris* as emerging multidrug-resistant type of *Candida* that poses a serious global threat. Apart from sever pathogenicity caused by *Candida auris,* it spreads easily in health care settings showing resistance to Fluconazole and Amphotericin-B approximately 93% and 35% respectively and extensively drug resistant of about 4%. [6,9]

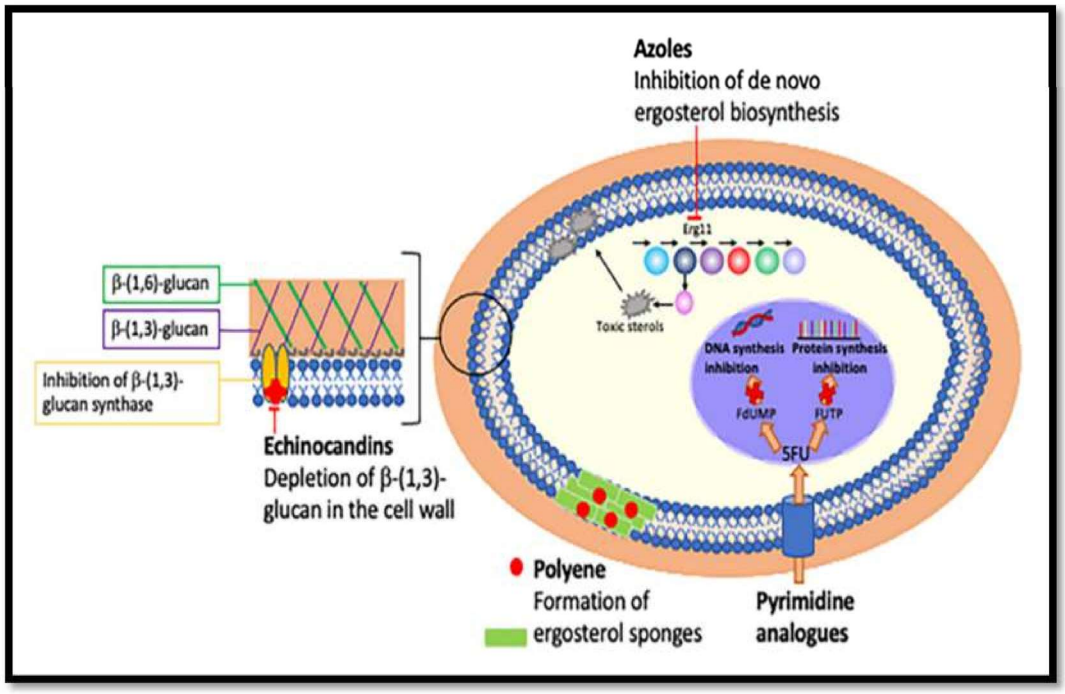


Figure 9: Mechanism and site of action of antifungal drugs

The above figure is the diagrammatic representation of mechanisms of antifungals at various sites. Like echinocandins act on β (1,3) glucan interlinks in the cell wall which play a vital role in maintaining the skeleton of the organism. Azoles inhibit the ergosterol biosynthesis which is a key component in cell wall synthesis. [49]

Table 5: Enumerates the most common antifungal drugs their site of action on the pathogen, resistance mechanism towards the antifungals and the most common species showing resistance to drugs. [49]

Drug class	Target pathway	Drug target	Mechanism of action	Mechanism of resistance	Species with reported resistance
Azoles (fluconazole, voriconazole, itraconazole, posaconazole, isavuconazole)	Cell membrane (Ergosterol)	Erg11p (lanosterol 14-ademethylase)	Inhibits de novo ergosterol synthesis thereby depleting membranes of ergosterol and causing accumulation of toxic sterol precursors	Increased drug efflux Mutations in Erg11p Overexpression of Erg11p Copy number variation Incorporation of non-ergosterol sterols into cell membranes	*C. albicans* *C. glabrata* *C. tropicalis* *C. dubliniensis* *C. parapsilosis* *C. krusei (intrinsic)* *C. auris (almost universal)*
Echinocandins (caspofungin, anidulafungin micafungin) Polyenes (amphotericin B)	Cell wall (b-1,3-glucan) Cell membrane (Ergosterol)	b-1,3-glucan synthase Sterols (ergosterol)	Inhibits b-1,3-glucan synthesis thereby disrupting cell wall stability Major: Sequesters ergosterol out of membranes. Minor: induces pore formation causing ion leakage	Mutations in FKS1/2 Incorporation of non-ergosterol sterols into cell membranes	*C. albicans* *C. glabrata* *C. auris* *C. albicans* *C. glabrata* *C. guillermondii* *C. krusei* *C. lusitaniae* *C. auris*
Pyrimidine analogues (5-fluorocytosine)	DNA synthesis, Protein synthesis	FUMP, FDUMP	Inhibits pyrimidine metabolism	Mutations in UPRT, FCY1, FCY2, FUR1	*C. albicans* *C. glabrata*

Antifungal resistance is an emerging problem worldwide, and this further complicates the selection of appropriate antifungal therapy. The term multidrug-resistant (MDR) *Candida* species is used to designate *Candida* species strains that are resistant to two antifungal drug classes, whereas the term extensively drug-resistant (XDR) *Candida* species can be used to designate *Candida* species strains that are resistant to ≥3 antifungal drug classes. [9].

Echinocandin resistance is almost exclusively due to point mutations in three hot spot regions in FKS1 or less frequently due to mutations in FKS2. The most frequently observed mechanism of azole resistance is reduced intracellular accumulation of drug through over-expression of efflux pumps (e.g., ABC or MFS transporters). Polyene resistance is due to incorporation of non-ergosterol sterols into cell membranes. 5FC resistance is mediated by point mutations in enzymes controlling its cellular uptake and conversion to 5FU: cytosine permease (FCY), cytosine deaminase (FCA1), and phosphoribosyl transferase (FUR1)

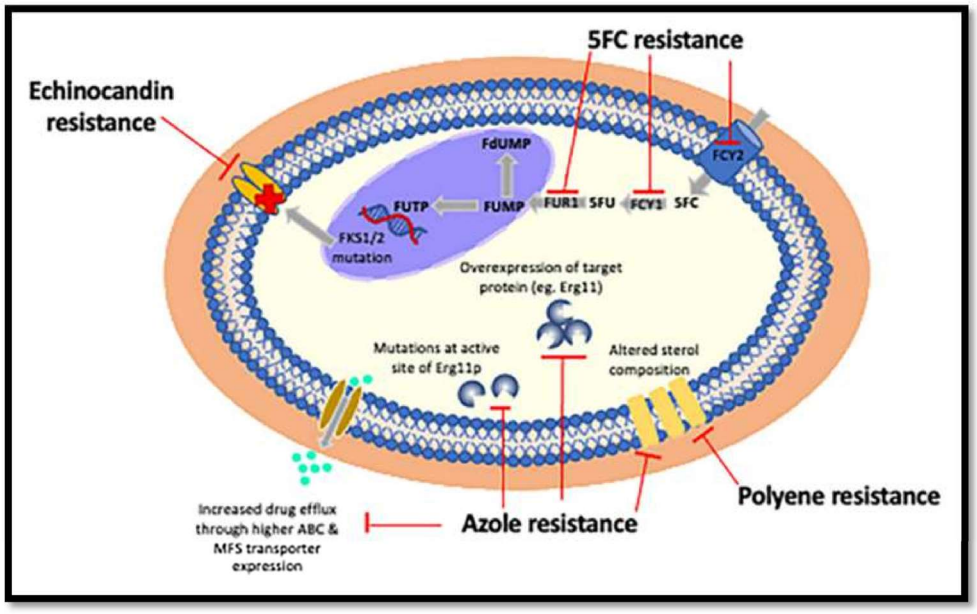


Figure 10: Mechanisms of resistance of currently using antifungal drugs.

Not only azole group of drugs but *Candida* also shows resistance to newer class of drugs echinocandins (Caspofungin, Micafungin, Anidulafungin) and other drugs like 5-flucytosine (5FC). The above figure is a representative model of resistance shown by the organism at various sites [49]

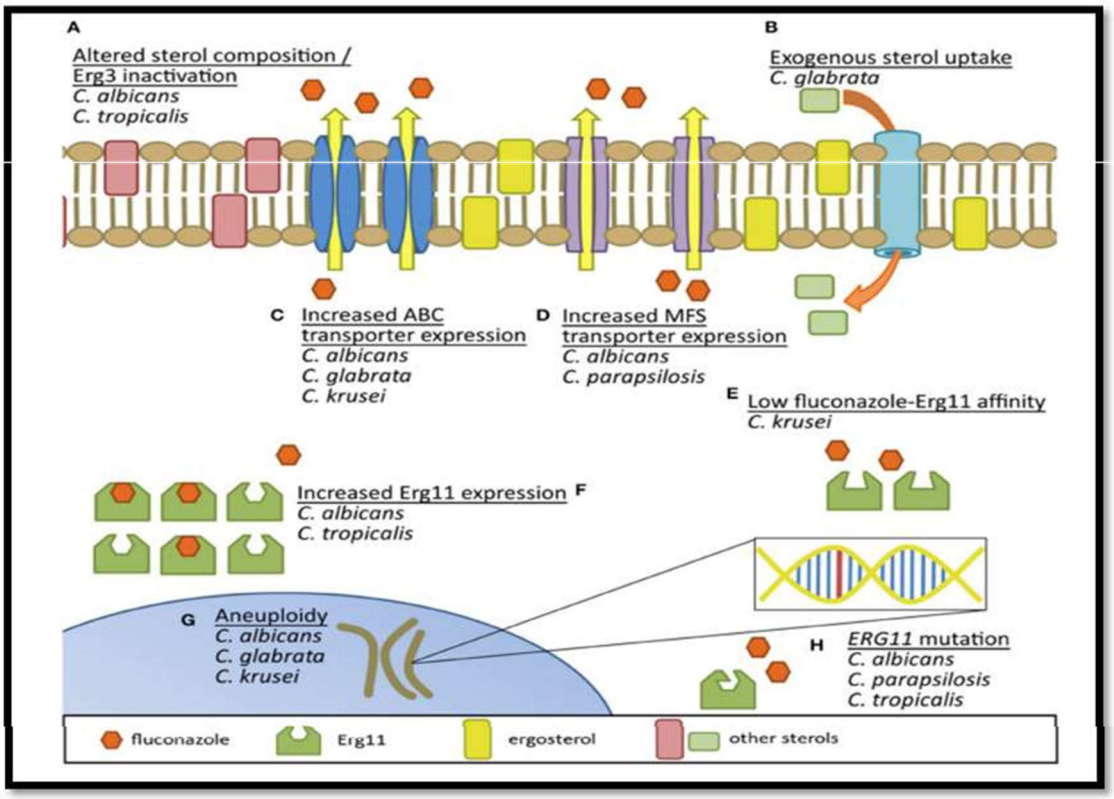


Figure 11: Various mechanisms of fluconazole resistance developed by *Candida species* [23]

The above figure represents various mechanisms developed by the pathogen against azole group of drugs, they are such as follows:

A. Erg3 inactivation results in utilization of alternative sterols in the yeast membrane.
B. Uptake of exogenous sterols helps circumvent endogenous sterol production inhibition by fluconazole.
C. ATP-binding cassette efflux pumps

D. major facilitator superfamily transporters reduce intracellular accumulation of azoles.
E. Inherently low affinity of fluconazole binding to species-specific Erg11 may decrease fluconazole's potential to inhibit the protein.
F. Increased expression of Erg11 protein can help overcome azole activity and
G. aneuploidy may promote genetic adaptation to azole exposure.
H. Mutations in ERG11 can also result in proteins with reduced affinity for fluconazole binding.

With the above detailed explanation and background of knowledge considering the critically ill neonates who were categorised basing on their mode of delivery, birth weight, term at birth, and along with the babies who were on long term antibiotic usage (> seven days), on mechanical ventilators (> seven days), centreline, UAV, UVC, TPN (>seven days) and finally number of days stay in hospital. [1,2,3]

All critically ill neonates with suspected sepsis especially late onset sepsis in attributed to fungal aetiology most common blood stream infection being candidemia according to the definitions term baby is more then thirty-six weeks of gestation, preterm birth means baby born between thirty-two to thirty-six weeks of gestation, very preterm means the neonate born before thirty-two weeks of gestation, and extremely preterm means neonate born between twenty-six to twenty eight weeks of gestation. Similarly, if birth weight is considered apt for birth weight is ≥2500 grams, low birth weight means < 2500grams, very low birth weight means <1500 grams, ELBW means <1000 grams. [2,3,50]

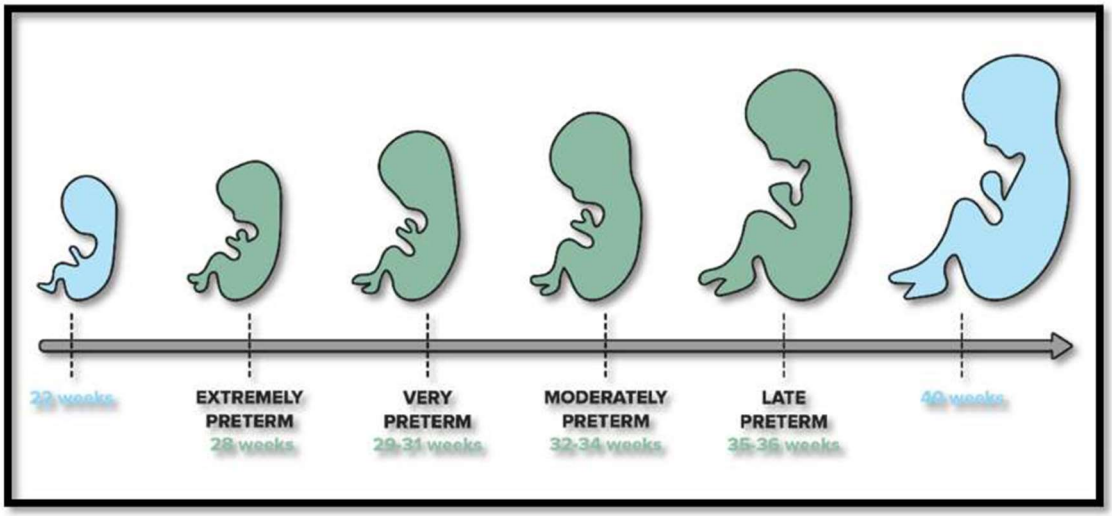


Figure 12: Is a pictorial depiction of neonate w.r.t gestational age

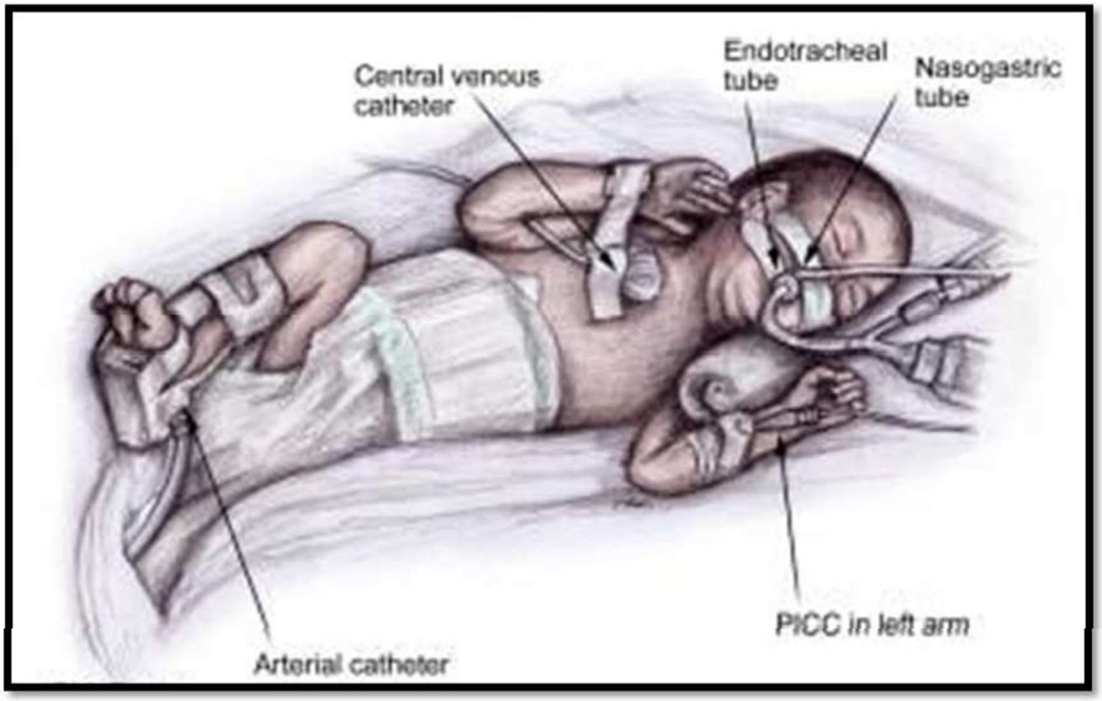


Figure 13: The above figure is a diagrammatic representation of external indwelling devices which can be one of the commonest routes causing late onset sepsis.

Figure 14: showing preterm extremely low birth weight neonate on mechanical ventilator and also UAC (umbilical arterial catheter) admitted in NICU (neonatal intensive care unit)

The commonly used anti-fungal empherical treatment in the NICU for suspected late onset sepsis of fungal etiology are Amphotericin-B (Lipid & deoxycholate formulations), Fluconazole and Echinocandins. And with rising incidence of fungal sepsis in critical care units fungal pathogens are also showing resistance to the commonly used and readily available empirical treatment such as azole group of drugs giving door way to higher and newer antifungals Echinocandins (Caspofungin, Micafungin, Anidulafungin) which are costlier drugs.

In late onset sepsis in critically ill neonates with Invasive candidiasis as a blood stream infection, blood cultures stand a gold standard for confirmation using conventional mycological standard procedures or else automation following the standard protocols.

Table 6: Diagnosis, clinical specimen and the advantages and disadvantages of the test used[19,33]

Diagnostic test	Specimen(s)	Advantages	Disadvantages
Fungal culture	Blood	Enables species identification and subsequent susceptibility testing	Slow (median detection time 2–3 days) Sensitivity suboptimal, particularly if high volume (≥60ml) and a fungal blood culture bottle are not employed
	Tissue and sterile body fluids	Enables species identification and subsequent susceptibility testing	Selective media, proper spreading of the sample and 3 days of incubation required for optimal performance
Microscopy	Cerebrospinal fluid, tissue and sterile body fluids	Highly sensitive, particularly if using fluorescent brightener staining	No species identification Lower sensitivity in absence of fluorescent brightener staining
Mannan antigen and antimannan antibody detection	Serum or plasma (EDTA) or cerebrospinal fluid	Increased diagnostic sensitivity when combined antigen and antibody testing is performed (although in neonates (in any sample) and in cerebrospinal fluid, antigen testing suffices	Heavy colonization (many non-sterile body sites culture positive for *Candida* spp. and/or with heavy growth in semi-quantitative culture) could cause positivity for blood testing
β-D-glucan detection	Serum or plasma (EDTA)	Pan-fungal marker	No separation between *Candida* spp. and other fungi Many sources for false positivity
PCR	Blood (EDTA	Rapid-tests Some commercial tests are FDA approved	Commercial tests are expensive May not detect all species

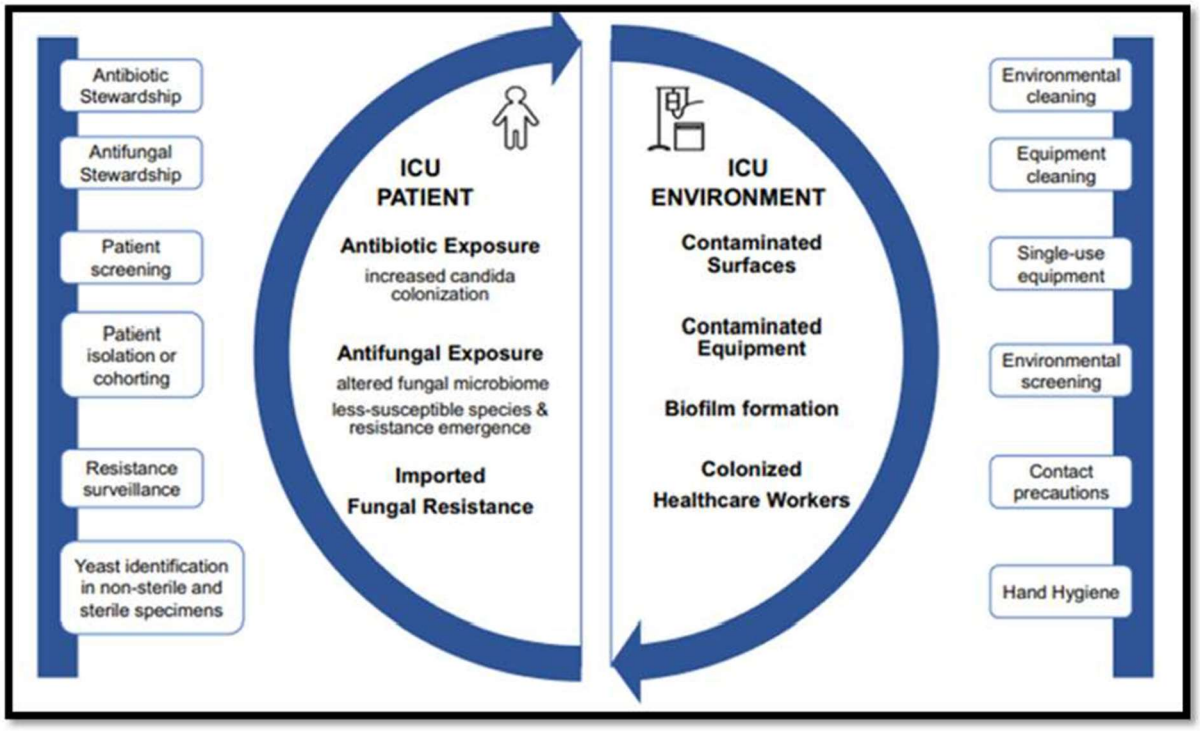


Figure 15: The following figure is the comparison of ICU patient microbiota with ICU environment and preventive strategies. [48]

As we can see in the above pictorial representation of figure 12 gives us the idea that prolonged stay in hospital especially in ICU which in turn leads to prolonged usage of antibiotics, prior exposure for antifungals, ICU environment like inadequate cleaning and inadequate disinfection pathogens colonizing on the hand surfaces of health care workers and the ability of pathogen with increased survival rates and property of biofilm formation are the leading causes to antimicrobial resistance (AMR) and long survival of organisms in the ICU's.

To deal with the above said problems strategies are well explained such as screening the patients depending upon the local epidemiology, isolation as and when needed, following antibiotic and antifungal stewardship programs, antibiogram dependent modification in empirical treatment, conducting resistance surveillance,

environmental screening and cleaning, proper disinfecting equipment, single use disposable equipment where ever needed, and strict hand hygiene measures needed.

Hence keeping in view all the current issues many studies have been conducted in India and also globally for incidence and prevalence of Candidemia and also many studies related to MDR stains of *Candida.*

The studies which show their results w.r.t *Candida* species:

Studies have been conducted in various parts of India and globally in regarding with emerging threat of *Candida* infections in blood steam of high-risk neonates and the epidemiological shift from *Candida albicans* to Non albicans *Candida* and their developing resistance to azole group of drugs which are used routinely as empirical therapy. [2,8].

The study done by *Uttam KG et al*[1], was a two-year retrospective observational study conducted in Kolkata have showed in their study 79 Fungal isolates of them 77 (97.7%) isolates are Non albicans *Candida* species isolates only 2 (2.30%) wee *Candida albicans*. , amongst all NAC species *C. pelliculosa* was highest (43%) where as in our study total of 63 positive fungal isolates were recorded of 230 total blood samples collected and *Candida albicans* being 16 of 63 constituting to 25.39% and NAC species being (n=47) 74.60% and most common fungal isolate are *Candida glabrata* (46%) .[1]

In 2019, in south India, Dharwad, Karnataka *Ananthaiah et al* [3]l isolated 71 positive blood culture of *Candida* species. Out of these 71 positive cultures maximum were Non-albicans *Candida* in which *Candida krusei* was isolates maximum i.e., 55 (constituting to 77%), next to which were *Candida albicans 7 (9.8%) followed by*

Candida tropicalis 3 (4.2%), Candida glabrata 2(2.8%), Candida rugosa 2(2.8%), Candida lipolytica 2(2.8%). [(2)]

In another study conducted by *Shettigar et al* [(14)] from A. J. institute of Medical Sciences, Mangalore, India showed Out of 54 isolated *of Candida* species, 35 were Non albicans *Candida* among 563 neonates admitted in the period of 3 years. And maximum isolates were *Candida krusei* accounting to 17 of 35 Non albicans *Candida.* And also showed that 51,43% of total Non albicans *Candida* were Fluconazole resistant. [(14)]

Raminder sandhu et al[(27)] from Haryana also showed that out of 45 pure isolates of *Candida* species 35 constitutes to Non albicans *Candida* and 10 constitute to *Candida albicans*. Most common isolates are *Candida krusei* and *Candida glabrata*. [(27)]

Similar study done by *Juyal et al* [(28)] from North India concluded that in 80.30% cases are of candidemia most prevalent being *Candida parapsiliosis* followed by *Candida tropicalis* constituting to 21.97%. 65.91% isolates were sensitive to Fluconazole and 96.21% to Amphotericin-B by disc diffusion method [(28)].

Goel et al [(36)] has done a study for antifungal susceptibility testing in *Candida* isolates from neonatal septicemia by comparison of results between Disc diffusion method and Broth micro dilution- MIC method for the isolates i.e., *Candida albicans* and all species of Non albicans *Candida* and concluded that 95.53% of Candida isolates were showing sensitivity to Fluconazole [(36)].

A comparative study of efficacy of echinocandins against fluconazole resistant isolates done by *Pfaller et al* [(32)] in Massachusetts, USA illustrated that among 162

Fluconazole resistant isolates of *Candida glabrata* 15 were resistant and 6 were intermediate to Echinocandins and rest were sensitive to echinocandins and also defines that Fluconazole resistant as a MIC value of ≥64 µg/ml, MIC of 0.25 µg/ml for Caspofungin and considered intermediate for Echinocandins if MIC value is of ≥0.5 µg/ml for Andulafungin and Caspofungin and MIC value of ≥0.25 µg/ml for Micafungin based on CLSI guidelines[32]

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, J. N. Med. College, KAHER, Belagavi

Source of the data:

The materials of the study constitute to the blood sample collected from all high-risk neonates admitted during the study period in NICU of Dr. Prabhakar kore charitable hospital, Belagavi from January 2021 to December 2021 received in the Department of Microbiology, J. N. Med. College, KAHER, Belagavi, were includedin the study.

Sample size: $n = \frac{z^2_{1-\alpha/2} pq}{(14\% \cdot p)^2} \times 1 \cdot 1 = \mathbf{90}$

Therefore n = $(1.96)^2$ x 70 x 30 / $(9.94)^2$ X 1.1 = 89.81

- **p= 70**,
- **q = 100- p = 30**,
- **14% of p = 9.94**,
- $z^2_{1-\alpha/2}$ **= 1.96**
- **From formula 1.1 = attrition**

INCLUSION CRITERIA: All the blood samples collected from high-risk neonates of NICU.

High risk criteria included in the study are:

- Low birth weight, very low birth weight, extremely low birth weight.
- Multiple gestation –twin gestation (1-1.8 kgs)
- Respiratory distress syndrome
- Birth asphyxia
- Neonatal seizures.
- PROM ≥ 18 hours
- FTND with early and late onset of sepsis.
- Instrumentation –Central line, long line, on life support, TPN, Transfusion

EXCLUSION CRITERIA:

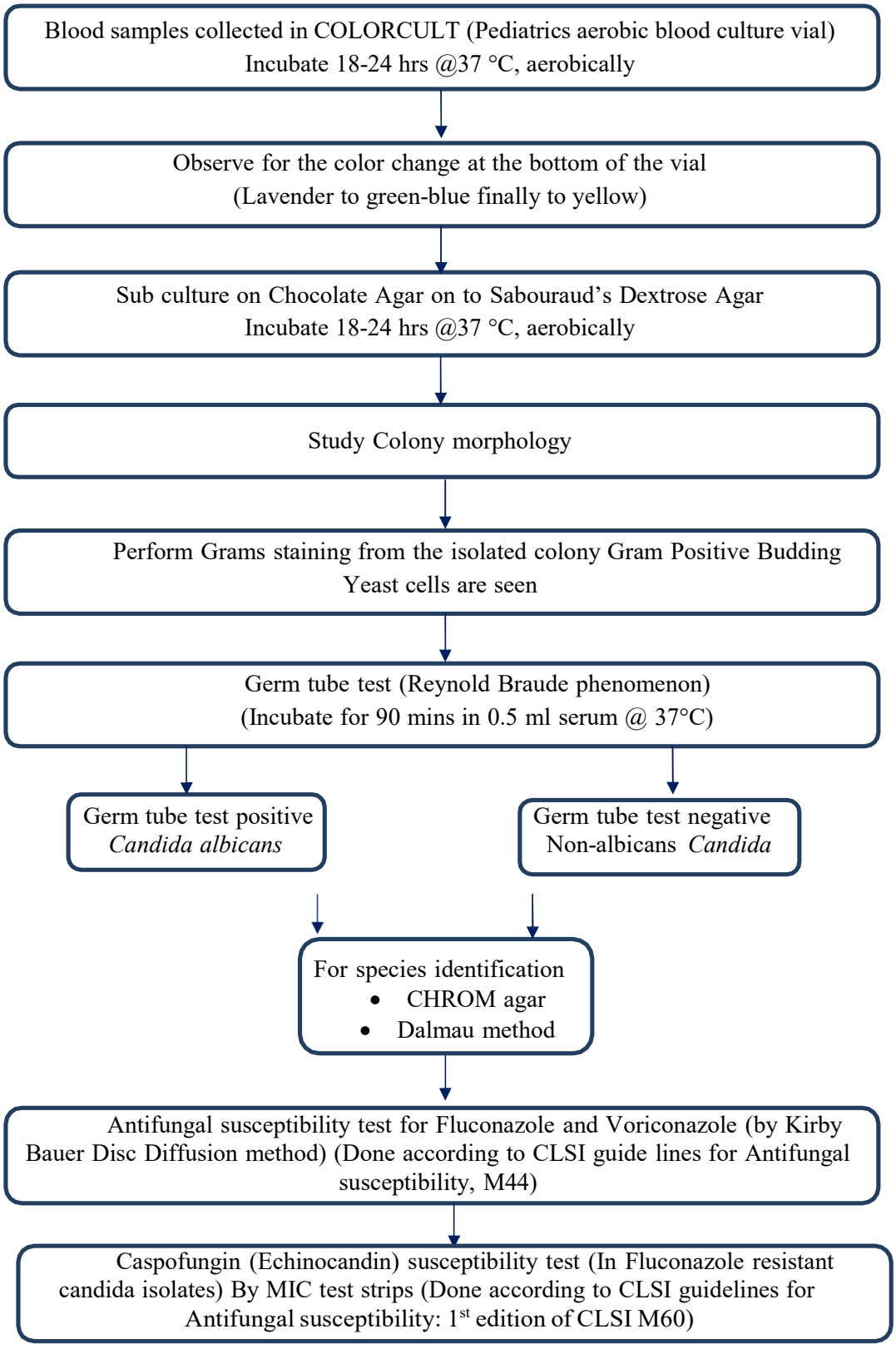
1) All the other clinical samples of high-risk neonates

2) Non-high-risk neonates admitted during the study period in NICU

NON-HIGH-RISK neonates:

- FTND with birth weight of 2.1kg only observation
- Babies with congenital anomalies –Hydrocephalus, Anal anomalies, Cleft palate, GI tract anomalies, Cardiac anomalies
- Neonates who underwent of surgeries (of any kind).
- Perinatal depression –weak cry/cry on stimulation –only observation
- Perinatal injuries-Breech presentation.
- Multiple gestation –twin gestation ≤34 weeks (2.1-2.3 kgs)
- Dehydration/ Inadequate feed/ Meconium-stained Liquor / Transient tachypnea / Hyperbilirubinemia / Hypoglycemia

METHODOLOGY followed: Plan of investigation



Materials used for the study according to the plan of investigation

Photo 1: On Aerobic pediatric color cult blood culture vials used in the study for the isolation of pathogenic organisms, showing lavender color at the bottom is the CO2 sensor –uninoculated (left), inoculated (right) showing the variation in color over a week time indicates growth.

Under aseptic condition 0.5 - 1ml of blood of high-risk neonates is collected and immediately is inoculated into the color cult aerobic blood culture vial provided on bedside and is given as gentle swirl. The constituents of this vial i.e., Resins/ adsorbent polymeric beads help in neutralizing many antibiotics along with β-lactam antibiotics, sodium polyanethol sulphonate inhibits the compliments and also inhibits the phagocytosis in turn enhances the growth of pathogens. 30ml of highly nutritive media is present in the vial to support the growth of both fastidious and non-fastidious organisms. Once the inoculated blood culture vial is received then it is incubated at 37°C aerobically. Subsequently change in color at the bottom of the vial is recorded and the subculture onto chocolate agar and Sabouraud's dextrose agar (SDA). After incubation before subculture a gentle swirl is given to homogenize the blood culture vial from which O.5cc of sample is withdrawn using a sterile 2cc single use

disposable syringe and inoculated on to freshly prepared media plates (both Chocolate agar and SDA). Inoculated plates are incubated at 37°C aerobically overnight / 18-24 hours. Next day colony morphology is studied and Gram's staining is performed to presumptive confirmation of the organism and for further proceedings. Note that is the CO2 sensor at the bottom of the vial is turned to bright yellow, then its time to discard the vial (according to the manufacturer instructions). Usually, blood is subculture serially three times. Results at the end of 48 hours and at the end of 5th day and the end of 7th day from the day of collection.

Media used are:

Table 7: List of media used and the purpose of using them.

Media	Purpose
Chocolate agar	• Used for isolation of pathogen (Both bacterial and fungal)
Sabouraud's dextrose agar	• Isolation of fungal pathogen • Antifungal susceptibility testing
Corn meal agar with tween 80	• For *Candida* species characterization
CHROM agar (Candida Differential Agar)	• For *Candida* species differentiation

Chocolate agar:

Chocolate agar is a non-selective enriched media used here to isolate pathogen from the clinical sample collected in color cult pediatric aerobic blood culture vial. Nutrient agar is prepared according to the manufacture's instruction i.e., 2.8gms in 100 ml of distilled water. Autoclave at 15 lbs. pressure at 121° C for 15mins. Bring down the temperature of the media up to 75-85 °C and maintain it in the water bath now add 5ml of sheep blood to the nutrient medium simultaneously giving a gentle swirl. Under sterile condition pour the media into sterile petri plates of 100 mm size to a thickness of 4mm of media w.r.t plate. Let the plates to cool down and the entire batch can rest overnight, next day check quality check is done and kept ready for use. Final pH is 7.2± o.2

Sabouraud's dextrose agar:

It is a selective medium used for isolation of yeast and molds. 6.5gms of SDA is dissolved in 100ml of distilled water. Autoclave at 15 lbs. pressure at 121° C for 15mins. Under sterile condition pour the media into sterile petri plates of 100 mm size to a thickness of 4mm of media w.r.t plate. Let the plates to cool down and the entire batch can rest overnight, next day check quality check is done and kept ready for use. Final pH is 5.6± o.2

The dextrose and low pH inhibit the growth of bacteria, enhancing the fungal growth.

Gram's staining:

A drop of normal saline is taken on the sterile grease free scratch free glass slide, using a sterile straight nichrome wire single isolated colony is selected and made a thin smear and heat fixed by flaming over the Bunsen burner. After fixing gentian violet was poured over the smear, care is taken to completely cover the smear. Primary stain was allowed to stand by for 1minute and washed under gentle running tap water. The Grams iodine was poured on the glass slide, and kept for 1minute, washed with tap water. Decolorization was done using 95% ethyl alcohol till no colored solvent flowed down the slide. The slide is then washed with tap water and counter-stained with safranin for 30 seconds and washed finally. Air dry the smear and observe and record under 100X oil immersion and record the results. Depending on which further plan of investigations are to be selected.

Germ tube test (Reynold's Braude phenomenon):

Germ Tube Test is a rapid and presumptive screening test which is used to differentiate *Candida albicans* from other yeast. This test is done after Gram's staining and confirming round to oval budding yeast cells which are gram positive and uniformly stained. A germ tube is a small hyphae like protrusion from the side wall of the yeast cell representing the increased synthesis of proteins an ribonucleic acid. Take 0.5 ml of serum in a 5ml sterile tube add a pinch of sucrose to this inoculate the isolated colony to be tested and incubate at 37 °C for 90 minutes. From this broth take 0.2ml and prepare a wet mount and observe under low power 10X and 40X for germ tube production. Basing on which the organism can be subdivided into albicans and non-albicans and further speciate. It is positive for *Candida albicans and Candida dubliniensis.*

Corn meal agar with tween 80:

Suspend 1.7 grams in 100 ml distilled water. If desired add 1% polysorbate 80. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Final pH is 6.0 ± o.2.

The main of using this media is to enhance the production of chlamydospores. Chlamydospore production is an accepted criterion for the identification of *Candida* species. Corn Meal Agar is a well-established mycological medium used for the cultivation of fungi and to study chlamydospores production of *Candida* species.

Used in Dalmau's method to record the characteristics and to speciate the *Candida* species. Isolated colony from SDA using a straight wire, and make a deep cut in the Corn Meal Agar plate. Place a flamed sterile coverslip over the line of inoculum. After incubation for 24-48 hours at 25-30°C, the streaks are examined microscopically, through the coverslip, using low and high-power objectives. *C.albicans* produces mycelium bearing ball-like clusters of budding cells and characteristics thick walled round chlamydospores.

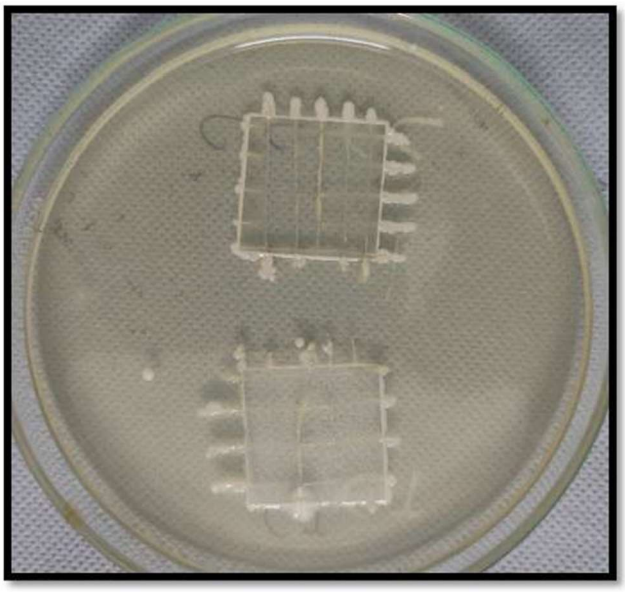


Photo 2: Showing the method to perform Dalmau's method to study the characteristics of the isolates- select the isolated colony and streak cutting the corn meal agar and cover the streak lines by a sterile coverslip as shown- incubate at 25°C and read after 36 hours of incubation.

CHROM agar:

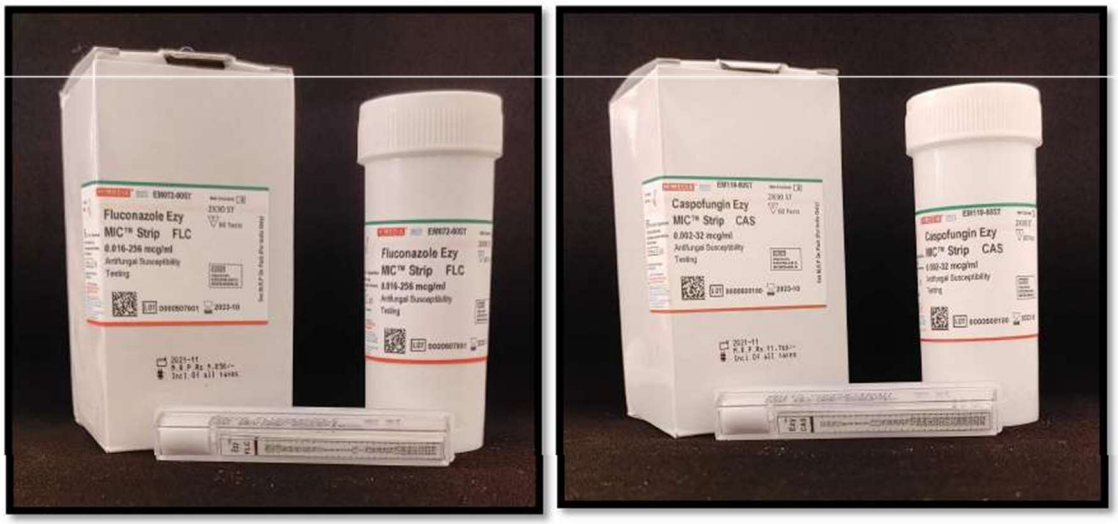
Hi-Crome *Candida* Differential Agar is recommended for rapid isolation and identification of *Candida* species from mixed cultures in clinical and non-clinical samples. 4.8 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Final pH is 6.3 ± o.2. *Candida* Differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* species namely *C. albicans, C. krusei, C. tropicalis and C. glabrata* on the basis of colouration and colony morphology. On these medium results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts.

Growth from chocolate agar and SDA are confirmed by performing Gram's staining, followed by GTT (germ tube test), Dalmau's method further on chromogenic media and antifungal susceptibility.

Antifungal susceptibility testing:

Antifungal susceptibility testing is done by both methods Kirby- Bauer's disc diffusion method and Epsilon meter MIC strip test method using SDA. One- two isolated colonies from the culture pate were inoculated into 2ml mycological peptone broth and incubated at 37° C for 2 hours. Turbidity was compared to that of 0.5 McFarland's standard (1.5×10^{8} CFU/ml). a sterile cotton swab was immersed, rotated in this inoculum, the swab was the pressed against the sides of the tube so as to remove excess inoculum. Then it is used to inoculate onto the SDA petri plate of 100 mm size by lawn culture in three different directions to ensure even and complete distribution of inoculum over the plate. The Antifungal discs are placed in 1 plate and MIC E-strips are placed in the other similarly lawn culture plate within 15 minutes of inoculation of the plates and are kept inverted and incubated at 37° C for 18- 24 hours

From Micro press and HI-media Commercially available Disc and MIC E-strips respectively were used. The strength of the disc and the gradient strip used and their zone of inhibition size interpretative standards were according to guidelines by CLSI M44-A2 and M60 respectively



<u>Photo 3:</u> MIC E-strip test for antifungal susceptibility testing- Fluconazole (FLC) 0.016 – 256 mcg/ml (left) & Caspofungin (CAS) 0.002-32mcg/ml (right) gradient strip.

CLSI document M44-A2 provides an established methodology for disk diffusion testing of *Candida* species. And CLSI document M60 provides and established documented reference for MIC by microbroth dilution break points, the reference ranges areas follow.

Table 8: showing reference range for antifungals MIC breakpoints-according to CLSI M60 [51]

Antifungal agent	Species	MIC Breakpoints and Interpretive Categories, µg/mL		
		S	**I**	**R**
Caspofungin	*C.albicans*	**≤ 0.25**	**0.5**	**≥ 1**
	C.glabarta	**≤ 0.12**	**0.25**	**≥ 0.5**
	C.krusei	**≤ 0.25**	**0.5**	**≥ 1**
	C.parapsiliosis	**≤ 2**	**4**	**≥ 8**
	C.tropicalis	**≤ 0.25**	**0.5**	**≥ 1**
Fluconazole	*C.albicans*	**≤ 2**	**--**	**≥ 8**
	C.glabarta	**--**	**--**	**≥ 64**
	C.krusei	**--**	**--**	**--**
	C.parapsiliosis	**≤ 2**	**--**	**≥ 8**
	C.tropicalis	**≤ 2**	**--**	**≥ 8**

NOTE:

Major remarks are given by CLSI guidelines w.r.t antifungal susceptibility testing [51]

- Caspofungin susceptibility testing in vitro has been associated with significant interlaboratory variability
- When testing Caspofungin, susceptible results may be reported as "susceptible"; however, laboratories should confirm "I" or "R" results by additional susceptibility testing with micafungin or anidulafungin
- DNA sequence analysis of FKS genes to identify resistance hot spot mutations in FKS1 (all *Candida* spp.) and FKS2 (*C. glabrata* only).
- Isolates of *C. krusei* are assumed to be intrinsically resistant to fluconazole, so their MICs should not be interpreted using this scale.
- When an isolate is identified as *C. glabrata* and the MIC is ≤32 µg/mL, it should be determined whether fluconazole is appropriate in the specific clinical context. If so, patients should receive a maximum dosage regimen of fluconazole. Expert consultation on selecting a maximum dosage regimen may be useful.
- Breakpoints may also be used for 48-hour readings if 24-hour growth control shows insufficient growth.

Photo 4 (Left) & Photo 5 (Right):

Showing the isolate susceptibility to E-strip method (MIC)

Isolate used here *Candida albicans* to demonstrate the sensitivity pattern for Fluconazole (Right photo) & Caspofungin (Echinocandins- left photo).

Table 9: Shows the drug and its MIC range

The gradient strip of:	Showing MICs in the range of:
Fluconazole	0.016– 256 mcg/ml
Caspofungin	0.002-32 mcg /ml

MIC strip is useful for quantitative determination of susceptibility of fungus to antifungal agents. The E-strip is predefined with quantitative gradient which is used to determine the MIC in mcg/ml of different anti-microbial agent against pathogens to be tested.

The strips give reproducible MIC value that are equivalent to standard reference MIC obtained by broth dilution performed as per guidelines with less effort.

Table 10: Showing reference range for antifungals Kirby-Bauer Disc diffusion method- M44- A2

Antifungal agent	Zone diameter in mm	Zone diameter in mm
Fluconazole (FLC)-10mcg	≥20 -Susceptible	≤14 - Resistant
Voriconazole (VRC)-1mcg	≥ 20- Susceptible	14 - Resistant

Table 11: Shows the drug and its concentration of Disc:

Anti-fungal Disc used	Concentration of drug
Fluconazole (FLC)	10mcg
Voriconazole (VRC)	1mcg
Amphotericin-B (AP)	50mcg

Note: As per CLSI guidelines Disc diffusion method is NOT the appropriate method for susceptibility testing for Amphotericin-B, best method is micro broth dilution method.

Candida albicans: The following pictures shows the characteristics

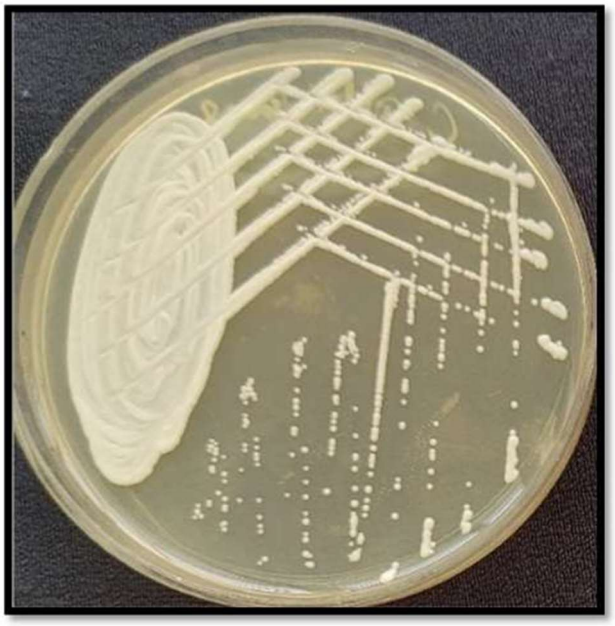


Photo 5:
Shows 1-2 mm whitish opaque convex moist pasty, smooth, cream-colored colonies on SDA Growth seen after 18-24 hours of incubation at 25°C.

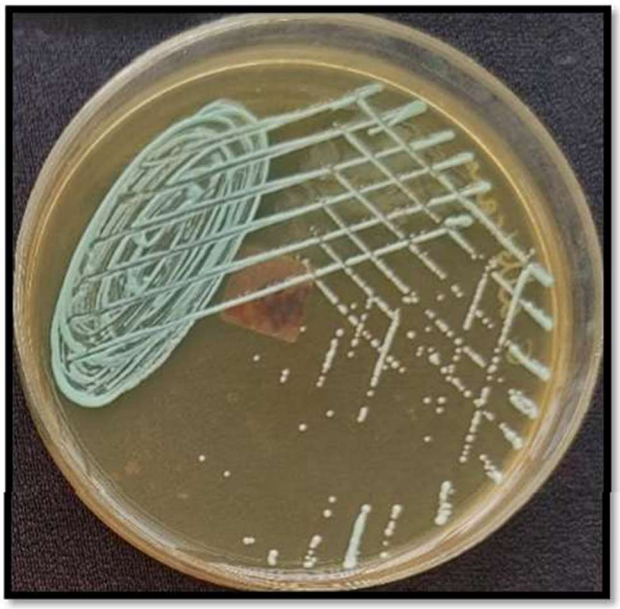


Photo 6:
On Chromogenic media: *Candida albicans* show Teal green color colonies.
Color reading done in 48hours of inoculation (incubation at 25°C)

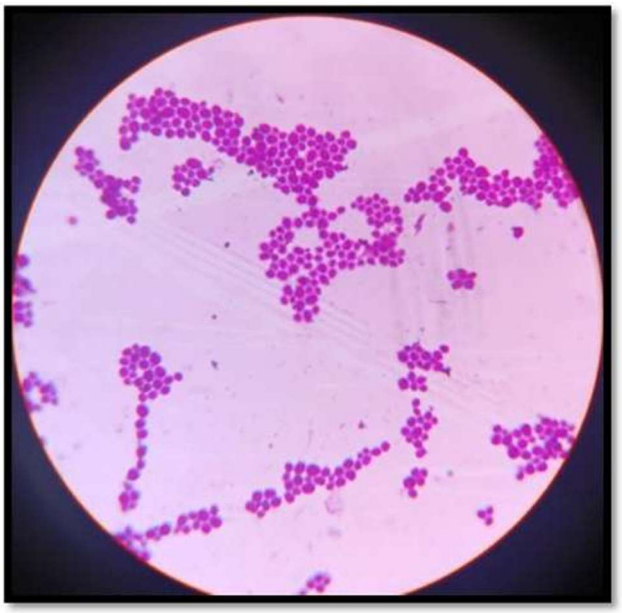


Photo 7: On Gram's staining (Hucker's modification):

Shows Gram positive budding yeast cells of approximately 3.5-7 X 4-8µm in size with the daughter budding cell at the broad base

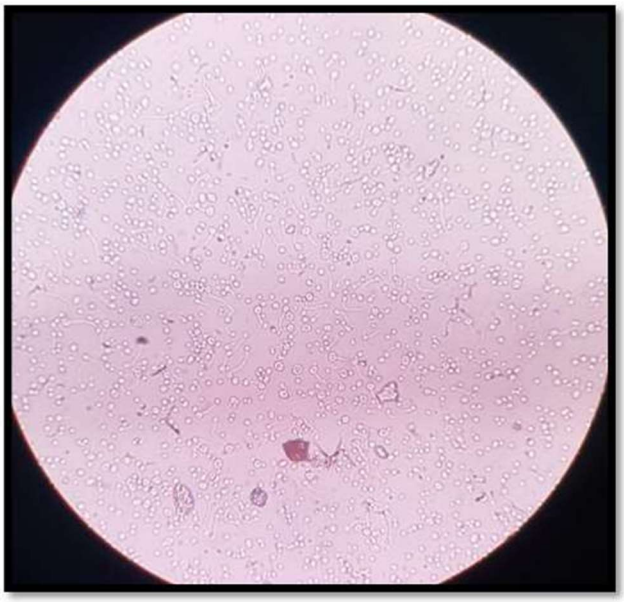


Photo 8: Germ tube test – Reynold's Braude phenomenon:

60-70% of *Candida albicans* shows Germ tube production- when isolated colony is inoculated in 0.5ml serum and incubate at room temperature for 90mins.

A germ tube is small extension hyphae like protrusion from the side wall of yeast cell measuring approximately 2-3µm.

*(At least 5 Germ tubes / 40X is considered to be positive)

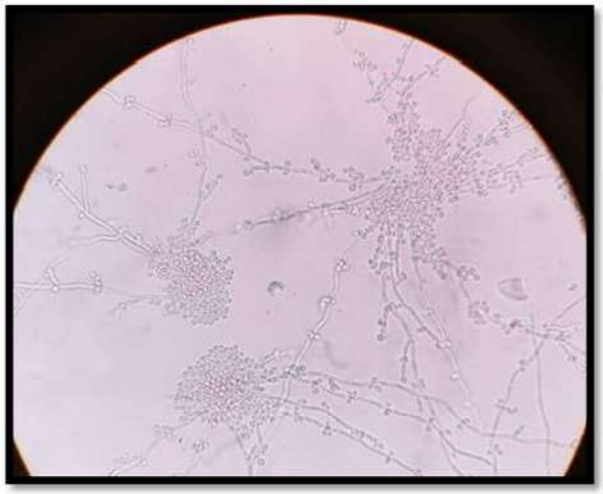


Photo 9:

On corn meal-Tween80 agar at 25 C for 72 hours shows pseudo hyphae forms with clusters of Blastoconidia at the septa. Large, thick walled, usually single terminal chlamydospores are characteristically seen in *C. albicans*

Photo 10:

On top Fluconazole (E-strip) is showing the MIC value = 0.75 i.e., pathogen is Sensitive, Below Caspofungin (E-strip) is showing the MIC value = 0.75 indicates that pathogen Resistance (refer to Table No – 8).

Candida glabrata: The following pictures shows the characteristics

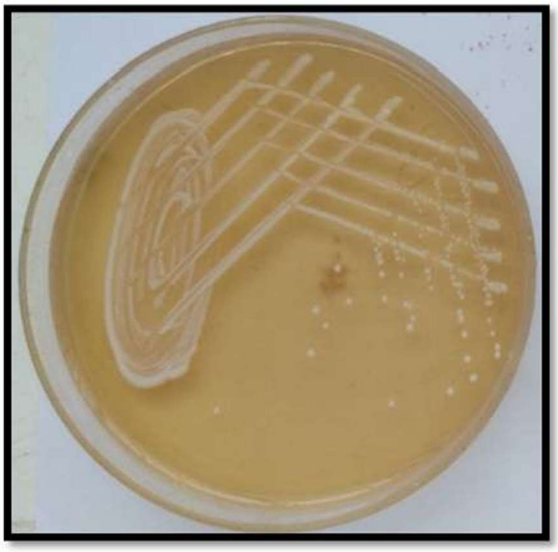


Photo 11:

Shows small approximately 1 mm whitish opaque convex, moist pasty, smooth, white to cream-colored colonies on SDA Growth seen after 18-24 hours of incubation at 25°C.

Photo 12:

On Chromogenic media: *Candida glabrata* show **Lavender color colonies**. Color reading done in 48hours of inoculation (incubation at 25°C)

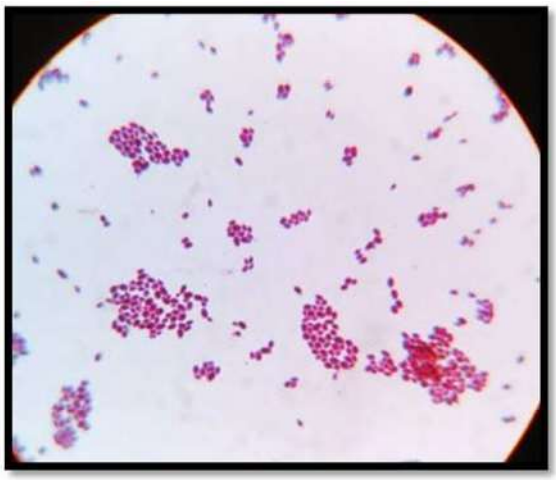


Photo 13:

Shows Gram positive budding yeast cells of approximately 2-3 X 3-4µm in size with the daughter budding cell at the broad base # Germ tube test is not produced hence Reynolds Braude phenomenon is NEGATIVE for non-albicans *Candida.*

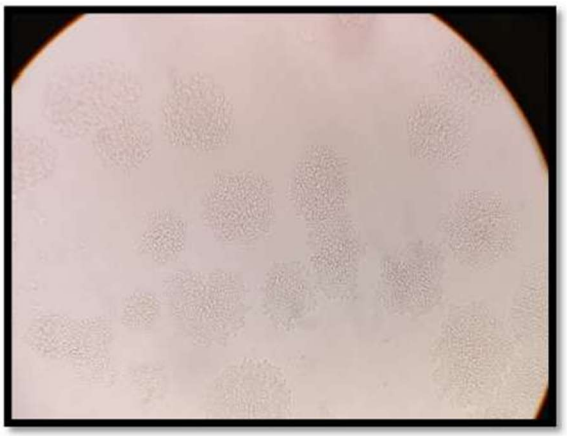


Photo 14: Dalmau's method:

On corn meal-Tween80 agar at 25°C for 72 hours, only small (2-3 X 3-4 µm), oval yeast cells with single terminal budding are seen. No pseudo hyphae are formed.

Photo 15:

On top is Fluconazole (E-strip) showing no zone of inhibition i.e., Resistance, below Caspofungin (E-strip) is showing MIC value =0.25 i.e., Resistant (refer to Table No – 8)

***Candida parapsiliosis*:** The following pictures shows the characteristics

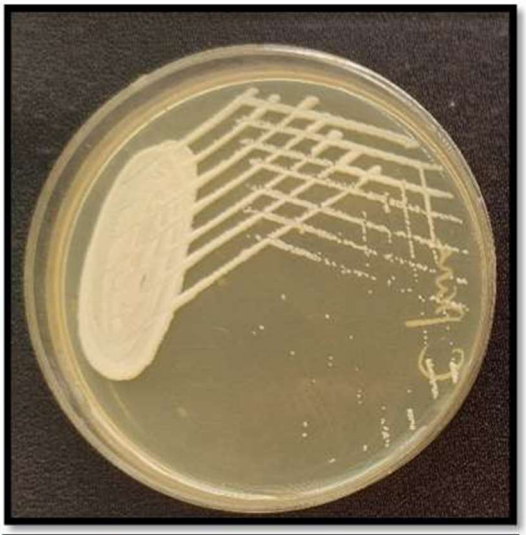


Photo 16:
Shows small approximately 1 mm whitish opaque convex, moist pasty, smooth, white to cream-colored colonies on SDA_Growth seen after 18-24 hours of incubation at 25°C.

Photo 17:
On Chromogenic media: *Candida parapsiliosis* show **white color colonies**.
Color reading done in 48hours of inoculation (incubation at 25°C)

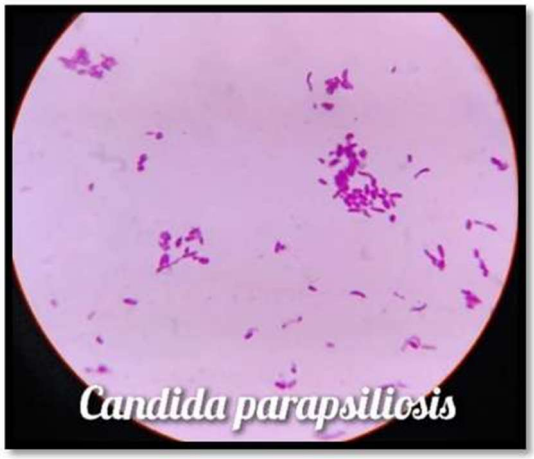


Photo 18:
Shows Gram positive ovoid budding yeast cells of approximately 2.4-4 X 3-8 µm in size with the daughter budding cell at the broad base

Germ tube test is not produced hence Reynolds Braude phenomenon is NEGATIVE for *C. tropicalis* (non-albicans *candida*).

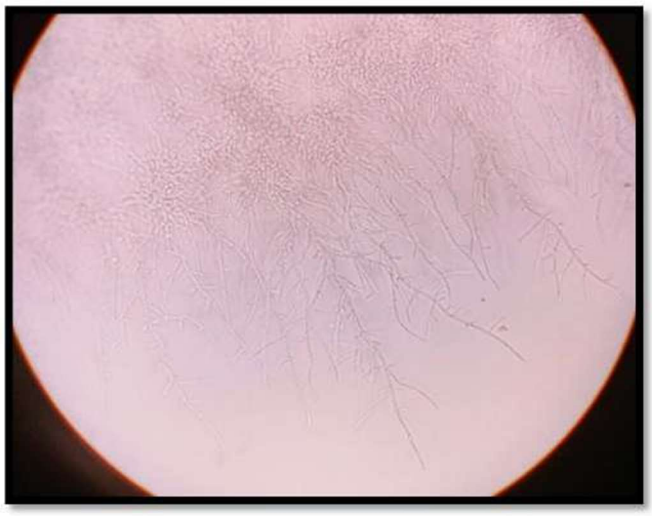


Photo 19: Dalmau's method:
On corn meal-Tween80 agar at 25°C for 72 hours, Blastoconidia are arranged singly or in small clusters seen along pseudo hyphae. Characteristic features are curved appearance and relatively short pseudo hyphae and few large hyphal elements called giant cells are seen.

Photo 20:
On top is Fluconazole (E-strip) showing the MIC value = 2 i.e., Sensitive, below Caspofungin (E-strip) showing the MIC value = 2 indicates Sensitive (refer to Table No – 8)

***Candida tropicalis*:** The following pictures shows the characteristics

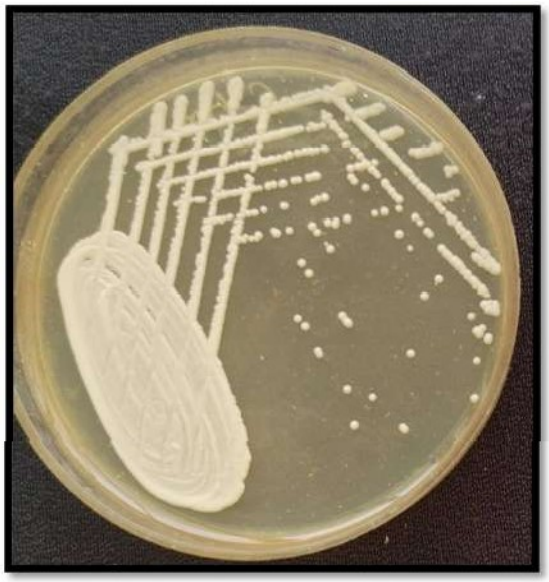


Photo 21:
Shows 1-2 mm whitish opaque convex moist pasty, smooth, cream-colored colonies on SDA Growth seen after 18-24 hours of incubation at 25°C.

Photo 22:
On Chromogenic media: *Candida tropicalis* show **metallic blue color colonies**. Color reading done in 48hours of inoculation (incubation at 25°C)

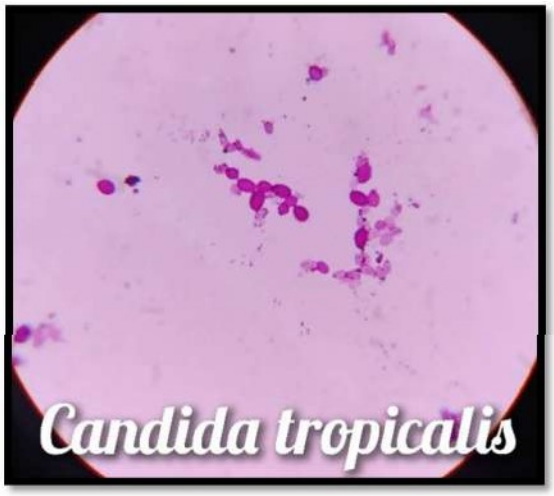


Photo 23: On Gram's staining (Hucker's modification):
Shows Gram positive budding yeast cells of approximately 3.5-7 X 5.5-10 µm in size with the daughter budding cell at the broad base.

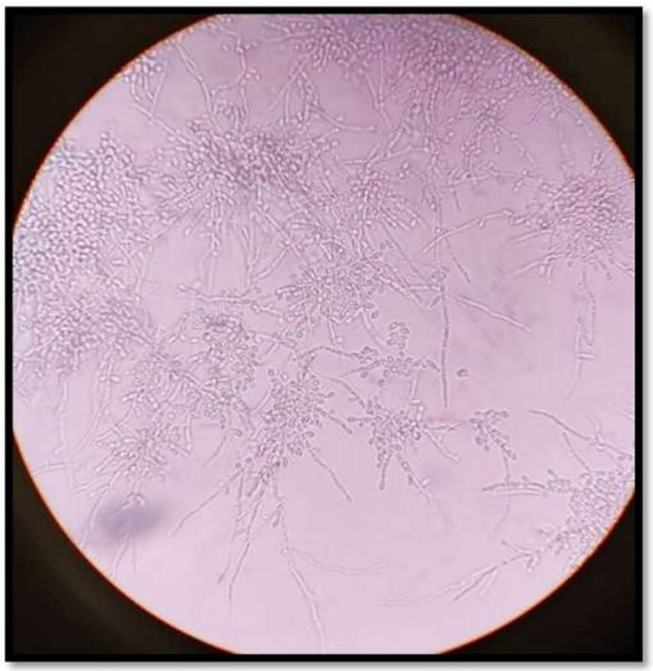


Photo 24:

Dalmau's method: on corn meal-Tween80 agar at 25 C for 72 hours, shows Blastoconidia arranged singly or in small groups all along graceful, long pseudo hyphae

Photo 25

On top is Fluconazole (E-strip) showing Resistance, Below Caspofungin (E-strip) showing the MIC value = 0.19 indicates Resistance (refer to table no – 8)

Candida krusei: The following pictures shows the characteristics

Photo 26:
Shows large 3 mm whitish opaque flat dry, cream-colored colonies on SDA Growth seen after 18-24 hours of incubation at 25°C.

Photo 27:
On Chromogenic media: *Candida krusei* show **dry pink color colonies**. Color reading done in 48hours of inoculation (incubation at 25°C)

Photo 28: (Hucker's modification):
Shows Gram positive oval to elongate budding yeast cells of approximately2-6 X 4-10 µm in size with thedaughter budding cell

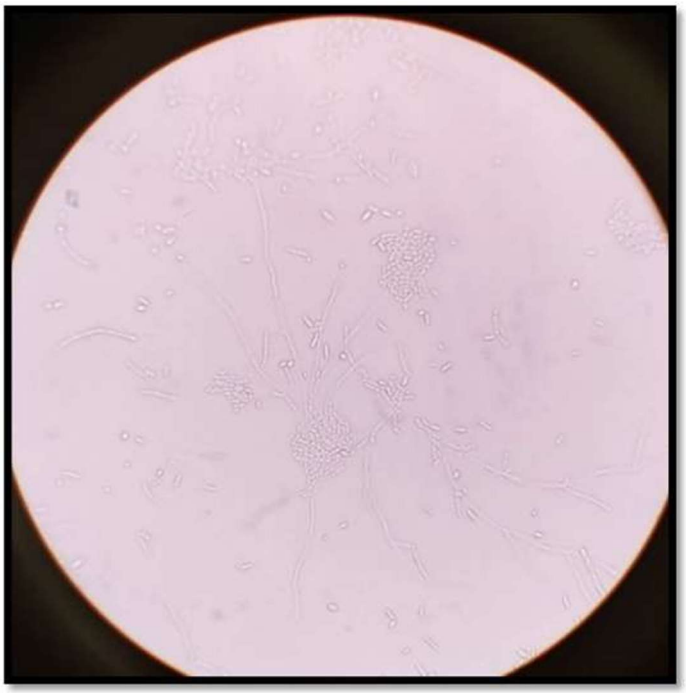


Photo 29:
On corn meal-Tween80 agar at 25°C for 72 hours, shows pseudo hyphae with Blastoconidia forming cross match sticks or tree like appearance

Photo 30:

On top is Fluconazole (E-strip) showing Resistance, bottom is Caspofungin (E-strip) showing the MIC value ≤0.25 indicates sensitive to echinocandin drug.

Candida kefyr: The following pictures shows the characteristics

Photo 31:

Shows small 1-2 mm whitish opaque smooth moist, cream-colored colonies on SDA
Growth seen after 18-24 hours of incubation at 25°C.

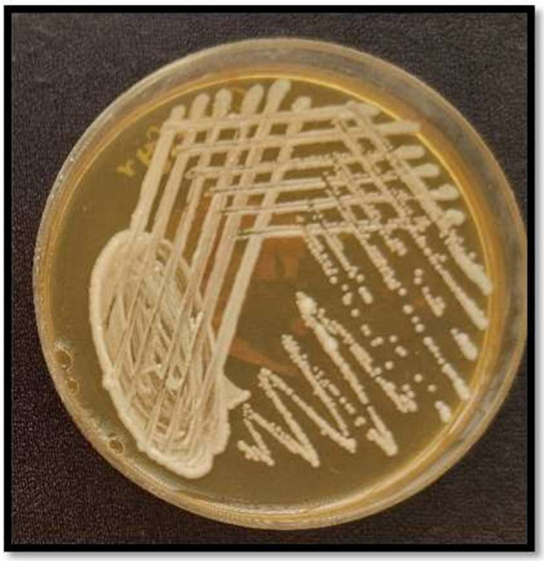


Photo 32:

On Chromogenic media: *Candida kefyr* show **cream to white color colonies**. Color reading done in 48hours of inoculation (incubation at 25°C)

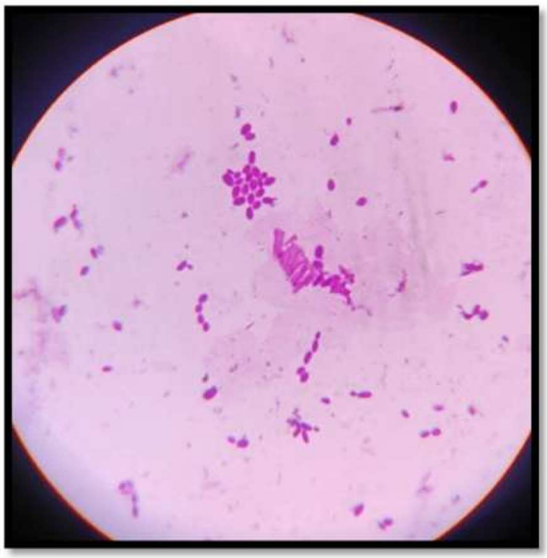


Photo 33:

Shows Gram positive budding yeast cells of approximately 3-8 X 5-12 µm in size with the daughter budding cell at the broad base

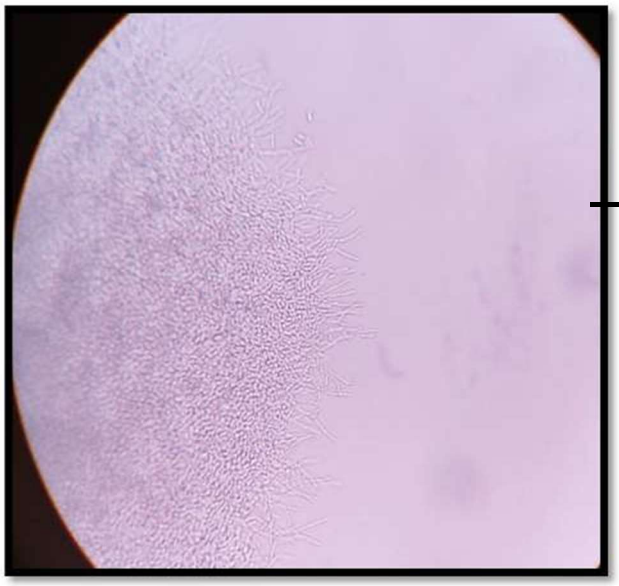


Photo 34:

On corn meal-Tween80 agar at 25°C for 72 hours, shows elongate Blastoconidia that characteristically line up in parallel, giving the appearance of logs in stream

Photo 35:

On top is Fluconazole (E-strip) showing sensitive, bottom is Caspofungin (E-strip) showing the MIC value = ≤0.25 indicates sensitive.

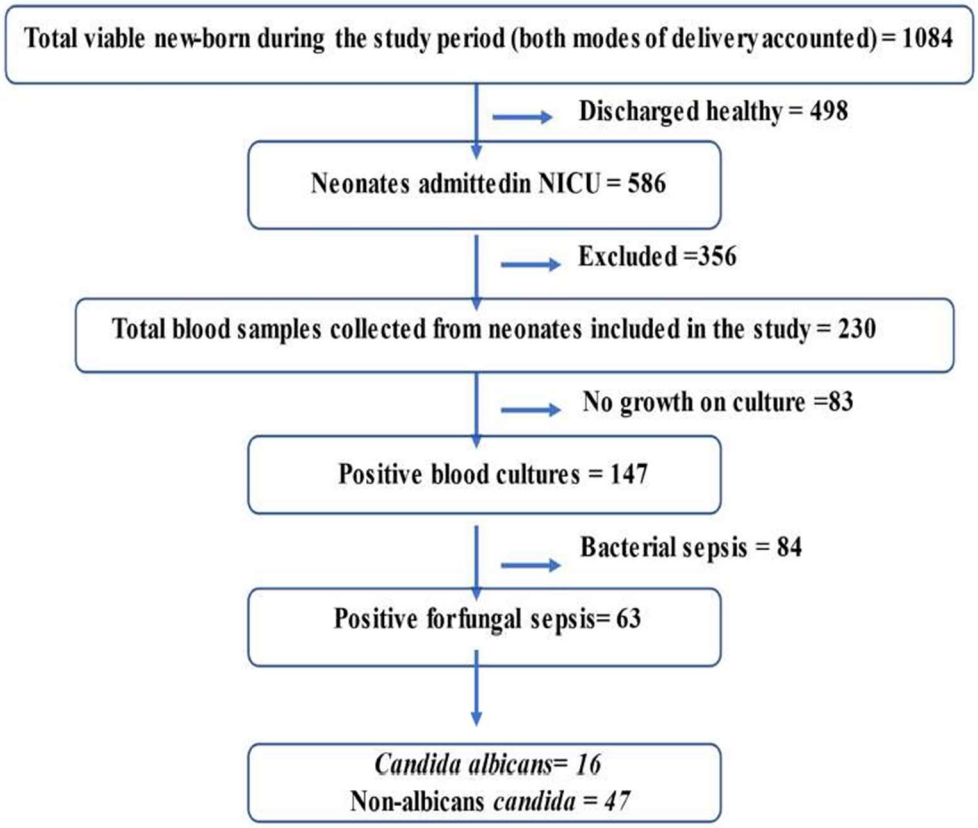
RESULTS

The present of blood stream infections of critically ill neonates caused due to Candida species was carried out in the Department of Microbiology, J. N. Medical college, Belagavi, over a period of one year from January 2021 to December 2021. All the high-risk neonates admitted during this period with suspected case of Candidemia and late onset sepsis of age group day 3 of birth to day 28 of birth, both the genders, both modes of delivery, and on long term treatment with antibiotics, neonates on mechanical ventilation, neonates on total parenteral nutrition, prolonged stay in NICU, neonates with birth asphyxia, and respiratory distress were included in the study.

Data was included in a predesigned proforma. It includes patient details such as Patients identification number, baby of mother's name, day of inclusion, gender, birth history, clinical presentation and assessment, Standard Microbiological diagnosis along with antifungal susceptibility.

Non-albicnas *Candida* are most prevalent isolates, *Candida glabrata* being the most common isolate(n=29) constituting to 46.03% of total fungal positive cultures. Followed by *Candida albicans* (n=16) i.e., 25.40 %.

Flow chart: (2)



Among total of 586 NICU admission 356 neonates were excluded, 230 blood samples were collected and further processed of which 63 are pure isolates of fungal sepsis. Sixteen are *Candida albicans* (n=16) accounting to 25.4% and forty seven are Non-albicans *Candida* (n=47) accounting to 74.6% of total isolates and resistance to azole drugs Fluconazole is seen in 53.38% and sensitivity to Echinocandins (Caspofungin) is seen in 79.36% of fungal isolates.

Graph 2: Prevalence of Candidemia in NICU.

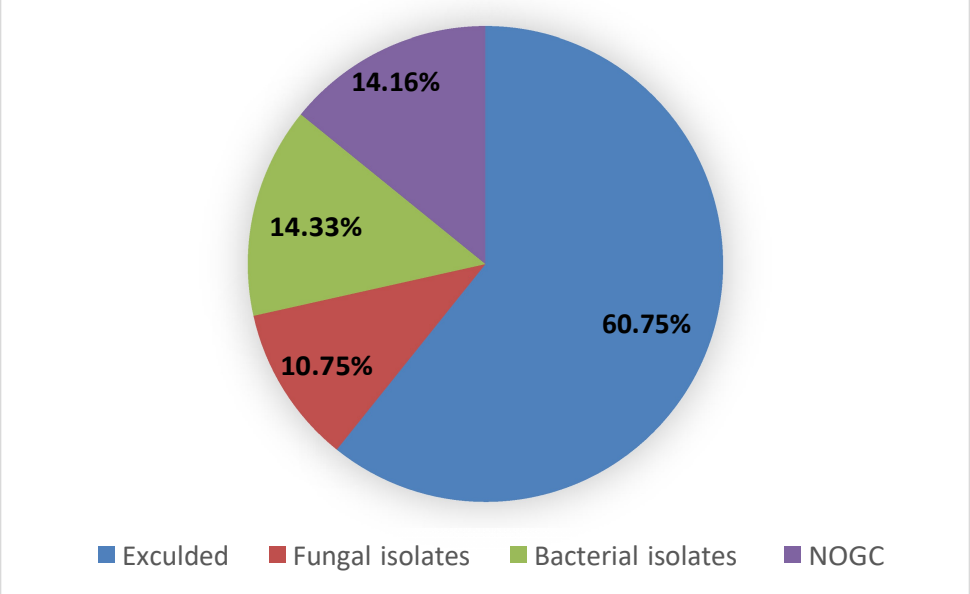


Table 12: Percentage of prevalence of sepsis related isolates in NICU

Excluded	60.75%
Fungal isolates	10.75%
Bacterial isolates	14.33%
NOGC	14.16%

The above pie graph and followed by table shows the total number of isolates in percentage. Neonatal candidemia accounts to 10.75% of total neonate admitted in NICU during study period, constituting to third most common blood stream infection

Graph

The percentage of *Candida* isolates (Total of 63 Fungal isolates)

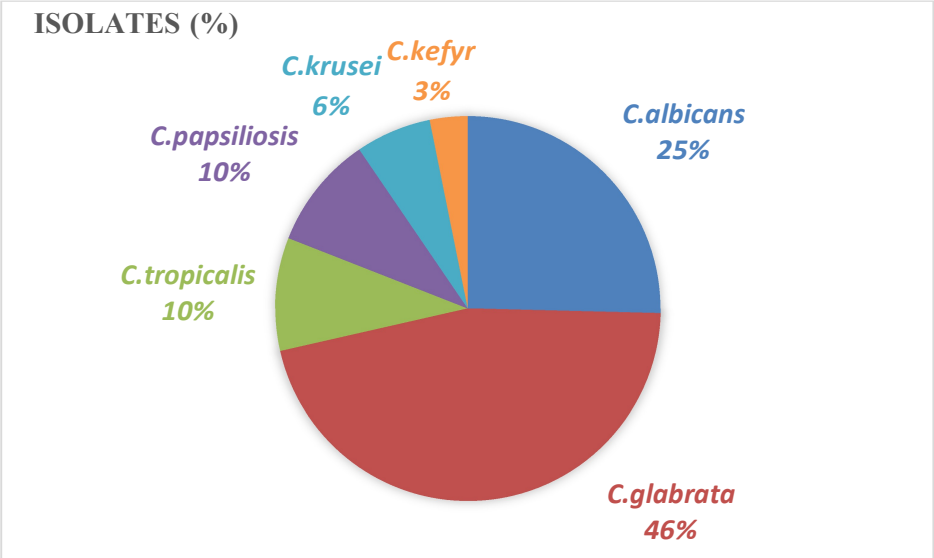


Table 13:

Total number and percentage of *Candida* isolates.

	ISOLATE	NO	(%)
ALBICANS (16/63)	*C.albicans*	16	25.40
NON-ALBICANS (47/63)	*C.glabrata*	29	46.03
	C.parapsiliosis	06	9.52
	C.tropicalis	06	9.52
	C.krusei	04	6.35
	C.kefyr	02	3.17
	TOTAL	**63**	**100**

The most common isolate in *Candida glabrata* (46.03%) followed by *Candida albicans* (25.40%). Ratio of Non-albicans to *Candida albicans* is 3:1

Graph

Gender wise distribution in percentage

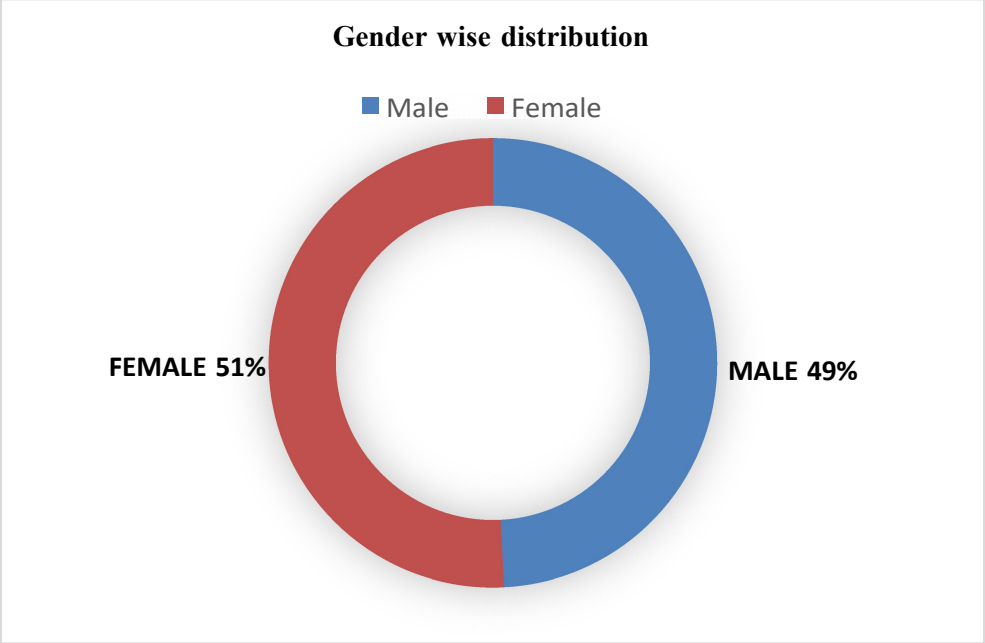


Table 14:

Distribution of fungal isolates gender wise (number and percentage)

GENDER	NUMBER	%
FEMALE	31	49.21
MALE	32	50.79
TOTAL	63	100.00

There were 31 male patients and 32 female patients. The total Male to Female ratio is approximately 1:1

Graph 5:

Mode of delivery -LSCS and NVD.

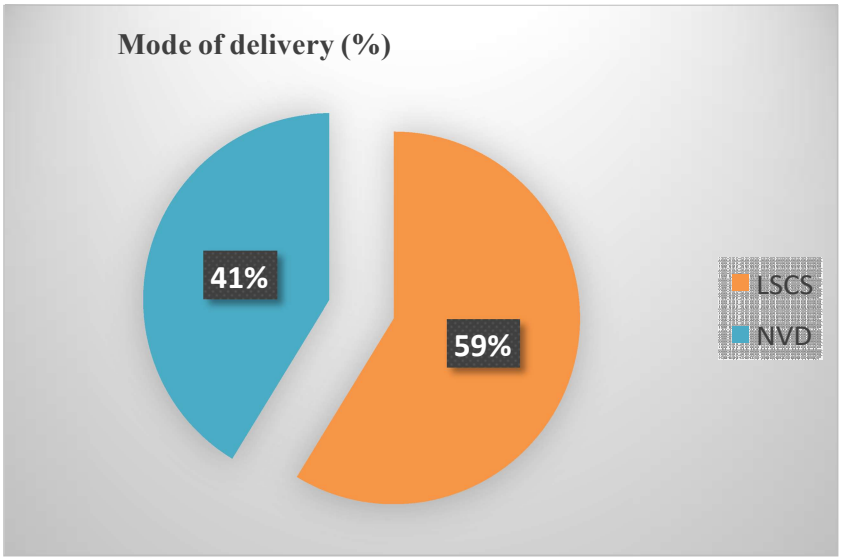


Table 15:

Distribution of fungal isolates w.r.t Gender and Mode of delivery.

GENDER	LSCS	NVD	TOTAL
MALE	19	14	33
FEMALE	18	12	30
TOTAL	37	26	63

Here in 37 (59%) neonates were born through LSCS and 26 (41%) through NVD. The ratio LSCS: NVD is 1.5: 1

Table 67:

Number of Fungal isolates with respective to gender

	FEMALE	MALE	TOTAL	p VALUE	INFERENCE
ALBICANS	5	11	16	0.0962	NS
NON ALBICANS	26	21	47		
TOTAL	**31**	32	63		

According the above table p value is 0.0962 indicates that candidemia is not significant with respect to gender

Graph 6:

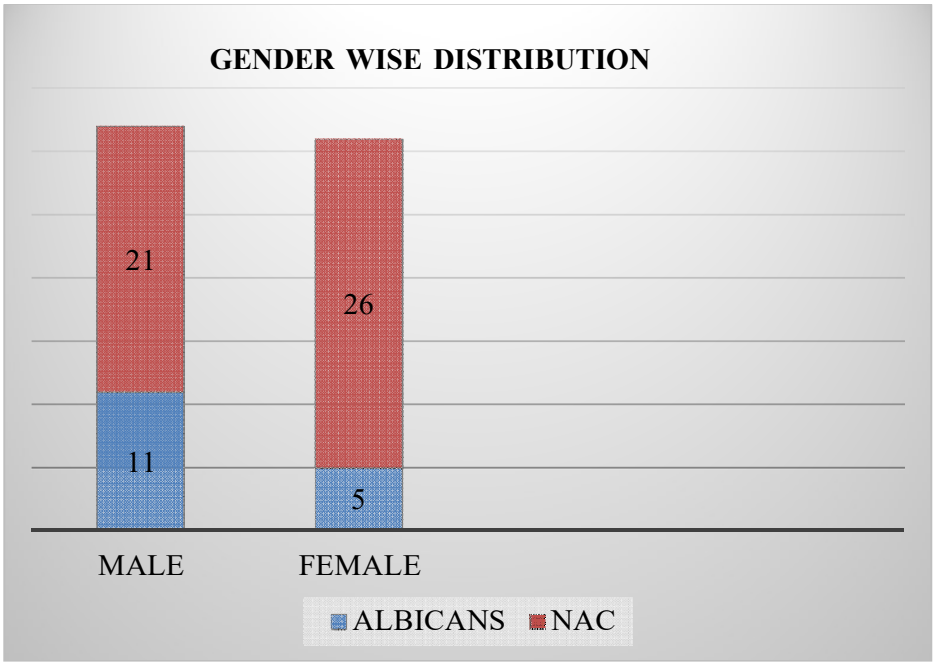


Table17:

Distribution of Fungal isolates with respective to Mode of delivery

	MODE OF DELIVERY				
	LSCS	**NVD**	**TOTAL**	**p VALUE**	**INFERENCE**
ALBICANS	7	9	16	0.2538	NS
NON ALBICANS	31	16	47		
TOTAL	38	25	63		

According the above table p value is 0.0962 indicates that candidemia is not significant with respect to mode of delivery.

Graph 7:

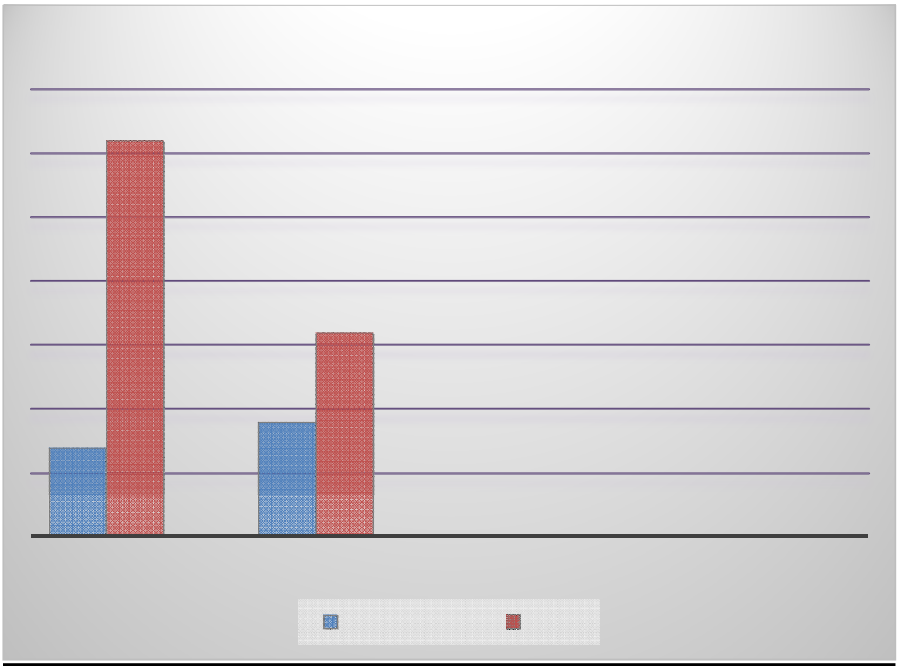


Table 18: Distribution of Fungal isolates with respect to Gestation age:

Gestation age	*C. albicans* (n=16)	Non-albicans *Candida* (n=47)		Total
Term	6	11	*C.glabrata = 5* *C.Krusei =2* *C.tropicalis=1* *C.parapsiliosis=1* *C.kefyr=1*	**17 (26.98%)**
Preterm	4	15	*C.glabrata =9* *C.Krusei =1* *C.tropicalis=3* *C.parapsiliosis=2*	**19 (30.15%)**
Very preterm	5	17	*C.glabrata =13* *C.Krusei =1* *C.parapsiliosis=2* *C.Kefyr =1*	**22 (34.92%)**
Extremely preterm	1	4	*C.glabrata = 2* *C.tropicalis=1* *C.parapsiliosis=1*	**5 (7.93%)**
Total	**16**	**47**		**63 (100%)**

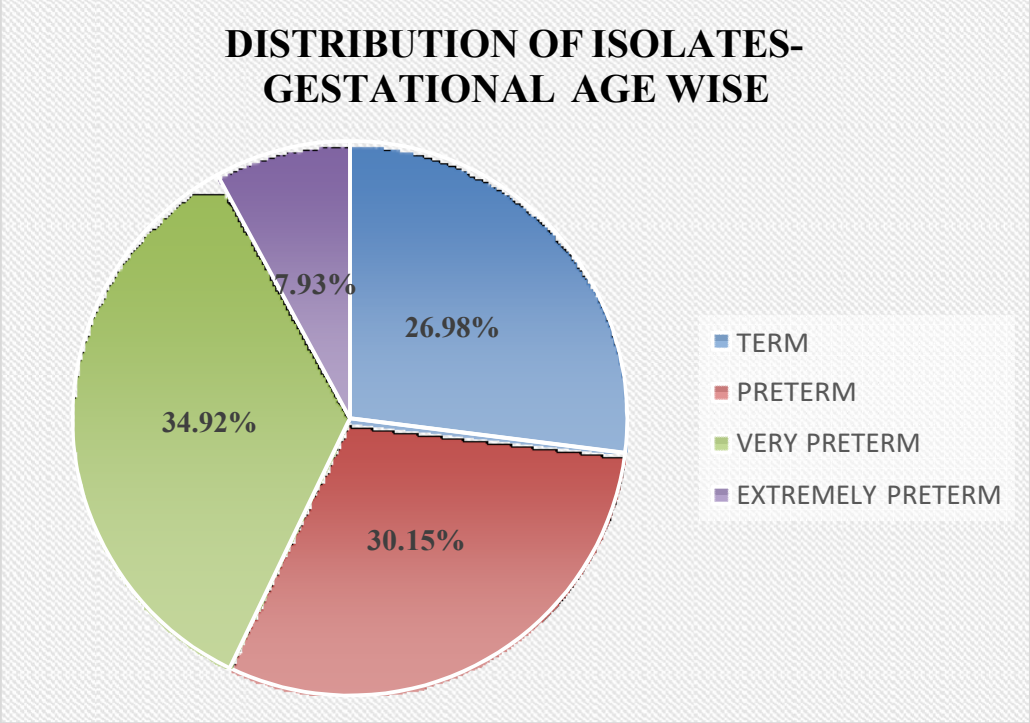
Thus, the above table shows more fungal isolates are associated with very preterm neonates, followed by preterm neonates.

Table 19: Distribution of Fungal isolates with respect to Birth weight:

Birth weight	*C. albicans* (n=16)	Non-albicans *Candida* (n=47)		Total
Normal weight	4	6	*C.glabrata=2* *C.parapsiliosis=2* *C.krusei=2*	**10 (15.87%)**
LBW	6	19	*C.glabrata=14* *C.tropicalis=3* *C.krusei=1* *C.kefyr=1*	**25 (39.68%)**
VLBW	6	19	*C.glabrata=11* *C.parapsiliosis=4* *C.tropicalis=2* *C.krusei=1* *C.kefyr=1*	**25 (39.68%)**
ELBW	--	3	*C.glabrata=2* *C.tropicalis=1*	**3 (4.76%)**
Total	**16**	**47**		**63 (100%)**

Thus, the above table shows more fungal isolates are associated with LBW and VLBW neonates

Graph 8:



Graph 9:

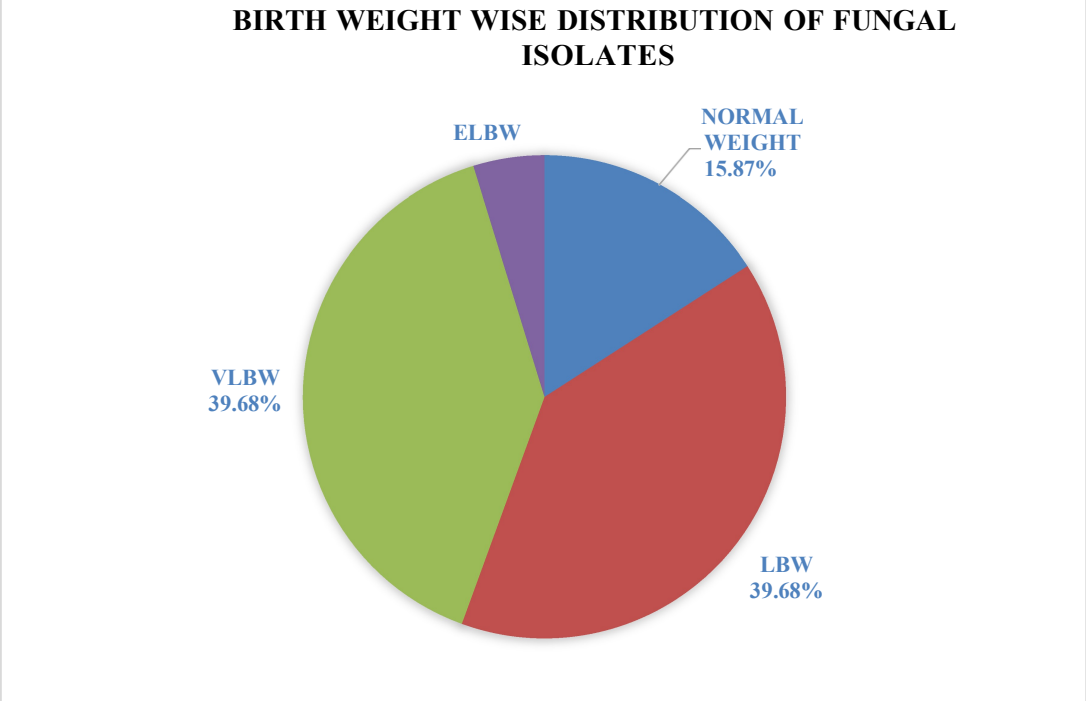


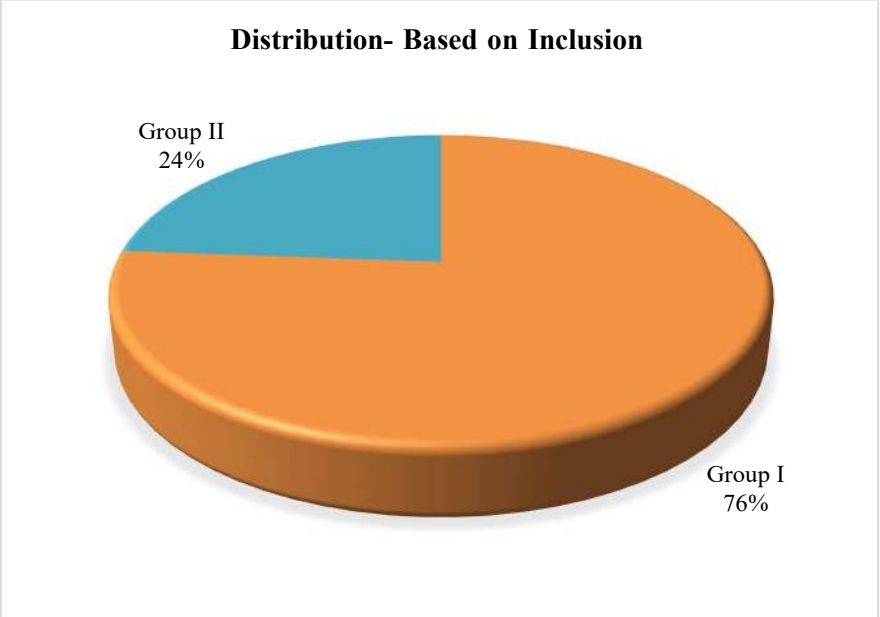
Table 72:

Number of Fugal isolates in 1st subculture

	1st SUBCULUTRE								
	C.albicans	*C.krusei*	*C. parapsilosis*	*C. tropicalis*	*C.glabrata*	*C.kefyr*	TOTAL	p VALUE	INFERENCE
ALBICANS	16	0	0	0	0	0	16		
NON ALBICANS	0	4	6	6	29	2	47	< 0.0001	HS
TOTAL	16	4	6	6	29	2	63		

According the above table p value is < 0.0001 indicates that candidemia is significant with respect to 1st sub culture, indicating that the fungal isolates correlate with day of inclusion and isolation

Graph 10: Distribution based on Day of Inclusion in the study



Group I represent the neonate included in the study from Day 3 (n=48) who were diagnosed with fungal sepsis in 1st subculture, Group II includes neonates who were diagnosed with fungal sepsis after few days of admission (n= 15) average day of inclusion being day 7

Table 21:

Susceptibility of fungal isolates to Fluconazole – By Kirby Bauer disc diffusion method.

	DISC DIFFUSION METHOD		
	FLUCONAZOLE		
	R	S	**TOTAL**
ALBICANS	6	10	16
NON ALBICANS	28	19	47
TOTAL	34	29	63

PERCENTAGE OF RESISTANCE = 53.97
PERCENTAGE OF SENSITIVITY = 46.03

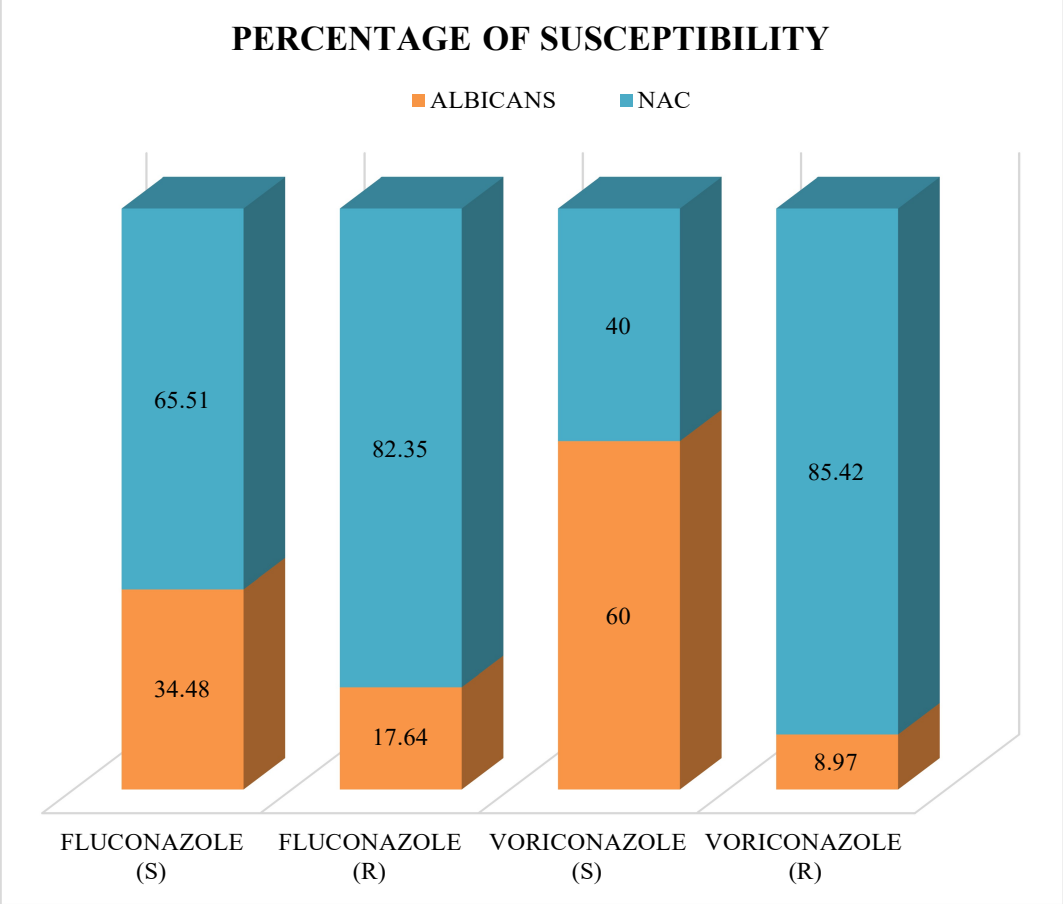
Table 22:

Susceptibility of Fungal isolates to Voriconazole – By Kirby Bauer disc diffusion method.

	DISC DIFFUSION METHOD		
	VORICONAZOLE		
	R	S	**TOTAL**
ALBICANS	7	9	16
NON ALBICANS	41	6	47
TOTAL	48	15	63

PERCENTAGE OF RESISTANCE = 76.19
PERCENTAGE OF SENSITIVITY = 23.80

Graph 11 : Distribution of isolates –disc diffusion method



Graph 12: Susceptibility pattern of both the drugs

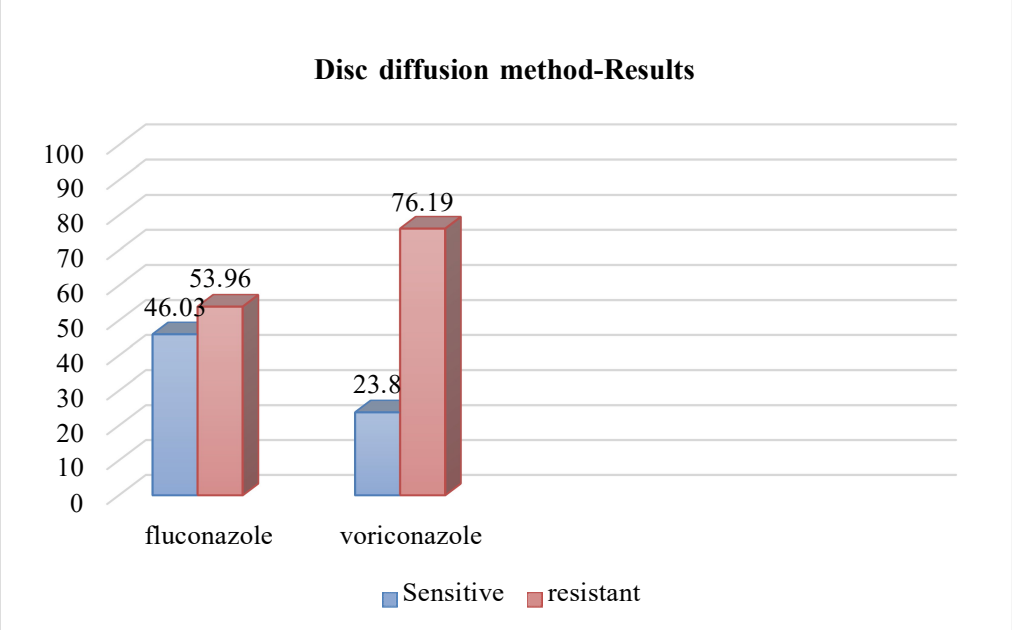


Table 23:

Fungal isolates susceptibility pattern to Fluconazole using MIC-E-strip method

	E TEST STRIP METHOD		
	FLUCONAZOLE		
	R	S	**TOTAL**
ALBICANS	5	11	16
NON ALBICANS	28	19	47
TOTAL	33	30	63

PERCENTAGE OF RESISTANCE = 52.38
PERCENTAGE OF SENSITIVITY = 47.61

Table 24:

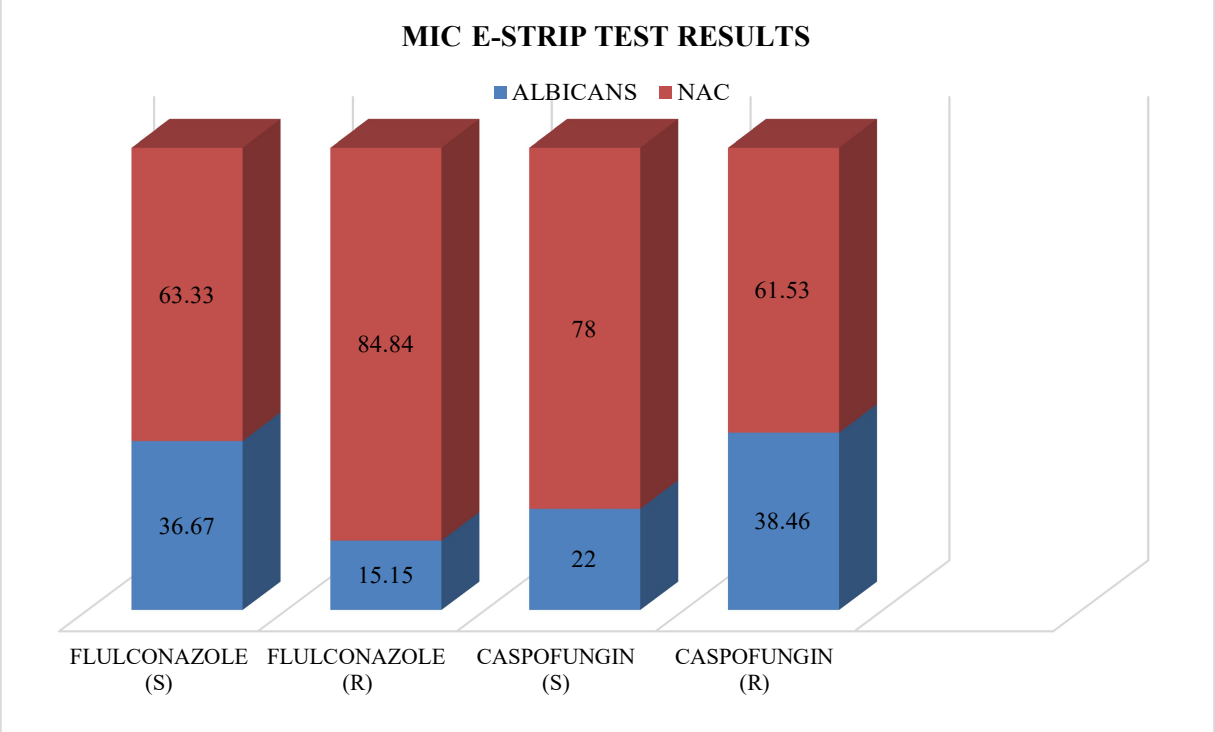
Fungal isolates susceptibility pattern to Caspofungin using MIC-E-strip method

	E TEST STRIP METHOD		
	CASPOFUNGIN		
	R	S	**TOTAL**
ALBICANS	5	11	16
NON ALBICANS	8	39	47
TOTAL	13	50	63

PERCENTAGE OF RESISTANCE = 20.63
PERCENTAGE OF SENSITIVITY = 79.36

Graph 13:

DISTRIBUTION OF ISOLATES –MIC E-STRIP TEST RESULTS



GRAPH 14:

PERCENTAGE OF ISOLATES –MIC E-STRIP TEST RESULT

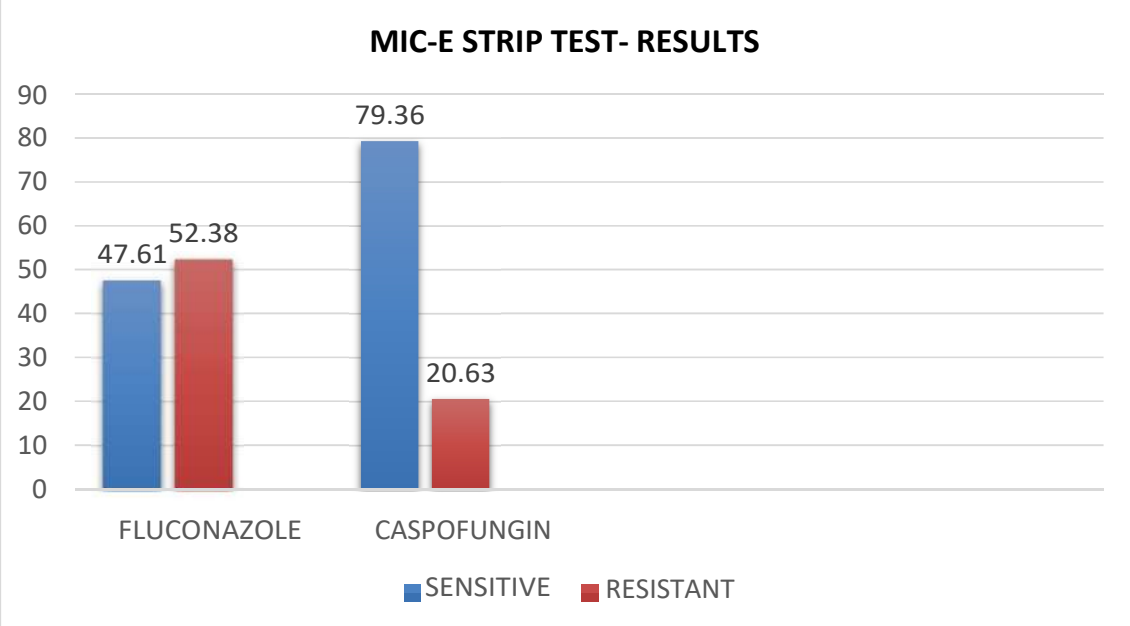


Table 77:

In the following table to find the agreement between disc diffusion method and e test strip method for fluconazole kappa statistic is calculated

	E TEST STRIP METHOD		
DISC DIFFUSION METHOD	**R**	S	**TOTAL**
R	33	1	34
S	0	29	29
TOTAL	33	30	63

p < 0.0001 (HS) **KAPPA = 0.9681**

THERE IS ALMOST PERFECT AGREEMENT BETWEEN THE TWO PROCEDURES

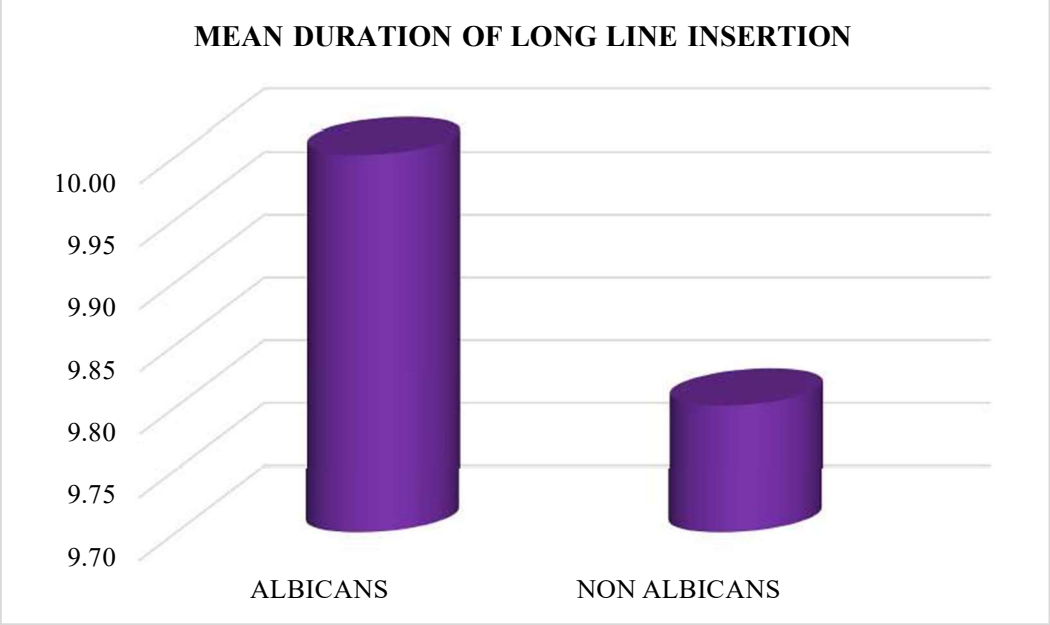
Table 78:

Fungal isolates in relation to duration of devices – Long line insertion

DURATION OF LONG LINE INSERTION								
ALBICANS				NON ALBICANS				
MEAN	S.D.	MIN	MAX	MEAN	S.D.	MIN	MAX	INFERENCE
10.00	6.48	0	20	9.80	3.98	0	20	NS

Graph 15:

Fungal isolates in relation to duration of devices – Long line insertion



The inference from the above graph is that *Candida albicans* are more associated with long line insertion than Non-albicans *Candida.*

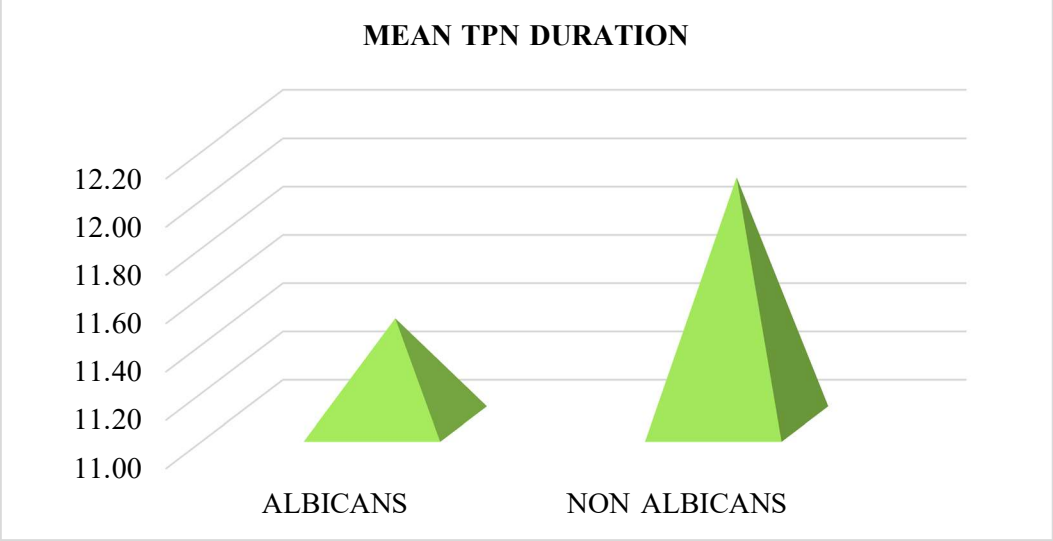
Table 79:

Fungal isolation in neonates with TPN duration

TPN DURATION							
ALBICANS				NON ALBICANS			
MEAN	S.D.	MIN	MAX	MEAN	S.D.	MIN	MAX
11.44	7.47	0	25	12.02	6.58	3	28

Graph 16:

Fungal isolation in neonates with TPN duration



The inference from the above graph is that Non-albicans *Candida* are more associated with increasing use of TPN (duration) than *Candida albicans.*

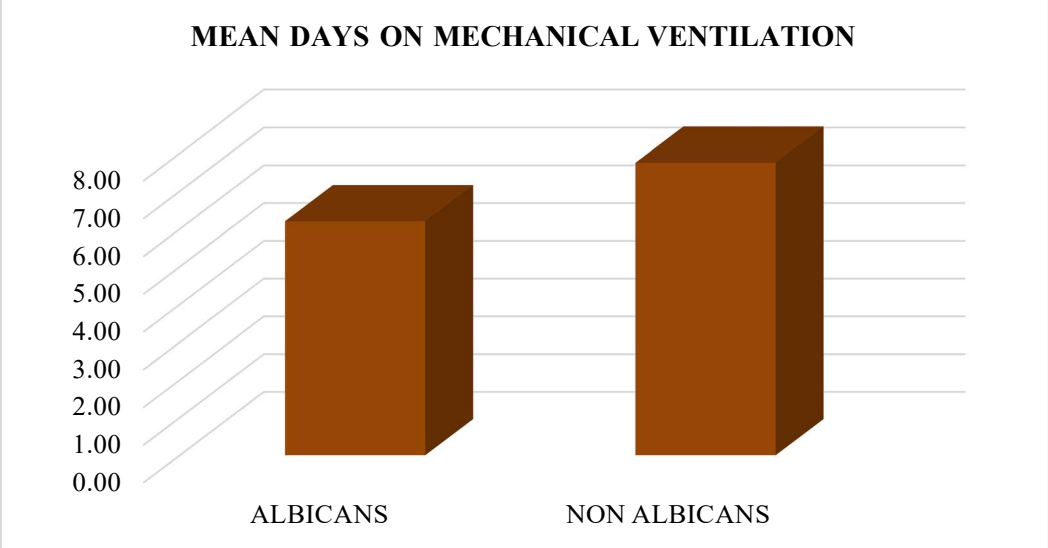
Table 80:

Fungal isolates in neonates with increasing days on mechanical ventilation.

DAYS ON MECHANICAL VENTILATION							
ALBICANS				NON ALBICANS			
MEAN	**S.D.**	**MIN**	**MAX**	**MEAN**	**S.D.**	**MIN**	**MAX**
6.20	5.17	2	15	7.75	6.85	1	25

Graph 17:

Fungal isolates in neonates with increasing days on mechanical ventilation.



The inference from the above graph is that Non-albicans *Candida* are more associated with mechanical ventilator use than *Candida albicans.*

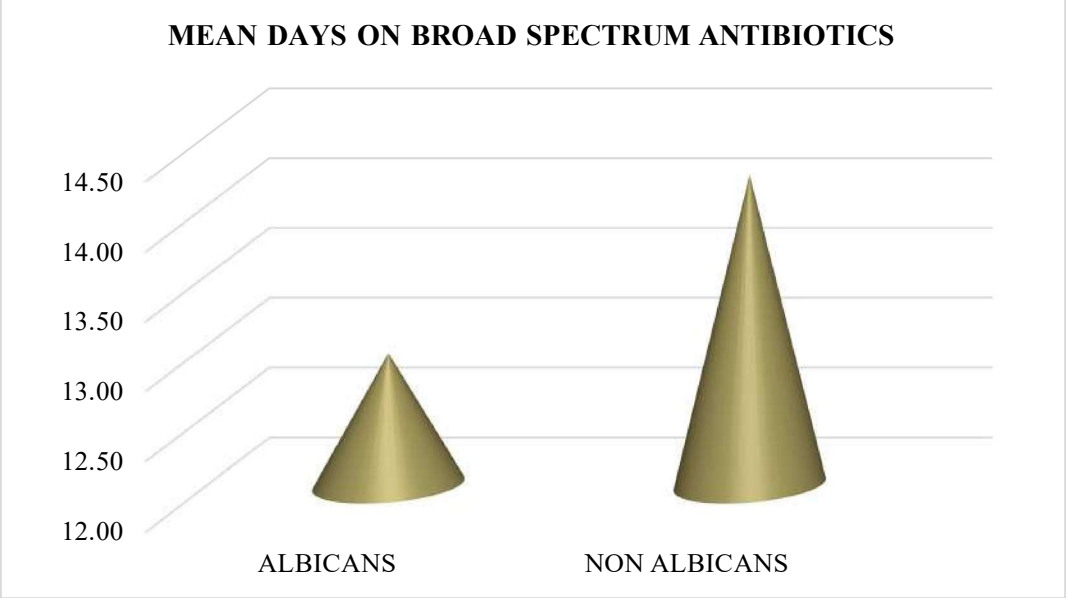
Table 81:

Number of Fungal isolates with increased usage of antibiotics

DAYS ON BROAD SPECTRUM ANTIBIOTICS							
ALBICANS				NON ALBICANS			
MEAN	S.D.	MIN	MAX	MEAN	S.D.	MIN	MAX
12.94	6.04	5	25	14.21	6.18	3	30

Graph 18:

Number of Fungal isolates with increased usage of antibiotics



The inference from the above graph is that Non-albicans *Candida* are more associated with increased use of antibiotics when compared to *Candida albicans*.

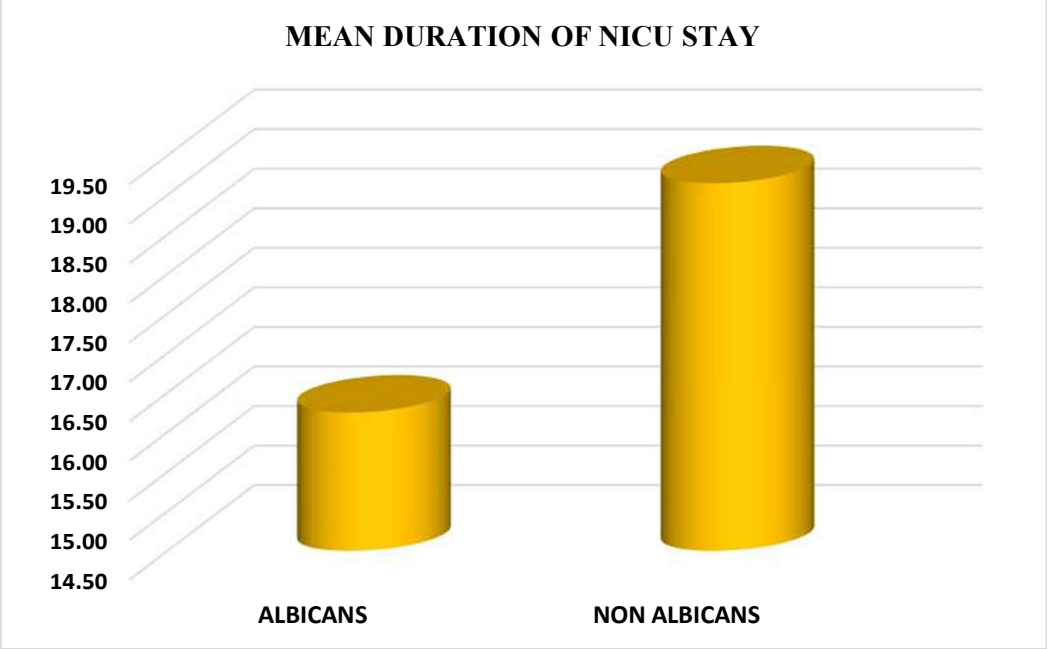
Table 82:

Number of fungal isolates with increasing duration of stay in hospital

DURATION OF NICU STAY							
ALBICANS				NON ALBICANS			
MEAN	S.D.	MIN	MAX	MEAN	S.D.	MIN	MAX
16.25	7.48	6	30	19.15	7.49	3	32

Graph 19:

Number of fungal isolates with increasing duration of stay in hospital



The inference from the above graph is that with increasing duration of stay in hospital (NICU) Non-albicans *Candida* are more associated than *Candida albicans*

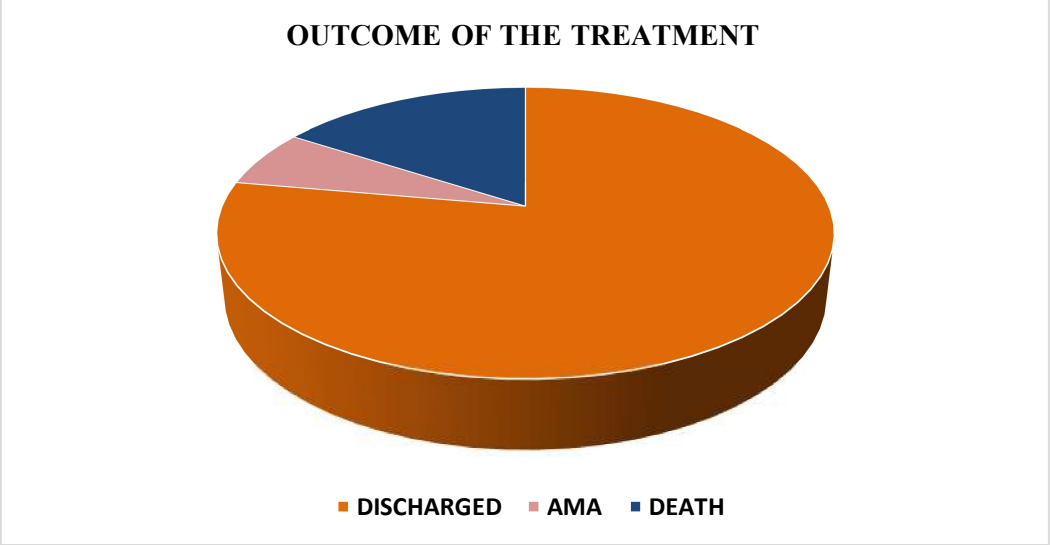
Table 83:

Morbidity and Mortality-outcomes number and percentage.

OUTCOME	NUMBER	%
DISCHARGED	49	77.78
AMA	4	6.35
DEATH	10	15.87
TOTAL	63	100.00

Graph 20:

OUTCOME chart



The above pie diagram represents the outcome in NICU

DISCUSSION

Invasive candidiasis in critically ill neonates is on rise, as it's becoming the third most common blood stream infection in ICU's especially NICU leading to increased morbidity and mortality. Added to this is the treatment is one of the biggest challenges in neonates. Epidemiological shift toward Non-albicans *Candida* is creating a menace globally. The real threat starts with the pathogens showing resistance to wards commonly used azole group of drugs in neonates such as Fluconazole and Voriconazole.

The current study is performed in the Department of Microbiology, J. N. Medical college for a period of 1 year, following standard mycological operating procedures conventionally and reported 63 Fungal isolates.

Clinically neonates who were admitted in NICU with signs and symptoms of late onset sepsis and high-risk neonates who are categorized under the inclusion criteria were taken into consideration in the study.

Our study reports 63 fungal isolates of 230 blood samples collected. Thus, we report a prevalence of 27.39 % of Candidemia in high-risk neonates.

In our present study 63 Fungal isolates were reported with age group from Day 3 to day 28 of birth in critically ill neonates. Male to female ratio is 1:1 and mode of delivery ration between LSCS and NVD being 1.5:1. Ratio of Non-albicans *Candida to Candida albicans* being 3:1 indicating the rise in Non-albicans *Candida* and is risk factors associated with the development of candidemia in high-risk neonates like prematurity, LBW, VLBW, prolonged stay in hospitals, neonates on

mechanical ventilation and external devices used. And rising resistance to Fluconazole used as empirical treatment is also recorded.

Our study and the data obtained correlated well with the studies conducted by *Uttam KG et al [(1)], Ananthaiah et al[(2)], Sundaram SC et al[(3)],Fu et al[(4)], Shettigar CG et al[(14)], Bhattacharjee P[(26)], Raminder sandhu et al[(27)], Juyal et al[(28)], Banarjee et al[(30)], Ballot DE et al[(31)], and Goel et al[(36)]* and many more .

The study done by *Uttam KG et al [(1)]*, was a two-year retrospective observational study conducted in Kolkata have showed in their study 79 Fungal isolates of them 77 (97.7%) isolates are Non albicans *Candida* species isolates only 2 (2.30%) wee *Candida albicans* ,amongst all NAC species *C. pelliculosa* was highest (43%) where as in our study total of 63 positive fungal isolates were recorded of 230 total blood samples collected and *Candida albicans* being 16 of 63 constituting to 25.39% and NAC species being (n=47) 74.60% and most common fungal isolate are *Candida glabrata* (46%) .

A similar study by *Anantaiah et.al [(2)]*., have also shown increased epidemiology in non-albicans species constituting to (n=64) 90.14% and *Candida albicans* are were less compared to NAC species that is (n=7) 9.85%. According to their study most predominant non-albicans species is *Candida krusei* is 77.46%.

Sundaram SC et al[(3)] study isolated 73 out of 212 positive blood stream infections of which 63 isolates (86.3%) were non-albicans *candida.*

Similarly, many studies have sown increasing number of fungal isolates and predominantly non-Albicans *Candida* species were recorded which exactly corelates with the local epidemiology of tertiary care center NICU and the data from out study.

Table 32:

Comparison of data of prevalence of fungal isolates and most predominant species.

Study conducted & year	Total Fungal isolates	C.albicans	NAC species	Predominant fungal isolate
Uttam KG et al (2022)	79	2 (2.30%)	77(97.70%)	*C.peliculosa*
Anataiah et al (2019)	71	7(9.85%)	64(90.14%)	*C.krusei*
Sundaram et al (2018)	73	10(13.69%)	63 (86.30%)	*Not mentioned*
Shettigar et al (2018)	54	19(35.18%)	35(64.81%)	*C.krusei*
Bhattacharjee P et al (2016)	70	34(48.57%)	36(51.42%)	*C.tropicalis*
Sandu et al (2015)	45	10(22.22%)	35 (77.77%)	*C.krusei*
Banaerjee et al(2014)	80	22(27.5%)	58(72.5%)	*C.glabrata*
Ballot et al (2013)	59	16(27.11%)	43(72.88%)	*C.parapsiliosis*
Juyal et al (2013)	132	27(20.45%)	105 (79.54%)	*C.parapsiliosis*
Our study	**63**	**16 (25.39%)**	**47 (74.60%)**	***C.glabrata***

As in the above table we can see there is rise in non-albicans *Candida* when compared to albicans infections, which corelates with the other studies and prevalence of NAC species is varies in studies from different geographical areas.

In our study male to female ratio is 1:1 and has no significance with fungal sepsis, when compared to *Uttam KG et al*[1] showed male to female ratio is 3:1, whereas *Anataiah et al*[2], *Sundaram et al*[3] also showed male to female ratio 1:1 and says that gender has no significance with fungal sepsis, indicating both the genders are at equal risk. Same is with the mode of delivery, our study and other studies conducted by *Anataiah et al*[2] , *Shettiger et al*[14] says that no significance for fungal sepsis with mode of delivery .

In NICU fungal sepsis is most encountered in high-risk neonates who less to gestational age and who are low in birth weight. In this context the above-mentioned studies along with our study recorded that fungal sepsis id more prevalent in premature low and very low birth weight babies.

Table 33:

Comparison of data of fungal sepsis in premature and preterm including LBW and VLBW neonates who are at high risk with our study to others studies which were conducted pan India and globally.

Risk factor	Pre term	LBW	VLBW
Uttam KG et al (2022)	66%	67%	25%
Anantaiah et al (2019)	71.6%	45.28%	31.37%
Fu et al (2018)	62%	--	58.3%
Shettigar et al (2018)	48.14%	12.96%	38.89%
Asish jain et al (2017	54.90%	33.33%	21.56%
Banaerjee et al (2014)	70%	64%	--
M.S.Hammoud et al (2013)	63%	16.7%	24.1%
Our study	**65%**	**39.68%**	**39.68%**

According the above statistics, invasive candidiasis in premature preterm infants from our study is 65% and low birth weight 39.68% and very low birth weight 39.68% which is in close agreement with *Uttam et al*[1] from Kolkatta west Bengal, *Anantaiah et al*[2] Dharward Karnataka, *Shettigar et al*[14] from Mangalore, *Fu et al*[4], China, M.S. *Hammoud et al*[34], Kuwait. Pre mature neonate who are less to their gestation age are at high risk when compared to term babies.

Apart from preterm LBW, VLBW the other risk factors as explained such as neonates on mechanical ventilators, on TPN, and on devices like central lines, UVC and UAC, long line tips, neonates admitted in NICU with increased duration of hospital stay and prolonged usage of antibiotics are at potentially higher risk for fungal sepsis or late onset sepsis.

Table 34:

Comparison of data with different risk factors

Risk factor (Variables)	Mechanical ventilation	TPN	Broad spectrum antibiotics	Devices used	Duration of stay in NICU(>14days)	Respiratory distress
Uttam KG et al (2022)	61%	76%	100%	--	--	36%
Anantaiah et al (2019)	13.2%	100%	100%	19%	83.01%	--
Fu et al (2018)	85.7%	92.9%	87.5%	59.6%	66.2&	--
Shettigar et al (2018)	53.70%	37.03%	59.26%	66.67%	77.78&	12.96%
Sundaram SC et al (2018)	--	50.68%	91.18%	--	58.9&	68.5%
Banaerjee et al(2014)	15%	--	70%	30%	--	--
M.S.Hammoud et al (2013)	90.7%	40.74%	74.1%	81.5%	--	--
Juyal et al (2013)	49.24%	55.30%	61.36%	64.39%	--	63.64%
Our study	**27%**	**98.4%**	**98.4%**	**77.78%**	**71.42%**	**85.71%**

Almost all the studies show similar association with the Risk factors as mentioned in neonates such as TPN, mechanical ventilation, prolonged usage of antibiotics, neonates on external devices like long line insertion, UAC/UVC and signs and symptoms of sepsis like respiratory distress.

Comparison of our study with the other studies and data with respect to susceptibility to azole group of drugs and echinocandins, basing on our tertiary care center antifungal prophylaxis, test was done for Fluconazole and Voriconazole by Kirby Bauer disc diffusion method and for Fluconazole and Caspofungin by E-strip test method. And all the studies focused on the increasing trends of NAC species followed by decreased susceptibility to empirically used drugs such as Fluconazole.

Our study showed of all isolates 53.97% resistance to fluconazole and 76.19 % resistance to Voriconazole by Disc diffusion method and resistance to Caspofungin is shown by 20.63% by E-strip method, which is in contrast with *Uttam et al*[1], *and Ananthaiah et al*[2], showing 0% resistance to voriconazole, 5% resistance to Caspofungin where as our study showed 79.36% sensitivity for Caspofungin. *Sandu et al* [27] showed 70% sensitive to Fluconazole and voriconazole, but 60% resistance showed by Non-albicans *Candida.*

In the other study done by *Shettigar et al*[14] in 2018 from Mangalore showed Fluconazole sensitivity 55.56%. Similar study from Uttarakhand conducted by *Juyal et al* [28] in 2013 showed 65.91% sensitivity to Fluconazole. From Varanasi in 2017 Basu et al [21] did a similar study in their tertiary care center which shows 100% sensitivity to Caspofungin and Voriconazole with increased resistance of Fluconazole

Jain et al [17] study done at Bundelkhand Medical College, Madhya Pradesh, India in the year 2017 showed that among all isolates of *Candida* species including albicans and non-Albicans 53.21% resistance to Fluconazole with shows a close agreement with our study which also showed 53.97% resistance to Fluconazole. Similarly, a study from Kolkata in 2016 done by *Bhattacharjee P et al* [26] showed increased resistance to Fluconazole in fungal isolates constituting to 61.11% which is in close agreement with our study.

Out of 33 Azole resistant *Candida* isolates of 20 were sensitive to Caspofungin and 10 isolates were resistant is recorded in our study, which infers that *Candida* species are slowly developing resistance to Echinocandins also.

Whaley et al [23] from Memphis, TN, USA had studied about the gene responsible for increased resistance shown by *Candida* isolates especially NAC species, also concluded that *C. glabrata* has highest incidence of azole resistance, which has an inherently property of less susceptibility to Azole drugs.

Another similar study done in India done by *Goel et al*[36], in 2009, PGIMS, Rohtak recorded that of total 67 isolates, antifungal susceptibility testing was done by two different methods i.e., disc diffusion method and broth micro dilution method in which they concluded saying small percentage of discrepancy was seen between the two methods used for detection of antifungal susceptibility. In contrast to this study our study has shown almost perfect agreement between the two procedures used i.e., Disc diffusion method and E-strip test method.

Al though there are many recent advances to decrease the morbidity and mortality of neonates there is increasing rates of fungal sepsis in neonates admitted to

NICU, and they are showing increased resistance to empirically used Azole drugs. Mortality rate in our study recorded is 10 deaths out of 586 admitted in NICU during the study period which is around 1.7%.

Table 35:

Comparison of data in regard with Morbidity and mortality

Study	Mortality rate (no. of death of total fungal positive neonates)
Uttam KG et al (2022)	32.91 % (26 0f 79)
Shettigar et al (2018)	31.48 % (17 of 54)
Sundaram SC et al (2018)	39.72 % (29 of 73)
Basu et Al (2017)	14.91 % (17 of 114)
Fu et al (2018)	14.3%
M.S.Hammoud et al (2013)	54%
D. E. Ballot et al (2013)	45.8%
Our study	**15.87% (10 of 63)**

Compared to others studies in mortality rate our study is nearly like *Basu et al* [21], from India & *Fu et al*[4], from China. Whereas others studies recorded very high mortality rate outcome compared to ours.

Tough above all studies used automation for rapid identification and to study antifungal susceptibility our study totally was relied on conventional standard mycological techniques for identification isolation and antifungal susceptibility reporting. Overall results and outcome are in very close similarity and agreement with the results of other studies who used automation.

CONCLUSION

- In this present study we are reporting the spectrum of *Candida* infection in high-risk neonates of NICU in our tertiary care hospital. We hereby report high burden of neonatal Candidemia, prevalence being 27.39% and predominantly due to non-albicans *Candida* species (74.60%) as well as an alarming increase resistance to azole group of drugs, Fluconazole resistance (53.97%) Voriconazole resistance (76.19%).
- And 79.36% sensitivity to Caspofungin.
- Prophylactic usage of Fluconazole as an antifungal drug is now questionable. Suggested for increased dose of fluconazole according to body weight.
- Echinocandins are the available group of drugs as life savior.
- There is no significant association of fungal isolates with respect to gender and mode of delivery indicates low or no risk for vertical transmission.
- Persistent Invasive Candidemia is most associated with prematurity, LBW and VLBW, TPN, prolonged stay in NICU, mechanical ventilation and usage of broad-spectrum antibiotics are the risk factors for fungal sepsis.
- Utmost care should be taken by all health care personnel (along with mother) to prevent horizontal transmission and cross contamination with the help of strict infection control protocols, appropriate hand hygiene, periodic environmental surveillance of air, water and NICU and change of indwelling devices as and when required can be suggested basing on our data analysis.

- Mother to child transmission while breast feeding in NICU should also be monitored to avoid horizontal transmission.

- Timely check on local epidemiology and antibiogram of NICU is needed for changing the protocol empirical or prophylactic antifungals along with antibiotics.

Strength of the study:

- Our data shows rise in Non-albicans *Candida* with respect to local epidemiology in our tertiary care hospital NICU.
- Increase resistance pattern of isolates was recorded.
- Tough conventional technique used timely reporting was done appropriately.
- Usage of Micro press aerobic Color cult bottles for sample collection for better isolation.
- For Fluconazole both Kirby Bauer Disc Diffusion method and E-strip method was used for susceptibility pattern
- Data statistically analyzed showed perfect agreement between both the methods implies that disc diffusion is still reliable and can be used instead of E-strip (in unaffordable conditions)
- For Caspofungin (Echinocandin) available was E-strip method was used for reporting.
- Depending on prevalence of local epidemiology of *Candida* species and their susceptibility pattern helped clinicians for changing the treatment as and when required.
- Environmental surveillance in both NICU and in Labour rooms was conducted and few protocols were amended in regard with disinfection and cleaning.

- Hand hygiene of the health care personnel was under continuous monitor and good practices were taught to them regularly.
- Efficacy of disinfectants were also done periodically.

Limitations of the study:

- Turnaround time was more when compared to automation
- Follow up with the patient was not done
- More study is needed for Echinocandins susceptibility
- Repeat blood sample was not done, discharge of the neonate was done based on the improvement symptomatically (clinical based).
- Sample size should be more to comment on significant p- values and to calculate odds ratio.
- All neonates were taken in consideration without segregation of inborn and out born neonates.
- Polymicrobial causes of death and along with other comorbidities, though less number were seen but was not included in our study.
- Multiple gestation, Birth asphyxia, Respiratory distress, Neonatal seizures in association of Candidemia were not commented.
- Tough many other parameters were noted still did not comment due to less sample size.

Future scope with this study:

- Automation like MALDI-TOF, VITEK-MS can be used to decreased turnaround time.
- Echinocandins susceptibility pattern should be under continues study, as MDR and XDR Candida are emerging, and we have restrictive antifungals to be used in neonates.
- Molecular diagnosis by PCR for detection of mutation in gene i.e., ERG 11 & FKS 1 (all Candida spp), FKS 2 (for Candida glabrata) which is responsible for resistance.
- Next generation sequencing can be done on isolates.

SUMMARY

The present study was conducted in the department of microbiology, J. N. Medical College, KAHER, Belagavi, from January 2021 to December 2021.

All the candida isolates from the from blood samples of high-risk neonates from NICU of Dr. Prabhakar Kore charitable hospital, KLE during the study period (January 2021 to December 2021) were included in the study.

- A total of **230 blood samples** were collected from high-risk neonates.
- **63 of 230** blood samples collected were Candida isolates constituting to **27.39%**.
- 63 isolates were processed according to the standard mycological laboratory procedures, conventionally. Isolation, identification, and antifungal susceptibility was done using both methods Kirby Bauer disc diffusion method for Fluconazole and voriconazole and MIC E-strip test method for Fluconazole and Caspofungin according to CLSI guide lines M44 and M60 respectively and reported.
- Out of 63 Candida isolates, Candida albicans (n=16) were 25.33% and **Non-albicans Candida (n=47) were 74.60%**
- The most prevalent Non-albicans Candida species in NICU is **Candida glabrata** (n=29) constituting to **46.03%** followed by *Candida parapsioliolsis (n=6),9.52%, Candida tropicalis (n=6),9.52%, next to them are Candida krusei and Candida keyfr* (n=4, n=2) 6.35%, 3.17% respectively.

- Kirby-Bauer's Disc diffusion results were 46.03% isolates were sensitive to fluconazole and 23.80% were sensitive to Voriconazole. 53.97% of isolates were resistant to Fluconazole and 76.19% were resistant to Voriconazole
- MIC E-strip test results were 52.38% isolates were resistant to Fluconazole.
- **79.36% isolates were sensitive to Caspofungin.**
- **Out of 33 Azole resistant *Candida* isolates of 20 were sensitive to Caspofungin and 10 isolates were resistant**
- The susceptibility of isolates to Fluconazole was compared between two methods (Disc diffusion and MIC E-strip test method) There is almost perfect agreement between the two procedures.
- There is no significant association of fungal isolates with respect to gender and mode of delivery.
- Hence, **Prevalence** of *Candida* infection in Blood stream infection in High-risk neonates of NICU is **10.75%**
- Mortality rate of neonates due to fungal sepsis is 1.7% (10 out of 586 admitted in NICU during study period).

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ANNEXURE I- PROFORMA

NAME:

IP.NO:

GENDER

MODE OF DELIVERY

ADRESS:

DATE OF ADMISSION:

DATE OF SAMPLE COLLECTION:

TIME OF SAMPLE COLLECTION:

NATTURE OF SAMPLE:

DIAGNOSIS:

Presenting complaints:

H/O any investigations:

If YES -specify (Type of investigation)

Antibiotic usage:

If YES, Duration

Current antifungal treatment:

Type of course and duration:

Any associated illness:

LAB DIAGNOSIS:

- GRAM STAINING
- CULTURE ON:

MEDIA USED	**INFERENCE**	
1. CHOCOLATE AGAR		
2. SABORAUDS DEXTROSE AGAR		
3. CORN MEAL AGAR		
4. CHROM AGAR		
5. SDA- ANTIFUNGAL SUSCEPTIBILITY TESTING (KIRBY-BAUER DISC DIFFUSION METHOD	FLUCONAZOLE	
	VORICONAZOLE	
6. SDA- ANTIFUNGAL SUSCEPTIBILITY TESTING (E-STRIP TEST METHOD)	FLUCONAZOLE	
	CASPOFUNGIN	

ANNEXURE II- CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

TITLE:" Prevalence of candidemia in high risk neonates of neonatal intensive care unit- A one year cross sectional study.

PURPOSE OF THE STUDY:
To isolate and identify the candida species in blood stream of high-risk neonates admitted in NICU during the study period and to test the antifungal susceptibility of the isolated organisms.

PROCEDURE:
You are requested to participate in this study which will provide appropriate and effective treatment. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge. The principal investigator of this study is Dr. ANUGULA. AMRITHA under the guidance of Dr. If you agree to enrol yourself in the study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

RISKS AND BENEFITS:
There are no risks involved and benefits to know about the causative organism and antifungal susceptibility of the same, so that appropriate treatment can be given.

ALTERNATIVES:
Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with Jawaharlal Nehru medical college. If you decide to participate you are free to with draw at any time.

PRIVACY AND CONFIDENTIALITY: All the information collected during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but you or the information provided by you during research will remain confidential. No information about you or information provided by you during this research will bed is closed to others without your written permission except:
1. In an emergency to protect your rights and welfare.
2. If required by law.

FINANCIAL INCENTIVES FOR PARTICIPATION:
You will not be paid/ offered any gifts/ incentives for participating in the study. You will not be reimbursed for expenses.

AUTHORIZATION TO PUBLISH RESULTS: When the results of research are published or discussed in a conference, no information will be displaced that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Question:
In case you have any questions related to the study, you can contact

1.
2.
3. In case you have any questions about your rights as a participant, you can contact Dr. Harsha Hegde, Chairperson, JNMC, IEC & Scientist D, ICMR, National Institute of Traditional Medicine, Ph No-9480422500.

S.no	IP.no	Name	Gender	lab no.	Day of inclusion	Gestational age	Mode of delivery	APGAR at 1min	APGAR at 5min	Birth weight	Respiratory distress	Apnoea	Birth asphyxia	Neonatal seizures	shock	Thrombocytopenia	Meningitis	DIC	Duration of Long line insertion	TPN duration	Non invasive Oxygen support	Days on mechanical ventilation	Days on Broad spectrum antibiotics	Transfusions	1st subculutre	2nd subculutre	Long line tip culture	ET tip culture	UAC/UVC tip culture	Duration of NICU stay	DISC DIFFUSION METHOD		E TEST STRIP METHOD		Fungal Isolate	Outcome	Cause of death
																															Fluconazole	Voriconazole	Fluconazole	Caspofungin			
1	1034467	b/o Uma manjunath	M	39056	3	Term	LSCS	7	9	Normal	√	0	0	0	√	√	0	√	10	15		10	15	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	15	R	R	R	R	C.glabrata	AMA	
2	1035093	b/o Devakka bharatesh	F	40843	3	Term	NVD	7	9	Normal	√	0	0	0	0	0	0	0		6			5	nil	C.albicans	C.albicans	NOGC	NOGC	NOGC	8	S	S	S	S	Candida albicans	Discharged	
3	1034219	b/o Ashwini sanjeev	F	51829	3	Term	LSCS	7	9	VLBW	√	0	0	0	0	0	0	0		5			5	nil	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	5	R	R	R	S	C.glabrata	Discharged	
4	1037083	b/o Meenaz twin -1	M	52293	3	Very preterm	LSCS	7		VLBW	√	√	0	0		√	0	0	15	20			15	RDP,PCV	C.glabrata	C.glabrata	C.glabrata	NOGC	NOGC	32	R	R	R	S	C.glabrata	Discharged	
5	1040330	b/o Pratiksha	M	54307	7	Preterm	LSCS	7	9	LBW		0	0	0	0	0	0	0		10			15	nil	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	22	S	R	S	S	C.glabrata	Disharged	
6	1041263	b/o Deepali	M	37667	7	Term	NVD	7	9	LBW		0	0	0	0	√	0	√	14	14			21	RDP,SDP,PCV,FFP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	28	R	R	R	S	C.glabrata	Discharged	
7	1041642	b/o Ruksar	M	37862	8	preterm	LSCS	7	8	Extremely LBW	√	√	0	0	√	√	√	√	20	25		25	25	RDP,PCV,FFP	C.tropicalis	C.tropicalis	C.tropicalis	C.glabrata	NOGC	25	S	R	S	S	C.tropicalis	AMA	
8	1046736	b/o Shanta patil	M	48084	1	Preterm	LSCS	7	9	LBW	√	0	0	0	0	√	0	0	10	15	15		21	RDP,PCV	C. krusei	NOGC	us	NOGC	NOGC	28	S	S	S	S	C. krusei	Discharged	
9	1049651	b/o Smita	F	42763	3	Very preterm	LSCS	7	9	LBW	√	√	0	0	0	0	0	0	14	5	3		10	nil	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	15	R	R	R	S	C.glabrata	Discharged	
10	1051240	b/o Sukanya	F	41017	7	Term	NVD	7	9	LBW	√	0	0	0	0	√	0	0	12	15	10		15	RDP	C. albicans	C. albicans	NOGC	NOGC	NOGC	15	S	R	S	S	C. albicans	Discharged	
11	1051622	b/o Zulekha	M	41027	3	Preterm	LSCS	7	9	Normal	√	0	0	0	0	√	0	0	0	7	6		10	RDP	C. albicans	C. albicans	NOGC	NOGC	NOGC	18	R	S	R	S	C. albicans	Discharged	
12	1051621	b/o Tasbiya	M	41029	3	Very preterm	NVD	3	7	VLBW	√	√	√	0	0	√	0	0	10	10	5		10	RDP	c.albicans	c.albicans	NOGC	NOGC	NOGC	22	S	S	S	S	C. albicans	Discharged	
13	1051283	b/o vidyashree	M	41125	7	Very preterm	LSCS	7	9	VLBW	0	√	0	0	0	√	0	0	20	25			20	RDP,PCV	C.albicans	C. albicans	C. albicans	NOGC	NOGC	30	R	R	R	R	C. albicans	Discharged	
14	1052567	b/o Akshata twin-1	F	43580		Very preterm	LSCS	8		VLBW	√	0	0	0	0	√	0	0	10	17	2		14	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	22	R	R	R	S	C.glabrata	Discharged	
15	1052675	b/o Lakshmi mallesh twin-1	M	41673	7	Very preterm	NVD	5	7	VLBW	√	√	0	0	√	√	0	0	14	15	15		20	RDP,PCV	C. albicans	C. albicans	MRSA	NOGC	NOGC	22	R	R	R	R	C. albicans	Discharged	
16	1051368	b/o Renuka milind twin-1	F	40999	3	Preterm	LSCS	6	9	LBW	√	√	0	√	√	√	√	√	20	22	20	2	20	RDP,SDP,PCV,FFP	C.albicans	C. albicans	NOGC	NOGC	NOGC	28	R	R	R	R	C. albicans	Discharged	
17	1055496	b/o Veena .k	M	43428	3	Term	LSCS	5	7	Normal	√	√	0	√	√	√	0	√	7	15	15	5	15	RDP,PCV,FFP	C.albicans	C. albicans	ccus	NOGC	NOGC	10	S	S	S	S	C. albicans	Death	Fungal sepsis
18	1054181	b/o Deepa basavalingayya	F	42052	5	Term	NVD	7	9	LBW	√	0	0	0	0	√	0	0	14	12	10		19	RDP	C.kefyr	C.kefyr	NOGC	NOGC	NOGC	20	S	S	S	S	C.kefyr	Discharged	
19	1054177	b/o Gufran	M	36914	3	Very preterm	NVD	7	8	VLBW	√	√	0	0	0	√	√	0	0	10	8		14	RDP,PCV	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	15	R	R	R	S	C.glabrata	Discharged	
20	1053401	b/o Pooja nivruti	M	53743	3	Term	NVD	5	6	Normal	√	0	√	√	0	√	0	√	14	15	18		12	RDP,PCV,FFP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	24	R	R	R	S	C.glabrata	Discharged	
21	1055837	b/o Radha	M	53647	3	Very preterm	NVD	6	8	VLBW	√	√	√	√	√	√	√	√	6	6	6	6	6	RDP,FFP	C. albicans	C. albicans	NOGC	NOGC	NOGC	6	S	S	S	S	C. albicans	Death	Fungal sepsis
22	1055136	b/o Lakshmi narayan	M	45124	7	Very preterm	LSCS	2	6	VLBW	√	√	0	√	0	√	0	0	12	25	25	15	25	RDP	C. albicans	C. albicans	C. albicans	Citrobacte	NOGC	25	R	R	R	R	C. albicans	Discharged	
23	1056312	b/o Mahadevi twin-1	F	43983	4	Very preterm	LSCS	6	9	LBW	√	√	0	√	0	√	√	0	10	10	9		21	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	30	R	R	R	R	C.glabrata	Discharged	
24	1056313	b/o Mahadevi twin-2	F	43985	4	Preterm	LSCS	6	9	LBW	0	0	0	0	0	√	0	0		3			12	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	25	R	R	R	S	C.glabrata	Discharged	
25	1056599	b/0 Manisha	M	52035	3	Term	LSCS	6	7	Normal	√	0	0	0	0	√	0	0		7	7	7	7	RDP	C. parapsilosis	C. parapsilosis	NOGC	NOGC	NOGC	7	S	R	S	S	C. parapsilosis	Discharged	
26	1056458	b/0 Ganga twin-2	M	51797	9	Very preterm	LSCS	7	9	VLBW	√	√	√	0	0	√	0	0	14	5	3		10	RDP	C. parapsilosis	C. parapsilosis	parapsilosis	NOGC	NOGC	28	S	R	S	S	C. parapsilosis	Discharged	
27	1057060	b/o Sushma	M	47317	3	Very preterm	LSCS	6	7	VLBW	√	√	0	√	√	√	0	√	3	3	3	3	3	RDP,PCV.FFP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	3	S	R	S	S	C.glabrata	Death	Fungal sepsis
28	1057874	b/o Rajashree	F	44493	3	preterm	LSCS	6	7	VLBW	√	1	√	√	√	√	0	√	7	7	7	4	7	RDP,FFP	C.glabrata	C.glabrata	NOGC	C.glabrata	NOGC	7	S	R	S	S	C.glabrata	Death	Fungal sepsis
29	1057706	b/o Veena..M	M	43578	3	Term	NVD	2	6	Normal	√	√	0	√	√	√	0	√		7	7	3	7	RDP,PCV,FFP	C.albicans	C. albicans	NOGC	cocus.aur	NOGC	7	S	S	S	S	C. albicans	Death	Fungal sepsis
30	1060565	b/o shilpa twin-1	F	42054	3	Preterm	NVD	2	6	LBW	√	√	0	0	√	√	0	√	12	24	24	12	24	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	24	R	R	R	R	C.glabrata	Death	Fungal sepsis
31	1060566	b/o shilpa twin-2	F	42056	5	Preterm	NVD	7	6	LBW	√	√	0	0	0	√	√	√	7	10	7		10	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	20	R	R	R	S	C.glabrata	Discharged	
32	1058441	b/o sangeeta	M	54309	3	Preterm	NVD	6	7	VLBW	√	1	0	√	0	√	√	0	9	5	5		12	RDP,PCV	C. tropicalis	C. tropicalis	NOGC	NOGC	NOGC	15	S	S	S	S	C. tropicalis	Discharged	
33	1059034	b/o shobha	M	54383	3	Term	LSCS	7	8	LBW	√	0	0	√	0	√	√	0	12	0			15	RDP	C. albicans	C. albicans	NOGC	NOGC	NOGC	15	S	R	S	R	C. albicans	Discharged	
34	1058995	b/o Kaveri twin-2	F	47101	2	Very preterm	LSCS	6	8	VLBW	√	√	0	0	0	√	0	0	5	7	5		10	RDP	c.glabrata	c.glabrata	NOGC	NOGC	NOGC	12	R	R	R	S	C.glabrata	Discharged	
35	1065726	b/o laxmi umesh	M	42273	3	Term	NVD	5	7	LBW	√	0	√	√	√	√	0	√	12	15	12		17	RDP,PCV	C. tropicalis	C. tropicalis	NOGC	NOGC	NOGC	20	S	R	S	S	C. tropicalis	Discharged	
36	1065523	b/o shaila triplet -3	M	45780	3	Very preterm	LSCS	6	7	VLBW	√	√	0	0	√	√	0	0	7	12	5		13	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	28	R	R	R	S	C.glabrata	Discharged	
37	1064795	b/o preetika	F	46215	3	Preterm	LSCS	7	9	VLBW	0	√	0	0	0	√	0	0	7	12	5		12	RDP,PCV	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	28	R	R	R	R	C.glabrata	Discharged	
38	1074653	b/o Sujata	M	51019	3	Preterm	LSCS	7	8	LBW	√	√	0	0	0	√	0	0		7	3		7	RDP	C.glabrata	c.glabrata	NOGC	NOGC	NOGC	15	R	R	R	S	C.glabrata	Discharged	
39	1076213	b/o Pushpa twin-2	M	51583	3	Preterm	LSCS	7	9	LBW	0	√	0	0	√	√	0	√	10	20	7		18	RDP,PCV,FFP	C.glabrata	C.glabrata	C.glabrata	NOGC	NOGC	23	R	R	R	S	C.glabrata	Discharged	
40	1111691	b/o Radhika .H twin-1	F	55125	3	preterm	LSCS	2	5	Extremely LBW	√	√	√	0	√	√	0	√	7	28	28	15	28	RDP,PCV,FFP	C.glabrata	C.glabrata	NOGC	Klebsiella	NOGC	28	R	R	R	R	C.glabrata	Death	Fungal sepsis
41	1111696	b/o Renuka. A	F	48473	3	preterm	NVD	7	8	VLBW	√	√	0	0	0	√	0	√	0	5	5		10	RDP	C.albicans	C.albicans	NOGC	NOGC	NOGC	18	S	S	S	S	C. albicans	Discharged	
42	1098427	b/o Mayuri. T	F	47677	7	Term	LSCS	7	8	LBW	√	√	0	0	0	√	0	0	11	12	7		11	RDP, PCV	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	22	S	R	S	S	C.glabrata	Discharged	
43	1089067	b/o Deepa. G	F	47672	3	Preterm	NVD	7	8	LBW	√	0	0	0	0	√	0	0	5	5	5		11	RDP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	12	R	R	R	R	C.glabrata	Discharged	
44	1119959	b/o Mahemuda. A	F	50510	2	Very preterm	NVD	7	8	VLBW	√	√	0	0	√	√	0	√	9	28	30		30	RDP,PCV,FFP	C.krusei	C. krusei,	C. krusei	NOGC	NOGC	30	R	R	R	R	C. krusei	AMA	
45	1112921	b/o Vijayalakshmi. M	F	53411	5	Very preterm	LSCS	7	7	VLBW	√	√	0	0	√	√	0	0	10	10	7		12	RDP,PCV	C. parapsilosis	C. parapsilosis	NOGC	NOGC	NOGC	18	R	R	R	S	C. parapsilosis	Discharged	
46	1074340	b/o Sarika. K	F	51021	3	Preterm	LSCS	7	8	VLBW	√	√	0	0	0	√	0	0	5	10	9		12	RDP	C. tropicalis	C. tropicalis	NOGC	NOGC	NOGC	15	S	R	S	S	C. tropicalis	Discharged	
47	1074310	b/o Ratna. Y twin-1	M	44131	3	Very preterm	NVD	7	8	Extremely LBW	√	√	0	√	√	√	0	√	13	17	17	3	17	RDP,PCV,FFP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	17	R	R	R	S	C.glabrata	AMA	
48	1091246	b/o Dinar. S	F	43973	7	Very preterm	VD	6	7	VLBW	√	√	0	√	0	√	0	0	15	10	7		15	RDP,PCV	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	25	R	R	R	S	C.glabrata	Discharged	
49	1062340	b/o Shilpa. W twin-1	F	51129	3	Preterm	LSCS	3	7	VLBW	√	√	0	√	√	√	0	√	8	12	12	5	12	RDP,PCV,FFP	C. parapsilosis	C. parapsilosis	ccus	NOGC	NOGC	12	R	R	R	R	C. parapsilosis	Death	Fungal sepsis
50	1074324	b/o Shobha. K	M	36746	3	Preterm	VD	7	8	LBW	√	0	0	0	0	√	0	0		10	5		13	RDP,PCV	.C.albicans	C. albicans	NOGC	NOGC	NOGC	12	S	S	S	S	C. albicans	Discharged	
51	1091229	b/o Renuka. P	M	53229	2	preterm	VD	6	7	VLBW	√	√	0	0	√	√	0	0	7	7	2		7	RDP	C. parapsilosis	NOGC	NOGC	NOGC	NOGC	10	S	R	S	S	C. parapsilosis	Discharged	
52	1092346	b/o Pooja. M twin-2	F	48039	2	Very preterm	LSCS	6	9	LBW	√	√	0	0	√	√	0	√	7	27	27	5	27	RDP,PCV,FFP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	27	R	R	R	S	C.glabrata	Death	Fungal sepsis
53	1091236	b/o Rjeswari. K	M	51149	2	Preterm	LSCS	7	7	LBW	0	√	√	0	√	√	0	√	10	10	4	1	10	RDP,PCV,FFP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	10	R	R	R	S	C.glabrata	Death	Fungal sepsis
54	1056620	b/o Shilpa. W	F	36914	3	Preterm	LSCS	7	8	LBW	0	√	0	0	0	√	0	0	12	7	0		12	RDP	C. tropicalis	C.tropicalis	NOGC	NOGC	NOGC	14	S	R	S	S	C. tropicalis	Discharged	
55	1065030	b/o Rukmini. H	F	46065	3	Preterm	VD	7	8	LBW	√	0	0	0	0	√	0	0		5	3		5	RDP, PCV	C. albicans	C. albicans	NOGC	NOGC	NOGC	10	S	S	S	S	C. albicans	Discharged	
56	1062071	b/o Saraswati. G	F	46475	7	Term	VD	6	8	LBW	√	0	0	0	0	0	0	0	6	15	15		23	RDP, PCV	C. tropicalis	C. tropicalis	ter	NOGC	NOGC	25	S	S	S	S	C. tropicalis	Discharged	
57	1063308	b/o Nirmala . M	M	46895	8	Very preterm	VD	7	8	VLBW	0	√	0	0	0	√	0	0	17	15	0		15	RDP, PCV	C.glabrata	C.glabrata	C.glabrata	NOGC	NOGC	22	R	R	R	S	C.glabrata	Discharged	
58	1054925	b/o Roshini. P	M	42133	3	Term	LSCS	7	8	LBW	√	0	0	0	0	√	0	0	7	10	10		11	RDP, PCV	C.glabrata	C. albicans	NOGC	NOGC	Klebsiella	14	R	R	S	S	C. albicans	Discharged	
59	1052567	b/o Akshata twin-2	F	46903	7	Very preterm	LSCS	6	8	VLBW	√	0	0	0	0	√	0	0	8	12	10		12	RDP, PCV	C.albicans	C.kefyr	NOGC	NOGC	NOGC	15	S	R	S	S	C.kefyr	Discharged	
60	1063868	b/o Sheetal. G	M	46831	10	Term	LSCS	6	8	Normal	√	0	√	0	0	√	0	0	10	10	10	3	14	RDP	C. parapsilosis	C. parapsilosis	parapsilosis	cinetobact	NOGC	15	S	S	S	S	C. parapsilosis	Discharged	
61	1064997	b/o Goura. B triplet-3	F	48144	5	Preterm	LSCS	6	7	VLBW	√	√	0	0	0	√	0	√	8	10	7		15	RDP,PCV,FFP	C.glabrata	C.glabrata	NOGC	NOGC	NOGC	25	R	R	R	S	C.glabrata	Discharged	
62	1125195	b/o Sneha rani	F	48150	3	Term	LSCS	6	7	Normal	√	0	0	0	0	0	0	0		3	3		5	nil	C krusei	C. krusei	NOGC	NOGC	NOGC	10	S	S	S	S	Candida krusei	Discharged	
63	1054175	b/o Deepa. T	F	46993	3	Term	VD	4	6	Normal	0	0	√	√	0	√	0	0		7	3		11	RDP	C.krusei	C krusei	NOGC	NOGC	NOGC	12	S	R	S	S	Candida krusei	Discharged	