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RESEARCH ARTICLE

Evaluation of Antifungal susceptibility in Neonatal intensive care unit in Ain Shams University

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Abstract

Neonatal infection is common in developing countries and a major cause of neonatal deaths**.**The incidence of the various fungal pathogens has increased dramatically over the past few decades **.**Candida spp. isthe most common of these pathogens. These infections are often severe, rapidly progressive, difficult to diagnose and refractory to therapy. The combination of suppressed host defense and exposure to multiple risk factors is responsible for their emergence.

Introduction:

Pediatric fungal infections are a substantial problem and account for substantial morbidity and mortality in young patients. Despite recent advances in diagnostic tools and treatments, invasive fungal infection in the pediatric population remains a major problem. Candidiasis, is the most common type of fungal infection seen in children. Other types of invasive fungal infections, such as Aspergillosis and Zygomycosis are less common **(1)**

Despite advances in perinatal care, neonatal sepsis is still a significant cause of morbidity and mortality in neonates **(2)**. Over time, the causative organisms of neonatal sepsis have changed; thus, an institutional survey for infectious diseases is essential for the prevention and treatment of neonatal invasive infections. This is particularly important because regional differences in the specific causative organisms of neonatal sepsis have been previously reported **(3).**

Colonization and infection of infants with antibiotic-resistant organisms such as methicillin-resistant S. aureus (MRSA) is a major concern in neonatal nurseries/ICUs where outbreaks can occur. Other organisms such as Serratiamarcescens have been implicated, and potentially devastating outbreaks can occur with Salmonella **(4).**Candidaemia with bothalbicans and non-albicans species is a particular problem in infants in neonatal ICU. Mortality increases as the time of gestation decreases **(5).**

Blood culture remains the golden standard of diagnosis in invasive Infection; other rapid diagnostic tests have been studied to try to rationalize empirical therapy. C-reactive protein (CRP) is relatively insensitive and non-specific, but useful if measured sequentially **(6).**

Central venous catheters and the duration of their use are very important risk factors for late onset sepsis in NICU patients, particularly in very low birth weight infants **(7).**Most catheter related infections are caused by CONS or other organisms colonizing the skin around the catheter exit site. Organisms colonizing the exit site can migrate along the external or internal surface of the catheter and enter the blood stream. Other catheterrelated infections are caused by translocation across the GI tract epithelium of pathogens such as Candida spp. **(8).**

The relationship between catheter manipulations, catheter colonization with CONS, and catheter associated blood stream infections have been studied. Birth weight < 1000 grams, catheter hub colonization, disinfection of the catheter hub, and blood sampling through the catheter were risk factors for catheter related infections.

Furthermore, Antibiotics and the duration of antibiotic use are risk factors that are frequently associated with blood stream infection in preterm infants (**9)** Similarly, The use of antibiotics for more than 5 days was associated with an

increased risk of candidemia .The use of 3rd generation cephalosporins has been linked to the emergence of extended spectrum B-lactamase producing KlebsiellaPneumoniae (**10).**

Candida species are the leading cause of invasive fungal infections in hospitalized neonates. Candida was found to be the third most common bloodstream isolate in children. The most common isolate was coagulase negative staphylococci CONS (43%), followed by enterococci, Candida species, and Staphylococcus aureus (9% each) **(11)**.Invasive Candida infection (ICI) in neonates requires specific definitions, evaluation, and treatment for this specific immunocompromised patient group with the unique risk factors of an immature system, including the skin and gastrointestinal tract, requiring intensive care and receiving multiple medications affecting colonization, proliferation, dissemination and infection **(12).** Oral thrush, vulvovaginitis and Napkin dermatitis are other forms of candidal infection in neonates **(13).**

The source of candidaemia is a subject of debate. Some authors suggest that it is the GI tract (endogenous acquisition) and others believe it to be healthcare workers' hands or catheter-related (exogenous acquisition). Identifying the source of candidaemia is important due to the implications for preventive strategies. If the gut is the primary source of candidaemia, attempts aimed at reducing gut colonization, such as the control of exposure to broad-spectrum antimicrobials, and the use of antifungal prophylaxis, may have an impact in reducing the incidence of candidaemia In contrast, implementation of intensive program to maximize compliance with hand hygiene recommendations, and adherence to current recommendations for placement and care of CVCs, should be implemented to control exogenous sources **(14**); (**15)**. Surveillance sites in which isolates were indistinguishable to the blood isolate. Surveillance sites in which isolates were indistinguishable to the blood isolate in 15 patients with C. albicanscandidaemia, from May 2004 to October 2005 in Hospital das Clı´nicas of University of Sao Paulo, Institute Emı´lioRibas and Hospital Geral de Itapecerica da Serra, Barazil **(15)**

Mortality: Invasive candidiasis (IC) is the fourth most common blood stream infection and is associated with the second highest case fatality rate in neonates and children with bloodstream infections **(5).**

Aspergillosis and Zygomycosis: Fungal skin infections other than cutaneous candidiasis in neonates are critical to consider. Aspergillus and Zygomycetes pathogens, while rare, they are a serious clinical challenge. For example, Aspergillosis is reported with increasing frequency in neonates **(12).**Aspergillusspecies are ubiquitous environmental molds that are easily transmitted through the air. In neonates, especially in preterm infants, cutaneous and invasive Aspergillosis can be associated with high morbidity and mortality rates, which is further compounded by the paucity of data regarding treatment strategies in this patient population. For the treatment of Aspergillus infections, the recommended primary treatment is voriconazole, although there are no pharmacokinetic studies to help guide dosing in neonates **(1).**

Amphotericin B is a naturally occurring, polyene macrolide antibiotic produced by Streptomyces nodosus. In spite of its toxic potential, amphotericin B is the drug of choice for the treatment of life threatening, systemic mycoses. The drug is also sometimes used in combination with flucytosine so that lower levels of amphotericin B to be less toxic **(16).**Several amphotericin B molecules bind to ergosterol in the plasma membranes of sensitive fungal cells. There, they form pores (channels) that require hydrophobic interactions between the lipophilic segment of the polyene antibiotic and the sterol (Fig. 4). The pores disrupt membrane function, allowing electrolytes (particularly potassium) and small molecules to leak from the cell, resulting in cell death (**16).**

5 Fluorocytosine(5-FC)enters fungal cells via a cytosine-specific permease an enzyme not found in mammalian cells. 5-FC is then converted by a series of steps to 5-fluorodeoxyuridine 5-monophosphate. This false nucleotide inhibits thymidylate synthase, thus depriving the organism of thymidylic acid an essential DNAcomponent.The unnatural mononucleotide is further metabolized to a trinucleotide (5-fluorodeoxyuridine 5'-triphosphate) and is incorporated into fungal RNA, thus disrupting nucleic acid and protein synthesis. Amphotericin B increases cell permeability, allowing more 5-FC to penetrate the cell. Thus, 5-FC and amphotericin B are synergistic. This combination of drugs is administered for the treatment of systemic mycoses and for meningitis caused by Cryptococcus neoformans and Candida Albicans**(17).**

Azoles are predominantly fungistatic. They inhibit C-14 alpha -demethylase (a cytochrome P450 enzyme), thus blocking the demethylation of lanosterol to ergosterol the principal sterol of fungal membranes. This inhibition disrupts membrane structure and function and, thereby, inhibits fungal cell growth. The selectivity of ketoconazole toward its target is not as precise as those of later azoles. For example, in addition to blocking fungal ergosterol synthesis, the drug also inhibits human gonad and adrenal steroid synthesis, leading to decreased testosterone and cortisol production. In addition, ketoconazole inhibits cytochrome P450 dependent hepatic drug metabolizing enzymes **(16).**

Voriconazole;Like the other Azoles act on ergosterol biosynthesis at the C-14 demethylation stage, a three step, oxidative reaction catalyzed by the cytochrome P-450 enzyme 14alpha-sterol demethylase (P- 450Dm) The resulting ergosterol depletion and accumulation of lanosterol and other 14-methylated sterols interferes with the 'bulk'

functions of ergosterol as a membrane component: it disrupts the structure of the plasma membrane, making it more vulnerable to further damage, and alters the activity of several membrane bound enzymes, such as those associated with nutrient transport and chitin synthesis. Severe ergosterol depletion (>99%) may additionally interfere with the hormone like functions of ergosterol, affecting cell growth and proliferation **(16).**

Resistance to antifungal can be visualized as a gradually evolving process wherein different mechanisms may appear during the course of chemotherapy. Studies so far suggest that antifungal resistance in Candida is a multifactorial phenomenon **(18,19).**

Subjects and methods: The present study was conducted in the neonatal intensive care unit, faculty of medicine; Ain shams University, in the period from February 2011 till august 2011. The present work was approved by ethical committee. An informed verbal consent was taken from parents of neonates before enrolment in the study. Patient selection:160 neonate admitted to the neonatal ICU were recruited for the study. All patients admitted to the NICU were included provided they fulfill the following criteria Gestational age more than 30 week and Body Wt more than 1.5 kg.**The neonates included in this study were divided into the following groups:** **Group I:** 80 Neonates with no sepsis. CRP< 6 **and** **Group II:** 80 Neonates presenting with sepsis. CRP > 6 **Laboratory investigations:** including CBC with differential leucocytic count was done using ColterT660 GEN-S (Corporaton,USA) , CRP on admission, it was measured by latex agglutination test (Omega diagnostic, UK 1/ 2008).Blood culture for neonates with CRP>6.**Blood culture formula**:Trypcase, L-cystine, Dextrose, yeast extract, Na thioglycolate, Resazurin, AgarCultures were done for samples using Brilliance Candida agar (chromogenic media) and determination of the resultant colonies by chromogenic identification method **(Milne,1996).**

Oxoid Brilliance Candida Agar: contains two chromogenic substrates, which are cleaved by enzymes possessed by certain Candida species; hexosaminidase and alkaline phosphatase. The action of the enzymes on the chromogens results in a build-up of color within the colony. The color produced depends on which enzymes the organisms possess **(20).**

Candida Tropicalis, C. albicansand C. dubliniensisall possess hexosaminidase which results in green colored colonies; however, other metabolic reactions of C. tropicalisproduce a localized drop in pH which results in dark blue colonies. Alkaline phosphatase activity in C. krusei results in a brown or pink pigmentation, whilst C. glabrata, C. kefyr, C.parapsilosis and C. lusitaniae appear as a variety of beige/brown/yellow colors due to the mixture of natural pigmentation and some alkaline phosphatase activity. It was feasible to differentiate these species by color and colony morphology **(20).**

Antifungal susceptibility testing using Disc diffusion method on Mueller Hilton agar .The antifungal drugs tested were Fluconazole, Nystatine and Voriconazole**(21).**

Antifungal disk diffusion susceptibility testing of yeasts: Approved guideline M-44 A, CLSI, USA**:**The method described here is only for testing Candida species.Medium:Mueller Hilton agar + 2% glucose and 0.5 µg methylene blue dye (GMB); pH 7.2-7.4. There should be no excess moisture on plates. Storage of antifungal discs at 4C in the refrigerator. Turbidity standard for inoculum preparation 0.5 McFarland standard. **(22)**

Inoculum preparation: Streaked on to SDA plate and incubated at 37C to obtain a pure culture of Candida species. 5 colonies of approximately 1 mm diameter from a 24-hour culture was picked and suspended in 5 ml of sterile normal saline. Vortexed for 15 seconds, turbidity was adjusted visually/ spectrophotometrically at 530 nm to 0.5 McFarland standards (1 x 106 to 5 x 106 cells/ml) – this produced semi-confluent growth with most Candida species.

Inoculation of test plates: Sterile cotton swab stick was dipped into suspension, rotated several times and pressed firmly against inside wall of the tube above fluid level to remove excess fluid. It was evenly streaked over the entire agar surface 3 times, each time at an angle of 60C, to ensure an even distribution of inoculum **Application of disks to inoculated plates:**Antimicrobial discs were dispensed onto the surface of an inoculated agar plate by means of a sterile forceps. They must be pressed down. The discs were evenly distributed on the plate, no closer than 2.5 cm from centre to centre; 5 discs can be put on 1 plate. Plates were inverted and incubated at 37C within 15 minutes.They were Read at 20-24 hours, when semi-confluent growth has formed. In case of insufficient growth, they were read at 48 hours. Zone of inhibition was measured at the point where there is prominent reduction in growth **(22).**

CLSI micro-broth dilution method for filamentous fungi: Approved guideline M-38A, CLSI,USA **.**This method is used for testing filamentous fungi that cause invasive infections, including Aspergillus species, Fusarium species, Rhizopus species, Pseudallescheriaboydii, and mycelial form of Sporothrixschenckii. Although other opportunistic melanizedmoulds have been evaluated, caution should be used in interpreting the MIC results for them. The method has not been used for the yeast forms of dimorphic fungi like Blastomycesdermatitidis, Coccidioidesimmitis, Histoplasmacapsulatum, Penicilliummarneffei or S. schenckii.**(22) .Procedure**Broth medium and buffers are similar to M-27-A2. Micro-titter plates similar to M-27A-A2 were used for preparing drug dilutions. Growth-control well

(no antifungal) incorporated for each isolate were tested.**Inoculum preparation:**Fungi were grown on potato dextrose agar for 7 days at 35C. Fusarium was incubated at 35C for 18-72 hours and then at 25C for 7 days. The 7-days-old cultures were covered with approximately 1 ml sterile normal saline.Suspension was prepared by gently probing the colonies with the tip of a transferable pipette. 1 drop of 20 was added to facilitate the preparation of aspergillus conidia. The resulting mixture of spores and hyphae was withdrawn and transferred to a sterile test tube. Heavy particles were allowed to settle for 3-5 minutes. The upper homogenous suspension was transferred to another sterile tube, cap was tightened and vortexed for 15 seconds. The densities of conidia were adjusted to an optical density (OD) that all range from 0.09-0.11 (80%-82%) for Aspergillus species and S. schenckii and 0.15-0.17 (68%-70% transmittance) for Fusarium species, P. boydii and Rhizopus species. These suspensions were dissolved 1:50 in the standard medium (RPMI-1640). The end result corresponds to 0.5 x 10CFU/ml. The rest of the steps are similar to M-27-A2. Incubation: Rhizopus species were examined at 24 hours, while the rest of the isolates at 48 hours except P. boydii, which was examined at 72 hours.Growth was scored with a mirror as follows: (1) Optically clear (2) Slightly hazy (3) Prominent decrease in turbidity (4) Slight reduction in turbidity (5) No turbidity.For Amphotericin B, Itraconazole and the newer azoles score of 0 as was taken as MIC while for Flucytosine, Fluconazole and Ketoconazole Score of 2 is taken as end point.

(Statistical analysis: Statistical presentation and analysis of the present study was conducted, using the mean, standard error, unpaired student t-test and chi-square by SPSS V17. (Statistical program for social science version 17) **(23)**

1. Mean =**Chi-square** the hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship. Pearson chi-square and likelihood-ratio chi-square. Fisher's exact test and Yates' corrected chi-square are computed for 2x2 tables.

Analysis of variance [ANOVA] tests. According to the computer program SPSS for Windows. ANOVA test was used for comparison among different times in the same group in quantitative data. SE_2= Standard error of the second group. Unpaired Student T-test was used to compare between two groups in quantitative data.

Results: A total number of 160 neonates were enrolled in this study they included in to 2 groups. **Group I**: 80 patients with negative CRP and **Group II**: 80 patients with positive CRP. They all were subjected to history taking, clinical examination, Laboratory investigation in the form of CBC with differential leucocytic count, CRP, Blood culture for group II, culture for all collected samples on scabrous agar and selective chromogenic media (Brilliance Candida agar), and Antifungal susceptibility testing using Disc diffusion method on Mueller Hilton agar .The antifungal drugs tested are Fluconazole, Nystatine and Voriconazole.

Samples collected were Blood culture91,34.60%, Bronchial secretions77, 29.28%, Napkin dermatitis swab45,17.11%,Oral swab8,3.04% ,Umbilical Catheter1,0.38%, Umbilical Swab36,13.69%,Urinary catheter1,0.38%,Wound Swab4,1.52% with Total263

This tableshows that the majority of the positive cultures were blood culture (58/186) that means blood stream fungal infection is common in NICU ,and the most common isolate from the blood cultures was AspergillusNiger (15/58 =25.86%)., the second common site of infection is respiratory tract infection (48/186) followed by Napkin dermatitis (41/186).

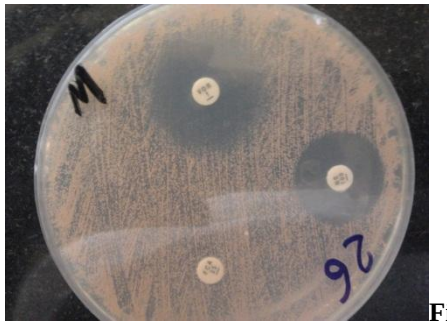

Fig. (1): Antifungal susceptibility testing using Disc diffusion method shows large inhibition zone around Voriconazole disc and another small one around Nystatin disc.

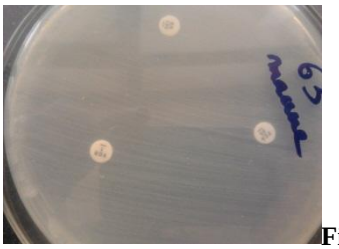

Fig. (2): Antifungal susceptibility testing using Disc diffusion method on Mueller Hilton agar shows resistance to the 3 antifungal drugs.

(a) 

(b)

Fig. (3):Microbroth dilution test showing (a) fungi resistant to Miconazole (Pink), and sensitive to Ketoconazole and voriconazole (Blue).(b) fungi resistant to ketoconazole (Pink), and sensitive to voriconazole and Amph.B (Blue).

Table (1): Number and percentage of isolated strains

Culture results	**N**	**%**
Negative	77	29.28
Candida Albicans	31	11.79
Candida Glabrata	29	11.03
Candida krusei	5	1.90
Candida Parapsilosis	23	8.75
Candida Stellatoidea	2	0.76
Candida Tropicalis	3	1.14
Orange Candida colony	1	0.38

AspargillusFumigatus	9	3.42
AspargillusFumigatus with penicillium	5	1.90
AspargillusNiger	31	11.79
AspargillusNigerwith Penicillium	8	3.04
AspargillusFlavus	21	7.98
AspargillusFlavuswith Penicillium	3	1.14
Fuserium Sp.	9	3.42
Mycelial colony	1	0.38
Penicillium colony	3	1.14
Unidentified	2	0.76
Total	263	100.00

Table (2):Culture results according to the site of infection

Culture results		**Samples**						
		Blood culture	**Bronchial secretions**	**Napkin dermatitis swab**	**Oral swab**	**Umbilical Swab**	**Wound Swab**	**Total**
Candida Albicans	N	5	7	10	3	5	1	31
	%	8.62	14.58	24.39	75.00	16.13	25.00	16.67
Candida Glabrata	N	5	4	12	0	7	1	29
	%	8.62	8.33	29.27	0.00	22.58	25.00	15.59
Candida krusei	N	1	1	1	0	2	0	5
	%	1.72	2.08	2.44	0.00	6.45	0.00	2.69
Candida Parapsilosis	N	1	9	5	1	6	1	23
	%	1.72	18.75	12.20	25.00	19.35	25.00	12.37
Candida Stellatoidea	N	0	0	1	0	1	0	2
	%	0.00	0.00	2.44	0.00	3.23	0.00	1.08
Candida Tropicalis	N	0	2	1	0	0	0	3
	%	0.00	4.17	2.44	0.00	0.00	0.00	1.61
Orange Candida colony	N	0	1	0	0	0	0	1
	%	0.00	2.08	0.00	0.00	0.00	0.00	0.54
AspargillusFumigatus	N	6	2	0	0	1	0	9

Culture results		Samples						
		Blood culture	Bronchial secretions	Napkin dermatitis swab	Oral swab	Umbilical Swab	Wound Swab	Total
	%	10.34	4.17	0.00	0.00	3.23	0.00	4.84
AspargillusFumigatus with penicillium	N	3	0	1	0	1	0	5
	%	5.17	0.00	2.44	0.00	3.23	0.00	2.69
AspargillusNiger	N	15	7	5	0	4	0	31
	%	25.86	14.58	12.20	0.00	12.90	0.00	16.67
AspargillusNigerwith Penicillium	N	6	0	1	0	1	0	8
	%	10.34	0.00	2.44	0.00	3.23	0.00	4.30
AspargillusFlavus	N	7	9	2	0	2	1	21
	%	12.07	18.75	4.88	0.00	6.45	25.00	11.29
AspargillusFlavus with Penicillium	N	3	0	0	0	0	0	3
	%	5.17	0.00	0.00	0.00	0.00	0.00	1.61
Fuserium Sp.	N	3	3	2	0	1	0	9
	%	5.17	6.25	4.88	0.00	3.23	0.00	4.84
Mycelial colony	N	0	1	0	0	0	0	1
	%	0.00	2.08	0.00	0.00	0.00	0.00	0.54
Penicillium colony	N	2	1	0	0	0	0	3
	%	3.45	2.08	0.00	0.00	0.00	0.00	1.61
Unidentified	N	1	1	0	0	0	0	2
	%	1.72	2.08	0.00	0.00	0.00	0.00	1.08
Total	N	58	48	41	4	31	4	186
	%	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table (3):Comparison between group I (CRP –ve) and Group II (CRP +ve) as regards culture results.

Organism		Groups					
		Group I		Group II		Total	
		N	%	N	%	N	%
Negative		9	11.25	21	26.25	30	18.75
Positive		71	88.75	59	73.75	130	81.25
Total		80	100.00	80	100.00	160	100.00
Chi-square	X^2	5.908					
	P-value	0.015*					

Table (4):Comparison between positive and negative cultures as regards someCBC parameters among group I.

Group I	Culture results							
	Negative			Positive			T-test	
	Mean	±	SD	Mean	±	SD	t	P-value
WBC	15687.500	±	2293.119	16425.352	±	5252.393	-0.391	0.697
Neutrophils	27.000	±	35.366	22.537	±	37.147	0.323	0.747
Lymphocytes	6.150	±	2.096	24.241	±	32.846	-1.549	0.126
Hgb.	28.325	±	38.368	23.363	±	32.616	0.401	0.690

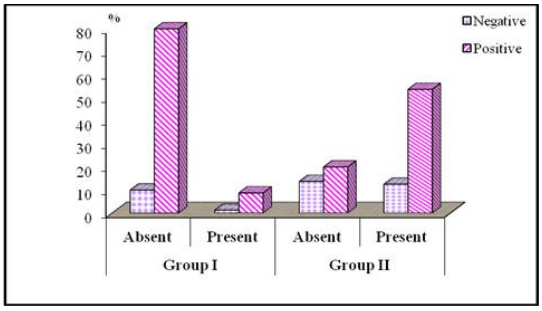

Fig. (4): Comparison between the number of positive and negative cultures as regard the presence and absence ofendotracheal tube (ET tube) in both group I and II

Table (5): Detailed results of susceptibility of the isolated Candida species to different antifungal drugs
R: Resistant, **S:** Sensitive, **Int:** Intermediately sensitive

Table (5):shows results of susceptibility of the isolated Candida species to Voriconazole in details.

culture results	Voriconazole							
	R		S		Int		Total	
	N	%	N	%	N	%	N	%

ulture results	Nystatine							
	R		S		Int		Total	
	N	%	N	%	N	%	N	%
Candida Albicans	6	19.35	21	67.74	4	12.90	31	32.98
Candida Glabrata	17	58.62	11	37.93	1	3.45	29	30.85
Candida krusei	5	100.00	0	0.00	0	0.00	5	5.32
Candida Parapsilosis	19	82.61	2	8.70	2	8.70	23	24.47
Candida Stellatoidea	1	50.00	1	50.00	0	0.00	2	2.13
Candida Tropicalis	0	0.00	0	0.00	3	100.00	3	3.19
Orange Candida colony	1	100.00	0	0.00	0	0.00	1	1.06
Total	49	52.1277	35	37.234	10	10.6383	94	100
Candida Albicans	10	32.26	19	61.29	2	6.45	31	32.98
Candida Glabrata	9	31.03	14	48.28	6	20.69	29	30.85
Candida krusei	3	60.00	0	0.00	2	40.00	5	5.32
Candida Parapsilosis	14	60.87	6	26.09	3	13.04	23	24.47
Candida Stellatoidea	1	50.00	1	50.00	0	0.00	2	2.13
Candida Tropicalis	0	0.00	3	100.00	0	0.00	3	3.19
Orange Candida colony	0	0.00	1	100.00	0	0.00	1	1.06
Total	37	39.3617	44	46.8085	13	13.8298	94	100

R: Resistant, **S:** Sensitive, **Int:** Intermediately sensitive

Table (5):Shows results of susceptibility of the isolated Candida species to Fluconazole in details

culture results	Fluconazole							
	R		S		Int		Total	
	N	%	N	%	N	%	N	%

Candida Albicans	10	32.26	21	67.74	0	0.00	31	32.98
Candida Glabrata	20	68.97	8	27.59	1	3.45	29	30.85
Candida krusei	5	100.00	0	0.00	0	0.00	5	5.32
Candida Parapsilosis	18	78.26	2	8.70	3	13.04	23	24.47
Candida Stellatoidea	1	50.00	1	50.00	0	0.00	2	2.13
Candida Tropicalis	3	100.00	0	0.00	0	0.00	3	3.19
Orange Candida colony	1	100.00	0	0.00	0	0.00	1	1.06
Total	58	61.7021	32	34.0426	4	4.25532	94	100

R: Resistant, **S:** Sensitive, **Int:** Intermediately sensitive

Table (6):CLSI microbroth dilution for MIC evaluation in filamentous Fungi

Isolate no.	Voriconazole S:0.5-4 R>4	Itraconazole S:0.2-0.5 R>0.5	Amp B S:0.5-4 R>4
Asp. niger	S	R	R
Asp. Fumigates (blood cult)	R	R	R
Asp. flavus	S	S	R
Penicilluim	S	R	R
Fusaruim	R	R	R
Unidentified mycelial growth	R	R	R

R: Resistant; **S:** Sensitive

Discussion:

Pediatric fungal infections represent a substantial problem accounting for significant morbidity and mortality in young patients. Despite recent advances in diagnostic tools and treatments, invasive fungal infection in the pediatric population remains a major problem. Candidiasis is the most common type of fungal infection seen in children. Other types of invasive fungal infections, such as Aspergillosis and Zygomycosis are less common**. (1).**

This study attempted to determine the antifungal susceptibility profile and to detect resistant strains of fungal species isolated from neonates admitted in Ain shams university Neonatal intensive care unit. **263** strains isolated from **91** bloodstream cultures,**77**Bronchial secretions aspiration, **45** Napkin dermatitis swabs, **8** Oral swabs, **1** umbilical catheter, **36** umbilical swabs, **1** Urinary catheter and **4** Wound swabs were studied.

Thepresent study revealed that Candida was the most common isolated genus (94/ 186) Candida Albicans was the most common (31/94).Similar results were reported by **(24,5)**as Candida Albicans was the most common invasive Candida species in pediatric patients (70% and 50%), Also **(25)** found that Candida Albicans is the most frequently isolated yeast (65.5%, P<0.05) in all clinical specimens taken from NICU except blood culture.In the present study Candida glabrata (29/94=30.85%) was the 2nd in order as it was the commonest isolated non-albicans species.The same results were reported by **(24),** they found that C.glabrata is the 2nd most common Candida species (44%). On the other hand **(26)** found that C. glabrata is infrequent isolated spp. In addition **(25)** report that Candida Parapsilosisis very common isolate especially from the blood (50%). Also, **(27,28)** found that Candida Parapsilosis (21%) and Candida Tropicalis (10%) were the most prevalent non albicans spp. The inconsistency in this area reflects even an intercenter variability possibly depending on the different strategies in culture surveillance, demographics of the admitted patient, feeding and antibiotic practices even the working staff because they are considered as a source of infection.

(7, 29, 30,26,1)found that the incidence of fungal infection especially the invasive one was inversely proportional to age and birth weight.As regard use of devices such as catheters and endotracheal tubes they destroy the natural barriers of the body and allows Candida spp. to penetrate, multiply and invade sterile body areas **(5).** In the present study there was a significant increase of the incidence of fungal infection in Group II neonates using endotracheal tube (P value 0.036) this was in alignment with **(31, 32, 10, 27, 30,29)**but disagree with multivariate analysis model of **(33).**

As regard the IV canula we found that there was absolute significant relation between using IV canula and increase incidence of fungal infection as all neonates with fungal infection were using IV canula.This finding is not surprising because Candida can adhere to platelets and fibrinogen on the surface of the catheters and form biofilms that may protect the organism from the immune response and antifungal agent **(34).**Also, the present study shows

significant increase in the incidence of fungal infection with prolonged hospitalization (P value 0.028). This is because longer NICU stay is associated with more prolonged therapeutic intervention and exposure to nasocomial infection **(28).**These findings were consistent with **(33)** (P value < 0.001)**, (27,28)** and disagree with **(31)** as they found children are relatively vulnerable even in relatively short stay and average duration of hospitalization of a week has been reported to be adequate time for infection.

Increase Neutrophilic count associated with the systemic fungal infection in our study (P value 0.030) in contrast **(1)** and **(26)** reported that neutropenia is a risk factor for fungal infection. This variation can be explained by the fact that both neutrophilia and neutropenia are a well- known laboratory finding in severe fungal infection. The present study tested the susceptibility of 263 isolate from 203 samples taken from 160 neonate in NICU to three antifungal drugs two of them are used systemically (Fluconazole and voriconazole) and the 3rd one is antifungal for local use only (Nystatine).

As regard Candida strains the findings were:Fluconazole:67.74% of C. Albicans were sensitive to fluconazole and 32.26% were resistant that means the majority of C. Albicans were susceptible to fluconazole on the same context **(35,36,37,38,39),** in contrast (**40)** found that C.Albicans strains were significantly frequently resistant to fluconazole than non albicans Candida species. This difference may be due to small number of isolate they used (142 isolate) **while** 27.59% of C. glabrata were sensitive to fluconazole and 68.97% were resistant that means the majority of C. Glabrata were resistant to fluconazole this was in agreement with **(35,25,41) ,** but these findings disagree with **(36**) as they found that 50-90% of C.glabrata were sensitive to fluconazole.100% of C.krusei were resistant to fluconazole. That means the majority of C. Krusei were resistant to fluconazole. In the same context **(35,42,36,25,41).**These can be explained by the naturally inherent resistance of C. krusei to fluconazole **(25).** In contrast **(40)** found that only 33.3% of C.Krusei were resistant to Fluconazole. 100% of C.tropicalis were resistant to fluconazolethat means the majority of C. tropicalis were resistant to fluconazole, In contrast **42,36,25,** found that the majority of isolated C. tropicalisspp. were sensitive to Fluconazole.8.70% of C. parapsilosis were sensitive to fluconazole and 78.26% were resistant that means the majority of C. parapsilosis were resistant to fluconazole this was disagree with **(36),** as they found that the majority of C. Parapsilosissinstive to fluconasole.

Voriconazole:61.29% of C. Albicans were sensitive to Voriconazole and 32.26% were resistant that means the majority of C. Albicans are susceptible to Voriconazole this was

in agreement with **(36,43, 45,44).**48.28% of C. glabrata were sensitive to Voriconazole and 31.03% were resistant that means the majority of C. Glabrata were sensitive to Voriconazole this was in agreement with **(44,43,45,36).**60% of C.krusei were resistant to Voriconazole. That means the majority of C. Krusei were resistant to Voriconazole. This finding disagree with **(36,43,45,44)** as they found that the majority ofC. Krusei were sensitive to Voriconazole. But we have to consider that C.krusei was more sensitive to Voriconazole than fluconazole.100% of C.tropicaliswere sensitive to Voriconazole this was in agreement with **(36).**

Nystatine: In this study C.Albicans was the most sensitive strain to Nystatine 67.74% followed by C.stellatoidea 50%. There is no enough studies tested this antifungal agent because of its local use as it is very toxic to be used systemically

Conclusion and recommendations:

Fungal infection is a serious problem in NICU, and the diagnosis of fungal infection should be considered in any neonate with sepsis and should be either confirmed or excluded. Neonates are very vulnerable group of patients so they are at high risk to develop serious invasive fungal infection and its complications.These infections are often severe, rapidly progressive, difficult to diagnose and refractory to therapy.

Risk factors for fungal infection include that the primary barriers of defense, such as the skin and mucosa, are anatomically more fragile in children than in adults and are therefore more easily colonized ,neonates generally have functionally immature phagocytes and T lymphocytes witch make the immune system week and easily invaded with fungi , the frequent use of broad-spectrum antibiotics especially when administered for more than a week, has been associated with elimination of protective bacterial flora, and give chance for opportunistic fungal infection, the use of devices such as catheters and endotracheal tubes destroys the natural barriers of the body and allows fungi to penetrate, multiply and invade sterile body areas.

On the other hand, the longer NICU stay the more exposure to the therapeutic intervention and exposure to nosocomial infection. Consequently we suggest that most of fungal infection in NICU are hospital acquired as we found that the incidence of infection does not show an increase with maternal PROM.Fungal infection should be considered in diagnosis of any neonate with severe illness and high CRP.We recommend specific culture sensitivity test as a golden tool to diagnose invasive fungal infection and to start treatment with the appropriate antifungal agent according to sensitivity resultsThe efficacy of the treatment should be assessed by the documentation of blood cultures returning sterileAccording to the WHO guidelines; the Empirical use of antifungal drugs should be . Rationalized as far as possible, in order to prevent rising of more resistant strains.There are Preventable risk factors

that can be manipulated to decrease the risk for fungal infection as prolonged use of multiple antibacterial drugs without culture sensitivity testing. Antimicrobial sensitivity testing should be a standardize guide to the judicious use ofa single effective antibacterial drug.The restricted use of invasive devices and the frequent change of various catheters are strongly recommended. Routine use of fluconazole can be replaced by voriconazole as we found that the isolated strains are more sensitive to voriconazole.Further prospective studies on other antifungal therapeutic agents as Itraconazole and Caspofungin are needed.

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