Clinical Research

Incidence of *Candida* species colonization in neonatal intensive care unit at Riyadh Hospital, Saudi Arabia

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ABSTRAK

Latar belakang: *Spesies Candida merupakan patogen yang didapat di rumah sakit yang penting terutama pada bayi yang masuk perawatan intensif neonatus (NICU). Penelitian ini dilakukan di NICU Rumah Sakit Arab Saudi, Riyadh, KSA untuk menganalisis pola kolonisasi Candida pada neonatus beserta menentukan faktor risiko potensial.*

Metode: *Kultur jamur surveilans mingguan dilakukan pada daerah anus, rongga mulut, umbilikus, dan liang telinga pada neontaus dilakukan saat lahir sampai pasien dipulangkan dari rumah sakit. Kolonisasi dianalisis berdasarkan waktu, tempat, spesies, berat lahir, dan usia gestasi. Sumber lingkungan potensial dan tangan dari petugas kesehatan juga dilakukan kultur jamur setiap bulan. Uji kepekaan antijamur juga dilakukan.*

Hasil: *100 subjek diajak untuk penelitian ini. Jumlah kolonisasi secara keseluruhan 51%. Kolonisasi awal didapatkan pada 27 neonatus (27%) sedangkan 24 neonatus (24%) didapatkan pada saat akhir perawatan di NICU. Daerah perianal dan rongga mulut merupakan daerah kolonisasi tersering. C. albicans merupakan spesies isolat terbanyak (56,8% dilanjutkan C. tropicalis (17,6%), C. glabrata (15,6%), dan C. krusei (2%). Dari 51 isolat Candida, 68,6% sensitif terhadap flukonazol, 80% terhadap itrakonazol, dan 64,7% terhadap ketokonazol, sedangkan hanya 33% yang sensitif terhadap amfoterisin B.*

Kesimpulan: *Candida merupakan penyebab umum infeksi pada bayi yang masuk ke NICU dan C. albicans merupakan spesies Candida tersering. Infeksi neonatus yang disebabkan spesies non-albicans timbul pada usia yang lebih lanjut selama dirawat di NICU.*

ABSTRACT

Background: *Candida* species are important hospital-acquired pathogens in infants admitted to the neonatal intensive care unit (NICU). This study was performed in the NICU of Saudi Arabian Hospital, Riyadh region, KSA to analyze patterns of neonatal *Candida* colonization as well as to determine the potential risk factors.

Methods: Weekly surveillance fungal cultures of anal area, oral cavity, umbilicus and ear canal of neonates were performed from birth until their discharge from the hospital. Colonization was analyzed for timing, site, species, birth weight and gestational age. Potential environmental reservoirs and hands of health care workers (HCWs) were also cultured monthly for fungi. Antifungal susceptibility of the identified isolates was also determined.

Results: One hundred subjects have been recruited in this study. The overall colonization rate was 51%. Early colonization was found in 27 (27%) neonates whereas 24 (24%) neonates were lately colonized during their stay in NICU. Colonization was more in preterm neonates than in full and post term. Perianal area and oral cavity were the most frequent colonized sites. *C. albicans* was the main spp. (58.8%) isolated from the neonates followed by *C. tropicalis* (17.6%), *C. glabrata* (15.6%), and *C. krusei* (2%). Of the 51 isolated *Candida* spp., 68.6% were sensitive to fluconazole, 80% to itraconazole and 64.7% to ketoconazole, while only 33% were sensitive to amphotericin B.

Conclusion: *Candida* has emerged as a common cause of infections in infants admitted to NICU, and *C. albicans* is the most commonly isolated candidal species. Neonatal infections caused by non- albicans species occur at a later age during their stay in NICU.

Keywords: *Candida* colonization, neonatal intensive care unit, pediatric patient

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Candida is a cause of neonatal infection in premature infants, especially for extremely low and very low birth weight infants.[1,2] *Candida* species have become important nosocomial pathogens in NICUs and are responsible for considerable morbidity and mortality, especially in preterm infants.[3,4] Although *C. albican*s has been the most frequently isolated species in colonized or infected neonates, colonization and infection with *non-C. albicans* spp., particularly *C. tropicalis,* and *C. parapsilosis*, has also increased dramatically.[3,5,6] In very low birth weight (VLBW) infants (birth weight less than 1,500 g), *C. albicans* is the third most common cause of neonatal late onset sepsis (LOS), which occurs after the first 72 hours of life. This was illustrated in a multicenter study from the National Institute of Child Health and Human Development (NICHHD) Neonatal Research Network that evaluated 6,956 VLBW infants (range of birth weight from 401 to 1,500 g) admitted over a two-year period from 1998 to 2000.[1] *C. albicans* was the causative agent in (6%) of first episodes of LOS following coagulase negative staphylococcus (48%), and *Staphylococcus aureus* (8%) in frequency.[2] In addition, *C. parapsilosis* was isolated as the causative agent in (4%) of the cases.

Transmission of the Candidal strains is either by vertical transmission from the mother or horizontal transmission from health care workers or the hospital environment.[7] Colonization with *Candida* has been identified as the major risk factor and a necessary first step in development of candidemia, providing a reservoir of the potentially invading *Candida* strains.[8,9] Molecular typing studies confirm that most invasive fungal infection arise endogenously and result from a colonizing *Candida* strain, rather than another completely different isolate.[10–12] In view of the risk of colonization, this study aimed to analyze patterns of *Candida* colonization in the (NICU) of Saudi Arabian Hospital as well as to determine the potential risk factors, the possible source of colonization and the susceptibility pattern of *Candida* isolates to different antifungal drugs.

METHODS

Cases

This study was conducted on a total of 100 neonates admitted to the NICU of Saudi Arabian hospital (Riyadh region, KSA) from September 2014 to October 2015. Neonates with an expected stay of more than one week in the NICU were enrolled in the study. Fungal surveillance cultures were obtained from the neonates, on admission and at weekly interval thereafter till NICU discharge. Fungal colonization was defined by a positive surveillance culture at any time during their stay in NICU or at baseline.[13] Early colonization was considered when *Candida* species was isolated from the initial cultures, while late colonization was considered when the initial cultures were negative and at least one subsequent culture was positive. Data were collected to assess possible risk factors for *Candida* colonization such as mode of delivery, gestational age, birth weight (BW), total parenteral nutrition (TPN), use of antibiotics, and other medications.

Samples collection and culture

Pre-moistened (with sterile normal saline) cotton-tipped swabs were used to obtain samples from the oral cavity, perianal area, ear canal, and umbilicus of the neonates' weekly from birth until discharge. Monthly swabs were also taken from potential environmental reservoirs such as benches, wash basin, and cubicles and from both hands of NICU health care workers (HCWs) rotating through the unit. The entire surface of the hand, the area under the fingernails, and between the fingers, were swabbed with a separate swab for each hand. All the samples were directly inoculated on sabouraud dextrose agar (Himedia laboratories, Mumbai, India) with 50 mg chloramphenicol/liter and 50 mg gentamicin/liter and incubated at 37°C for 48 hours.

Examination of the mycological growth

Identification of the isolated Candida strains

The conventional yeast identification methods based on morphology, Gram stained smears, sporulation and fermentation characteristics as well as the assimilation of a wide range of carbon and nitrogen sources were used. The isolates were tested to grow on media without different vitamins (thiamine, pantothenate, myo-inositole, pyridoxine, niacin, para aminobenzoic acid). The pathogenic potentialities of the yeast isolates were studied by testing protcolytic, lipolytic and haemolytic activity. The species were also determined by the germ tube test and the KJ3006 HiCanclicla Identification Kit (Himedia laboratories, Mumbai, India) according to manufacturer's instructions.

Confirmatory tests

Tween 80 oxgal-caffic acid (TOC) agar plates were streaked with a 48 hours-old yeast colony, covered with a sterile cover slip, incubated at 37°C for three hours and observed for germ tube production. TOC agar plates were incubated at 28°C for 2–3 days in the dark to promote the production of chlamydospores, hyphae and pseudohyphae. Ascospore formation and urea hydrolysis were also tested for the isolated strains to confirm the identification.

Antifungal susceptibility testing

Susceptibility testing of isolates was performed for amphotericin B, ketoconazole, itraconazole, fluconazole (Hi media laboratories, Mumbai, India) using the disk diffusion method according to the guidelines of Clinical Laboratory Standards Institute (CLSI).[14]

Statistical analysis

Quantitative variables were presented as medians or mean ± standard deviation (mean ± SD), whereas qualitative variables were described as number and percentages. Data were analyzed using Chi-square tests and Mann-whitney test, as appropriate. Univariate analysis for detection of risk factors was performed using Chi-square tests. A p value of <0.05 was considered statistically significant. All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) software version.

RESULTS

During the study period, *Candida* colonization was detected in 51 (51%) out of 100 neonates. Early colonization was found in 27 (27%)

Table 1. Characteristics of study neonates with early and late colonization

Characteristics	All neonates (n=100)	Early colonized (n=27)	Late colonized (n=24)
Sex			
Male: n (%)	52 (52)	17 (63)	10 (41.7)
Female: n (%)	48 (48)	10 (37)	14 (58.3)
Preterm n (%)	54 (54)	17 (63)	13 (54.2)
Fullterm n (%)	18 (18)	4 (14.8)	3 (12.5)
Postterm n (%)	28 (28)	6 (22.2)	8 (33.3)
Weight (kg)			
Mean±SD	2.27±0.82	2.38±0.86	1.86±0.7
Median	2.39	2.39	1.5
<1.5 kg: n (%)	24 (24)	6 (22)	13 (54)
>1.5 kg: n (%)	76 (76)	21 (77)	11 (45)
Delivery			
Vaginal	16 (16)	4 (14.8)	5 (20.8)
Cesarean section	84 (84)	23 (85.2)	19 (79.2)
PROM	4 (4)	2 (7.4)	3 (12.5)
TPN n (%)	31 (31)	5 (18.5)	11 (46)
Oral feeding n (%)	76 (31)	20 (74)	12 (50)
Antibiotic intake n (%)	89 (31)	24 (89)	21 (87.5)
Colonization Site			
Perianal	3 1 (60%)	16 (59%)	15(62.5%)
Oral	29 (56%)	16(59%)	13 (54%)
Ear	10 (19.6%)	5 (18.5%)	5 (21%)
Umbilicus	15 (29%)	7 (26%)	8 (33%)

PROM: premature rupture of membrane; TPN: total parenteral nutrition

neonates whereas 24 (24%) neonates were lately colonized during their stay in NICU. Three *Candida* species were isolated from the hands of 3 nurses on different occasions, while no *Candida* was isolated from the environmental samples. The characteristics of the neonates and those with both early and late colonization are presented in Table 1.

Twenty eight (54%) neonates were colonized with *Candida* at one site (15 initially and 13 during the stay), while 23 (45%) were colonized at two or more sites (12 initially and 11 during the stay). Perianal area and oral cavity were the most frequent sites of early and late colonization.

Fermentation reactions

In this study the *Candida* strains isolated from the study neonates in NICU were also identified by using fermentation tests. The results were negative in all isolates of *C. krusei*, except D-glucose while the different species of *Candida* were able to ferment a narrow range of sugars as described in Table 2.

Assimilation tests

In the present study, six species of *Candida* were identified; *Candida albicans, C. tropicalis, C. glabrata*, *C. krusei*, *C. lusitaniae* and *C. parapsiolosis*. The results of vitamin assimilation tests revealed that none of the isolates were capable of growing on melibiose, lactose, erthritol, methanol and inulin as carbon source (Table 3) as well as creatinine, nitrate and nitrite as nitrogen source (Table 3). The results of vitamin assimilation showed that *C. tropicalis* was unable to grow on medium without thiamine.

Confirmatory tests for identification of *Candida* strains

Germ tube test, hydrolysis of urea, Ascospore formation, and Chlamydospore formation along with assimilation and fermentation results confirmed the identification of the isolated *Candida* strains (Table 4).

Pathogenic potentialities of the isolated *Candida* strains

The pathogenic potentialities were tested for the isolated *Candida* strains and the results showed that the strains were able to grow at 37°C, which indicates their ability to grow at body temperature. Also, they could hydrolyze casein and patient's fats indicating their ability to produce proteolytic and lipolytic enzymes (Table 4).

Overall, *C. albicans* was the most frequent spp. (58.8%) isolated from the neonates in NICU, followed by *C. tropicalis* (17.6%), and *C. glabrata* (15.6%). However, while *C. albicans* was the predominant early colonizing spp. (92.6%), the most common *Candida* spp. lately colonizing the neonate was *C. tropicalis* (33.3%) followed by *C. glabrata* (29.2%) then *C. albicans* (20.8%). The three *Candida* spp. isolated from hands of nurses were two *C. glabrata* and one *C. tropicalis*.

Table 2. Fermentation reactions of Candida strains isolated from the study neonates in NICU

Physiological characteristics	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. lusitaniae*	*C. parapsilosis*
Fermentation Reactions						
D - glucose	+	+	+	+	+	+
D - galactose	+	+	-	-	+/-	+
Maltose	+	+	-	-	+	-
Sucrose	-	+/-	-	-	+	-
Trehalose	+	+	+	-	+	-
Melibiose	-	-	-	-	-	-
Lactose	-	-	-	-	-	-
Cellobiose	-	+/-	-	-	+	-
Raffinose	-	-	-	-	-	-
Inulin	-	-	-	-	-	-
Starch	+	+	-	-	-	-
D - Xylose	+	+	-	-	+	+

Table 3. Assimilation tests of substances as carbon source by Candida strains isolated from the study neonates in NICU

Physiological characteristics	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. lusitaniae*	*C. parapsilosis*
Assimilation Tests:						
D - Glucose	+	+	+	+	+	+
D - Galactose	+	+	-	-	+/-	+
L - Sorbose	+/-	+/-	-	+/-	+/-	+/-
D - Glucose amine	-	-	-	+	-	-
D - Ribose	+/-	+/-	-	-	+	+/-
D - Xylose	+	+	-	-	+	+
L - Arabinose	+/-	+	-	-	+/-	+
D - Arabinose	-	-	-	-	+/-	-
L - Rhamnose	-	-	-	-	+	-
Sucrose	+	+/-	-	-	+	+
Maltose	+	+	-	-	+	+
Trehalose	+	+	+	-	+	+
Cellobiose	-	+/-	-	-	+	-
Salicin	-	+/-	-	-	+	-
Arbutine	+	+	-	+	+	-
Melibiose	-	-	-	-	-	-
Lactose	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-
Inulin	-	-	-	-	-	-
Starch	+	+	-	-	-	-
Glycerol	+/-	+/-	+/-	+	+	+
Erythritol	-	-	-	-	+	-
Ribitol	+	+/-	-	-	+	+
Xylitol	+	-	-	-	+	-
L - Arabinitol	+	+	-	-	-	-
D - mannitol	+	+	-	-	+	+
D - Gulcitol	-	+	-	-	+	+
Myo - inositol	-	-	-	-	-	-
Succinate	+/-	+	-	+	+	+/-
Citrate	+/-	+/-	-	+/-	+/-	+/-
Methanol	-	-	-	-	-	-
Ethanol	+	-	-	+	+	+
Nitrate	-	-	-	-	-	-
Nitrite	-	-	-	-	-	-
Ethylamine	+	+	-	+	-	+
L - lysin	-	-	-	+	-	+
Creatine	+	-	+	-	-	-
Creatinine	-	-	-	-	-	-
Cadaverine	+	+	+	+	+	+
without thiamine	+	-	+	+	+	+
without pantothenate	+	+	+	+	+	+
without myo - inositol	+	+	+	+	+	+
without pyridoxine	+	+	+	+	+	+
without Niacin	+	+	+	+	+	+
without para amion-benzoic acid	+	+	+	+	+	+
Assimilation test of nitrogen source						
Nitrate	-	-	-	-	-	-
Nitrite	-	-	-	-	-	-
Ethylamine	+	+	-	+	-	+
L - lysin	-	-	-	+	-	+
Creatine	+	-	+	-	-	-
Creatinine	-	-	-	-	-	-
Physiological characteristics						
Cadaverine	+	+	+	+	+	+
without thiamine	+	-	+	+	+	+
without pantothenate	+	+	+	+	+	+
without myo - inositol	+	+	+	+	+	+
without pyridoxine	+	+	+	+	+	+
without Niacin	+	+	+	+	+	+
without para amion-benzoic acid	+	+	+	+	+	+

Of the 54 preterm neonates, 30 (55.5%) were colonized with *Candida*, which was more than colonization in full term neonates (7/18; 38.8%) and post term neonates (14/28; 50%). The most common *Candida* species isolated from the 30 colonized preterms was *C. albicans* (18; 60%) followed by *C. tropicalis* (7; 23.3%), *C. glabrata* (2; 6.7%), *C. lusitamae* (2; 6.7%), and *C. krusei* (1; 33.3%).

Table 4. Confirmatory tests for identification and pathogenic potentiates of Candida strains isolated from the study neonates in NICU

Candida strains	Germ Tube test	Hydrolysis of Urea	Ascospore formation	Chlamydospore formation	Casein hydrolysis	Produce proteolytic enzyme	Produce lipolytic enzyme	Growth at 37°C	Growth on Cycloheximide medium
C. albicans	+	-	-	Chlamydoconidia	+	+	+	+	+
C. tropicalis	-	-	-	Blastoconidia	+	+	+	+	+
C. glabrata	-	-	-	Blastoconidia	-	+	+	+	-
C. krusei	-	+/-	-	Blastoconidia	+	+	+	+	-
C. lusitaniae	-	-	-	Blastoconidia	-	+	+	+	-
C. parapsilosis	-	-	-	Blastoconidia	+	+	+	+	-

Table 5. Neonatal risk factors for *Candida* colonization of the study neonates

	Early Colonization		95% CI	p
	No (n=73)	Yes (n=27)		
Sex			0.219–1.340	0.182
Male	35 (48%)	17 (63%)		
Female	38 (52%)	10 (37%)		
Weight			0.553–4.982	0.363
≤1.5 kg	20 (27.4%)	5(18–5%)		
>1.5 kg	53 (72.6%)	22(81.5%)		
Preterm	37 (51%)	17(63%)		0.549
Fullterm	14 (19%)	4(15%)		
Postterm	22 (30%)	6 (22%)		
Delivery			0331–3.866	0.840
Vaginal	12 (16.4%)	4(15%)		
CS	61 (83.6%)	23 (85%)		
Late colonization				
Sex			0.683–4.375	0.245
Male	42 (55%)	10 (42%)		
Female	34 (45%)	14 (58%)		
Weight			0.058–0.437	0.001
≤1.5 kg	12(16%)	13 (54%)		
>1.5 kg	64 (84%)	11 (46%)		
Preterm	41 (54%)	13(54.2%)		0.653
Fullterm	15 (20%)	3(12.5%)		
Postterm	20 (26%)	8 (33.3%)		
Delivery			0.199–2.081	0.459
Vaginal	11 (14.5%)	5(21%)		
CS	65 (85.5 %)	19(79%)		
TPN	15 (20%)	11(46%)	1.289–9.184	0.011

CS: cesarean section; TPN: total parenteral nutrition

Neonatal risk factors related to early and late colonization as determined by univariate analysis are presented in Table 6. No risk factors was identified for early colonization, However, late colonization was significantly associated with BW <1.5 kg and total parenteral nutrition (Table 5).

Antifungal susceptibility testing

Candida albicans and *C. tropicalis* were found to be highly susceptible to azoles, especially to itraconazole in contrast to *C. glabrata* and *C. krusei*, which were totally resistant to azoles. Neonatal isolates of *Candida* species exhibited decreased susceptibility to amphotericin B where only 40% of *C. albicans*, 22% of *C. tropicalis* and none of *C. glabrata* and *C. krusei* isolates were sensitive to amphotericin (Table 6).

As regards to antifungal drug susceptibility of the 51 isolated *Candida* spp., 35 (68.6%) were sensitive to fluconazole, 41 (80%) to itraconazole and 33 (64.7%) to ketoconazole, while only 17 (33%) of *Candida* spp. were sensitive to amphotericin B.

As regards to the *Candida* species isolated from hands of nurses, *C. tropicalis* was sensitive to the azoles while the two *C. glabrata* were resistant to all antifungal drugs tested. These isolates gave the same pattern of susceptibility as that of the same spp. lately colonizing the neonates at the same week of sampling, suggesting a possible source of colonization.

DISCUSSION

Candida species generally colonize the skin, gastrointestinal tract, lower female genital tract, intertriginous areas (eg, groin and armpits), and the foreskin of the uncircumcised male. Similar to adults and older children, colonization usually precedes invasive fungal infection. Neonates are generally infected with a clone with which they had previously been colonized.[11,15] In infants admitted to the NICU, colonization occurs in 30% to 60% of patients. Many studies have shown that the rate of colonization is dependent upon the birth weight of the infant. A retrospective analysis of weekly surveillance fungal cultures of the skin, gastrointestinal tract, respiratory tract, and umbilicus performed in 50 extremely low birth weight (ELBW) infants (birth weight below 1,000 g) from birth to six weeks of age demonstrated colonization by a Candidal species in 62 percent of infants by six weeks of age.[16] Approximately 85 percent of the colonization occurred in the first two weeks of life. The skin and gastrointestinal tract were the first sites colonized, followed by the respiratory tract. Colonization was inversely related to gestational age. In a study of VLBW (birth weight below 1,500 g) infants, approximately 27 percent of patients were colonized with a candidal species, of which one-third developed mucocutaneous candidiasis and 8% invasive candidal infection[15]. The level of colonization is an important factor in developing invasive disease. The greater the density of organisms, the more likely it is that the organism

Table 6. Antifungal sensitivity of *Candida* species isolated from neonates in the NICU

Candida spp.	Fluconazole			Itraconazole			Ketoconazole			Amphotericin B		
	S n (%)	I n (%)	R n (%)	S n (%)	I n (%)	R n (%)	S n (%)	I n (%)	R n (%)	S n (%)	I n (%)	R n (%)
C. albicans (n= 30)	24 (80)	6 (20)	-	30 (100)	-		24 (80)	6 (20)		12 (40)	18 (60)	-
C.tropicalis (n=9)	9 (100)		- -	9 (100)	-		7 (78)	2 (22)		2 (22)	7 (78)	-
C.glabrata (n=8)	-	-	8 (100)	- -	8 (100)		-	8 (100)		-	1 (12.5)	7 (87.5)
C.krusei (n=1)	-	-	(100)		1 (100)		-	1 (100)			1 (100)	-
C.lusitaniae (n=2)	2 (100)	-	-	2 (100)	-		2 (100)	-		2 (100)	-	-
C.parapsilosis (n=1)	-	-	1 (100)	-	1 (100)		-	1 (100)		1 (100)	-	-

S: Sensitive; I: Intermediate; R: Resistant

will penetrate the host epithelial barriers, spread to the underlying tissue, and be disseminated through the blood stream.

In the present study about half of the 100 neonates admitted to the NICU were colonized with *Candida*. Early colonization was found in 27 (27%) neonates whereas 24 (24%) neonates were lately colonized during their stay in NICU. In a similar study done in during the same period by Mohamed et al[17] they found that 62.5% of patients have become colonized by fungi during their stay. Mahieu et al[4] also reported a much lower rate of early *Candida* colonization where only 1.2% of neonates were colonized. This notable difference might be attributed to their cultures included umbilical, ear, groin and axillary swabs, without culturing the oral mucosa which is a main site of early colonization. In addition, variation in the rate of colonization depends upon management protocol and nature and intensity of routine antifungal antiseptics measures applied in a particular setup.[5]

Fifty-four percent of study neonates were colonized with *Candida* at one site, while 45% were colonized at more than one site. Although the clinical relevance of *Candida* colonization at one site is limited, the relationship between multisite colonization and subsequent development of candidemia has been demonstrated by several investigators.[18,19]

Perianal area and oral cavity were the most frequently colonized sites (60% and 56%, respectively). This high rate of early gastrointestinal colonization is in accordance with observations of others who found rates up to 90% when rectal culture was done at birth.[1,16] Gastrointestinal tract can serve as a reservoir from where the fungus can spread, particularly if there is a breach in mucosal lining[20]. The critical role of such colonization was confirmed by Saiman et al[10] where GIT colonization was a risk factor for candidemia and molecular typing revealed that a high proportion of neonates had preceeding *Candida* colonization with the same clone that caused candidemia.

Overall, *C. albicuns* was the most frequent species (58.8%) isolated from the study neonates followed by *C. tropicalis* (17.6%), *C. glabrata* (15.6%), *C. lusitaniae* (4%), and *C. kmsei* and *C. parapsilosis* (2% each). In concordance with our findings is the study of Farmaki et al[6] where the most frequent isolates were *C. albicans* (42%), *C. tropicalis* (24%), *C. kmsei* (11%), *C. parapsilosis* (7%), and *C. glabrata* (7%) while *C. htsitanitie* was isolated in one neonate in association with *C. tropicalis*.

In early colonization, *C. albicans* was the most common spp. (92.6%), which agrees with that reported by Mahieu et al[4], where *C. albicans* was also the main colonizing spp. at birth (82%). On the other hand, the non-*C. albicuns* predominated in late colonization; *C. tropicalis* (33.3%), and *C. glabrata* (29.2%) while *C. albicans* represented 20.8%. In line with our findings, Mohamed et al[17] reported that the non- *C. albicans* were the most commonly acquired fungi during the stay in PICU; *C. glabrata* (14.3%), *C.tropicalis*, (12.5), and *C.krusei* (7.1%) vs *C.albicans* (8.9%). An increase in the frequency of the non-albicans spp. isolated from neonates with late colonization was also demonstrated by Farmaki et al representing 59%.[6]

In accordance with our study, other investigators reported that most of the neonates who exhibit early colonization are colonized by *C. albicans*, presumably as being the most frequent species that colonizes the maternal vagina.[4,6,21] In contrast, due to close relation of non- *C. albicans* species with NICU practices and materials, these strains more frequently colonize and infect neonates staying in the NICU for longer periods where horizontal transmission takes place.[6]

The increase in the non- *C. albicans* in several NICUs might also be attributed to the possibility of selection of less susceptible species by the extensive use of antifungal prophylaxis. Furthermore, the increasing use of several diagnostic and therapeutic interventions such as central venous catheter and parenteral nutrition might explain the shift away from *C. albicans* towards other species.[22]

The isolation of *C. tropicalis* in the present study is noteworthy, being more virulent than *C. albicans* and disseminated infection is associated with high mortality rates.[23] Likewise is the isolation of *C. glabrata* which has been reported as a significant nosocomial pathogen in pediatric patients causing high mortality due to the development of

a secondary resistance to fluconazole.[10] Although only one of each of *C. krusei* and *C. parapsilosis* was isolated, yet it also must be noted too. *C. krusei* has been increasingly incriminated in serious disseminated infections while *C. parapsilosis* is considered a relevant nosocomial pathogen and the most frequent *Candida* colonizing catheters owing to its ability of biofilm formation.[24]

The above data show that preterm neonates colonization (55.5%) was more than in full term neonates (38.8%) and post term neonates (50%) and *C. albicans* was the most common colonizing species (60%), followed by *C. tropicalis* (23%). In the study of Mendiratta et al,[5] colonization in preterm (33.9%) was significantly higher than in term neonates (10%) and *C. albicans* was also the main isolate (45.9%) followed by *C. tropicalis* (21.6%). Manzoni et al[3] also reported *C. albicans* as the most frequent isolated spp. (83.5%), followed by *C. parapsilosis* (15.9%) and *C. glabrata* (7.9%). Increased colonization in preterm has been attributed to relative immunodeficiency such as decreased function of neutrophils and relative quantitative deficiency of maternal IgG to *Candida*.[25]

No risk factors in the present study were associated with early colonization. In the study of Farmaki et al[6] and Mahieu et al,[4] vaginal delivery was identified as a risk factor for early colonization and most likely represents acquisition during delivery. Its in line with what was stated by Saiman et al[10] that delivery via cesarean section (CS) was protective from *Candida* colonization at birth. However, the association could not be detected in this study probably because more than 80% were born by CS.

Lower gestational age at birth was a significant risk factor for early neonatal colonization in the study of Mahieu et al.[4] Although, not considered as risk factor in the present study, early colonization was more associated with preterms. The increased colonization in neonates of low gestational age can be explained by vulnerability of this group to critical illness that subject them to more invasive devices in NICU and aggressive antibiotic use predisposing them to fungal colonization.

Low BW and total parenteral nutrition administration were identified as risk factors for late colonization in the present study. LBW was also demonstrated by Saiman el al[10] as a risk factor for *Candida* colonization and BW <1,500g was the only independent risk factor for late colonization in the study of Farmaki et al.[6] The longer stay of low BW neonates in the NICU might explain why they are colonized by *Candida* spp. more frequently than larger neonates. In addition, degree of prematurity, central venous catheters and other factors could all be contributory.[6] Total parenteral nutrition was significantly associated with *Candida* colonization. Saiman et al postulated that loss of normal GIT flora due to delayed enteral feeding may facilitate *Candida* species colonization.[10]

Antibiotics, especially third generation cephalosporin administration were found to be associated with colonization in neonates in many studies.[6,10,11] Antibiotics are known to suppress immune system[25] and administration to already compromised neonates promotes colonization,[5] in addition to inhibition of the endogenous microflora and unopposed flourishing of *Candida.*[26] However, an association could not be detected in the present study, as 89% of the study neonates have received third generation cephalosporin on admission.

Of the isolated *Candida* species, 68.6% were sensitive to fluconazole, 80% to itraconazole and 64.7% to ketoconazole, while only 33% were sensitive to amphotericin B. *Candida* isolates from NICU and PICU in the study of Kuzucu et al[12] showed a higher susceptibility pattern where 93% and 82% were susceptible to fluconazole and itraconazole, respectively, while all were sensitive to amphotericin B. These results come in line with the study of Farmaki el al[6] in NICUs, where *C.albicans* was highly susceptible to azoles. Kusucu et al[12] reported that the sensitivity of *C. albicans* isolated from patients in NICU and PICU was 86.7% and 73.4% to fluconazole and itraconazole, respectively.

About third of *Candida* species isolated in the present study were sensitive to amphotericin B which is inconsistent with the findings of Farmaki et al[6] and Kuzucu et al[12] where *Candida* isolates from NICU and PICU patients remained completely susceptible to amphotericin B. Only 40% of *C. albicans* and 22% of *C. tropicalis* isolated in the present study were sensitive to amphotericin B.

No *Candida* was isolated from the environmental surfaces in NICU, however from the hands of HCWs, one *C. tropicalis* and two *C. glabrata* were isolated. These isolates had the same antifungal susceptibility pattern as the same species lately colonizing the neonates at the same time of sampling, indicating a probable source of colonization. In the study of Saiman et al[10] 29% of the HCWs hands were positive for *Candida*, where *C. parapsilosis* was cultured from 19%, *C. albicans* from 5%, while *C. tropicalis* was cultured from <1%.

Huang et al[11] reported that acquisition of *C. tropicalis* very likely occurred in the NICU by cross-contamination. This was also suggested by Roilides et al[27] in a molecular epidemiologic study of an outbreak of infection caused by *C. tropicalis* in NICU. Mendiratta et al[5] reported no samples from NICU or hands of HCW were positive for *Candida*. Although, the examination of the hands of HCWs during the study period failed to document a common source of any *Candida* species, Farmaki et al[6] could not exclude transient hand carriage of personnel. They suggested nosocomial acquisition of *Candida* predominantly non-albicans *Candida,* as a larger percentage (14.2%) of neonates were colonized at late stage during their NICU stay than at an early stage (2.5%).

As horizontal transmission from colonized hands of HCWs or, less commonly, from contaminated or surfaces, has been reported especially in the sicker neonates who require more handling by HCWs.[10] Thus the importance of hand washing and compliance with guidelines for preventing nosocomial transmission must be emphasized in NICUs.

In conclusion, *Candida* has emerged as a common cause of infections in infants admitted to NICU, particularly preterm infants. Neonatal infections caused by non-albicans species occur at a later age during their stay in NICU and are more likely to be acquired from HCWs.

Most candidal infections are due to vertical transmission from the mother. *Candida* generally colonizes the skin, gastrointestinal tract, lower female genital tract, intertriginous areas, and the foreskin of uncircumcised males. The rate of colonization increases with decreasing birth weight. Infants admitted to the NICU, especially premature infants, are at increased risk for candidal infections

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Conflict of interest
The author confirms no conflict of interest in this study.

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