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Presence of *Malassezia* Species in Patients Admitted to the Neonatal Intensive Care Unit and Antifungal Sensitivity of *Malassezia furfur*

Yenidoğan Yoğun Bakım Ünitesinde *Malassezia* Türlerinin Varlığı ve *Malassezia furfur*'un Antifungal Duyarlılığı

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Abstract

Introduction: Prophylactic administration of fluconazole in very low birth weight newborns (<1,500 g) in the neonatal intensive care unit (NICU) greatly reduces the incidence of fungal infections. However, the number of cases of neonatal fungemia caused by *Malassezia furfur* (*M. furfur*), a lipid-dependent species of *Malassezia*, is increasing. The presence of antifungal resistance among yeasts may also cause treatment failure. Thus, we aimed to evaluate the rate of skin colonization by *Malassezia* species, factors associated with its colonization, and antifungal sensitivities of *M. furfur* strains in our hospital's NICU.

Materials and Methods: This study included 150 newborns admitted to the NICU. Swabs of the skin surface were collected on the day of hospitalization and inoculated on mDixon agar which was incubated for one week. Conventional tests and MALDI-ToF analysis were used to identify *Malassezia* species. Antifungal susceptibility tests were performed using RPMI-1640 medium enriched with fatty acids and resazurin (RPMI++).

Results: *Malassezia* species colonization was detected in 33.3% of the included newborns (n=50). *M. furfur* was the most frequently isolated strain (n=16, 32.0%), followed by *Malassezia sympodialis* (n=13, 26.0%), *Malassezia restricta* (n=9, 18.0%), *Malassezia obtusa* (n=6, 12.0%), and *Malassezia globosa* (n=6, 12.0%). *M. furfur* was isolated from 12 (19.04%) newborns receiving total parenteral nutrition (n=63) ($p<0.05$). *Malassezia* species colonization was observed in 39.2% of the fluconazole-naïve neonates and 10% of the infants in the prophylaxis group ($p<0.05$). Fluconazole demonstrated a high MIC_{90} value (32 µg/ml) for *M. furfur* strains.

Conclusion: *Malassezia*-associated infections may be masked because microbiological cultures for *Malassezia* species are not frequently performed. Thus, screening for *Malassezia* species in newborns admitted to NICUs and determining its antifungal resistance patterns will aid in establishing treatment procedures.

Keywords: NICU, *Malassezia furfur*, fluconazole, resistance

Öz

Giriş: Yenidoğan yoğun bakım ünitelerindeki (YYBÜ) çok düşük doğum ağırlıklı yenidoğanlarda flukonazol profilaksisi kullanımının mantar enfeksiyonu insidansını büyük ölçüde azalttığı gösterilmiştir. Bununla birlikte, *Malassezia*'nın lipid bağımlı türlerinden biri olan *Malassezia furfur*'un neden olduğu artan sayıda yenidoğan fungemisi olgusu bildirilmektedir. Mayalar arasında antifungal direncin varlığı da tedavi başarısızlığına neden olabilir. Bu çalışmanın amacı, hastanemiz YYBÜ'de *Malassezia* türleri ile deri kolonizasyonu oranını, ilişkili faktörleri ve *M. furfur* suşlarının antifungal duyarlılıklarını değerlendirmektir.

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Öz

Gereç ve Yöntem: Bu çalışmaya YYBÜ'de takip edilen 150 yenidoğan dahil edildi. Örnekler hastaneye yatış gününde deri yüzeyinden sürüntü yöntemi ile alınarak mDixon agar besiyerinde bir hafta süreyle kültüre edildi. *Malassezia* türlerini tanımlamak için geleneksel testler ve MALDI-ToF analizi kullanılmıştır. Antifungal duyarlılık testleri yağ asitleri ve resazurin ile zenginleştirilmiş RPMI-1640 besiyerinde (RPMI++) yapıldı.
Bulgular: *Malassezia* sp. kolonizasyonu 150 yenidoğanın 50'sinde (%33,3) bulundu. En sık *M. furfur* izole edilirken (16; %32,0), bunu *Malassezia sympodialis* (13; %26,0), *Malassezia restricta* (9; %18,0), *Malassezia obtusa* (6; %12,0) ve *Malassezia globosa* (6; %12,0) izlemiştir. Total parenteral beslenme alan 63 yenidoğanın 12'sinde (%19,04) *M. furfur* kültürü yapılmıştır (p<0,05). Flukonazol almayan yenidoğanların %39,2'sinde *Malassezia* sp. kolonizasyonu gözlenirken, profilaksi grubundaki bebeklerin sadece %10'unda gözlenmiştir (p<0,05). Flukonazolün *M. furfur* suşları için 32 µg/ml gibi yüksek bir MIC_{90} değerine sahip olduğu bulunmuştur.
Sonuç: Mikrobiyoloji laboratuvarlarında *Malassezia* kültürlerinin sıklıkla yapılmaması nedeniyle *Malassezia* ile ilişkili enfeksiyonlar maskelenecektir. Yenidoğan yoğun bakım ünitelerinde *Malassezia* taraması yapılması ve antifungal direncin mevcut olup olmadığının belirlenmesi, tedavi prosedürlerinin oluşturulması sürecinde yardımcı olacaktır.
Anahtar Kelimeler: YYBÜ, *Malassezia furfur*, flukonazol, direnç

Introduction

Neonatal infection or sepsis is a clinical syndrome characterized by the presence of systemic signs and symptoms of infection within the first month of life and the isolation of a specific agent from blood culture. It is diagnosed via a combined evaluation of clinical and laboratory data[1,2]. The mortality rates associated with neonatal infection vary across different units and time periods. Furthermore, it is inversely proportional to gestational age. The mortality rate is 2.5-3% in term infants and 24-54% in premature infants, depending on the causative agent. Bacterial infections are the most common cause of neonatal infections, and fungal infections are the third most common cause of late-onset neonatal infections. Although fungal infections occur in only 4-8% of newborns with extremely low birth weight (<1,000 g), the mortality rate can reach 30%. Invasive fungal infections are often attributed to *Candida* species. Nevertheless, there have been rare reports of *Malassezia* yeasts as the etiological agent[2,3]. Although fluconazole (FLZ) prophylaxis in these facilities considerably reduces the prevalence of fungal infections, there is a risk of mortality in the presence of antifungal-resistant strains. Very low birth weight, prematurity, use of intravenous lipid solutions, skin emollients, or broad spectrum antibiotics, gestational age, immunosuppression, and prolonged neonatal intensive care unit (NICU) stay are significant risk factors for the development of fungal infections[4-7].

Early diagnosis of neonatal infections is possible using culture-based methods. Approximately 94% of the pathogens can be identified within 36 hours via blood culture, which can guide empirical neonatal antibiotic therapy[8]. Although blood culture methods are commonly used for bacterial pathogens, mycotic agents can also be cultured on these media. Sabouraud dextrose agar is routinely used for yeast culture in clinical microbiology laboratories. Addition of lipids to the blood culture medium can help detect rare fungemia agents such as lipid-dependent *Malassezia furfur*[9].

The colonization of *Malassezia* species, a basidiomycetous yeast fungus, exhibits notable variations on the basis of factors such as geographical location, age, social behaviors, and high-risk populations. At the molecular level, colonization in newborns is of maternal origin and commonly occurs during breastfeeding[10]. *Malassezia* species colonization begins in the first week of life and causes clinical conditions such as cephalic pustulosis in newborns due to regionally elevated skin sebum levels[5,11,12].

Identification of antifungal resistance in *Malassezia* species will provide valuable guidance regarding the use of prophylactic antifungal administration in clinical settings. The RPMI-1640 medium is a standard medium utilized for antifungal susceptibility testing of yeasts and filamentous fungi that is recommended by the European Committee on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Institute (CLSI). However, *Malassezia* species do not grow on the RPMI-1640 medium due to lipid dependency and differences in growing time. Consequently, the standardization of antifungal drug susceptibility testing for *Malassezia* species is lacking, and definitive clinical breakpoints are yet to be established. Several reports have demonstrated the *in vitro* antifungal activities of amphotericin B and azole antifungals in *Malassezia* species. However, this inhibition has not been observed in echinocandins[13-15]. If the minimum inhibitory concentration (MIC) at which 90% of the isolates are inhibited (MIC_{90}) of various antifungals is high, it most likely reflects neonatal colonization. Although this approach was most prominently observed with *Malassezia pachydermatis*, it may be explained by the fact that *M. pachydermatis* does not require lipids for growth, resulting in its more frequent isolation[2]. In neonates, these data remain limited, irrespective of their admission to the intensive care unit[16].

The aim of this study was to determine the skin colonization rate of *Malassezia* species, factors associated with its colonization, and the antifungal sensitivity of *Malassezia furfur* in neonates admitted to our hospital's NICU.

Materials and Methods

This cross-sectional study was conducted in a single NICU at the Pamukkale University Hospital, and it included 150 infants. The demographic (e.g., sex and birth weight) and clinical (e.g., gestational age, age at the time of clinic admission, FLZ prophylaxis, total parenteral nutrition (TPN), and concomitant disease) data of the neonates were collected. Neonates with a compromised epidermal integrity or skin infection were excluded from the study. According to the birth weight, the newborns were grouped as follows: extremely low birth weight, <1,000 g; very low birth weight, <1,500 g; and low birth weight, <2,500 g[3].

Skin samples were collected from newborns admitted to the NICU[17-19]. Rayon swabs (plain sterile; Copan Diagnostics, Italy) soaked in 2 ml sterile saline were used for sampling by moving the swab back and forth over a 1-inch stroke area for 20 strokes, while constantly rotating the swab. The samples were transferred to the laboratory within 2 h and inoculated on mDixon agar with antibiotic (3.6% malt extract, 1% mycological peptone, 2% bovine bile, 1% tween 40, 0.2% glycerol, 0.2% oleic acid, 1.5% agar, and 0.02% chloramphenicol; pH 6.0). Subsequently, the medium was placed in a humid incubator for 2 weeks. Colony growth was checked at two-day intervals. *Malassezia* colonies were identified by conventional methods, including Gram staining, Tween assimilation tests, beta-glucosidase activity evaluation, and ability at 40 °C[20]. Additionally, *M. furfur* strains were confirmed using matrix-assisted laser desorption/ionization time of flight (MALDI-ToF) mass spectrometry (MALDI Biotyper, Bruker Daltonics, Bremen, Germany) with a log score of >2.0[21,22].

The optimized colorimetric microdilution broth (RPMI++) described by Leong et al.[13] was used to perform antifungal susceptibility testing for *M. furfur* strains. The basis of the broth medium was filtered RPMI-1640 with 0.165 M MOPS without sodium bicarbonate (Multicell, Wisent Inc., Canada). To obtain RPMI++, the following components were added: 2% glucose, 1% glycerol, 2% oleic acid, 0.5% Tween 60, 0.2 mg/ml sodium bicarbonate, and 12.5 µg/ml resazurin (pH 6.25)[13].

The antifungals tested in the assay were amphotericin B, itraconazole, posaconazole, voriconazole, ketoconazole (concentration range, 0.06-64 µg/ml), FLZ, and terbinafine (0.125-128 µg/ml). Dimethyl sulfoxide (Sigma) was used in 100X stock solutions of antifungals, which were stored at -80 °C. The final *M. furfur* inoculum concentration used for antifungal tests was adjusted to $5.0x10^3$. The suspensions were adjusted of the optical density at 630 nm by measurements (ELx808, BioTek, USA). To verify, a 10 µl sample of the diluted inoculum (1:10 ratio) was inoculated on mDixon agar and incubated at 33 °C for 4-7 days. After 48 h, the MICs were determined visually on the basis of the colorimetric color change. In accordance with CLSI standards, a 50% growth inhibition was considered the MIC value for azoles and echinocandins, and a complete growth inhibition was deemed the MIC value for amphotericin B[13,23]. *M. furfur* CBS 1,878 and *Candida parapsilosis* ATCC 22,019 were used as quality control strains for antifungal susceptibility testing.

Statistical Analysis

All statistical analyses were performed using MiniTab (Ver16.0; Cologne, Germany). Univariate analysis and Kruskal-Wallis test were used to evaluate the data. The results are presented as the mean, MIC at which 50% of the isolates were inhibited (MIC_{50}), and MIC_{90}. A p-value of <0.05 was considered statistically significant.

Results

A total of 88 males and 62 females were enrolled in the study, with a mean birth weight of 2,370.12±887.06 g (range, 520-3,850 g) and a mean gestational age of 34.90±4.25 weeks. A total of 40 infants (32.5%) were delivered via cesarean section. Sixty-three of the included newborns (42.0%) had a central venous catheter for TPN. Among the included infants, 77 (51.3%) were low birth weight, 39 (26.0%) were very low birth weight, and 22 (14.7%) were extremely low birth weight. Thirty (20.0%) infants were administered FLZ prophylaxis on the sampling day. The samples were obtained from 114 infants (76.0%) who were <2 days old, 16 infants (10.7%) who 2-7 days old, and 20 infants (13.3%) who were >7 days old.

Malassezia species were isolated from the samples of 50 newborns (33.3%) admitted to the NICU. The most prevalent isolate was *M. furfur* (n=16, 32.0%), followed by *Malassezia sympodialis* (n=13, 26.0%), *Malassezia restrica* (n=9, 18.0%), *Malassezia obtusa* (n=6, 12.0%) and *Malassezia globosa* (n=6, 12.0%). Table 1 presents the prevalence of *Malassezia* sp. colonization among newborns in relation to various factors. *Malassezia* sp. colonization significantly increased (p<0.05) in infants with a lower age (number of days) at the time of admission to the clinic. Furthermore, *Malassezia* species colonization in the newborns with low birth weight was higher than expected (p<0.05).

Malassezia furfur was isolated more frequently in neonates who received TPN (n=12/63, 19.04%) than in those who did not receive TPN; there was no such difference among the other species (p<0.05). Table 2 presents the distribution of different *Malassezia* species colonizing neonates according to various factors. *M. furfur* was isolated from all newborns admitted to the NICU who were delivered via cesarean section. *M. furfur* was isolated in three of the 30 newborns (10.0%) who were

administered FLZ prophylaxis; no other species were isolated. *Malassezia* species (*M. furfur*, n=13) was isolated in 47 newborns (39.2%) who were not administered FLZ (Table 1). This difference in colonization rate was statistically significant (p<0.05).

Antifungal susceptibility assessment using the RPMI++ medium, in which the color change due to reduction of resazurin was assessed, revealed sharp values for the interpretation of MIC values. The mean MIC values of amphotericin B, ketoconazole,

Table 1. Factors associated with *Malassezia* species colonization in newborns

Variables		n (%)	Odds ratio	Relative risk	p
Sex	Male (n=88)	29 (33.0)	1.04	0.973	0.907
	Female (n=62)	21 (33.9)			
TPN	Present (n=63)	24 (38.1)	1.44	1.27	0.292
	None (n=87)	26 (29.9)			
Delivery type	Cesarean (n=123)	40 (32.5)	1.22	1.14	0.203
	SVB (n=27)	10 (37.0)			
Gestational age, week	38-42 (n=49)	14 (28.6)	0.72	0.80	0.389
	<38 (n=101)	36 (35.6)			
Birth weight, g	2,500-4,000 (n=12)	26 (33.8)	-	-	0.069
	1,500-2,500* (n=77)	18 (46.2)			
	1,000-1,500** (n=39)	3 (13.6)			
	<1,000*** (n=22)	3 (25.0)			
Sampling day#	<2 (n=114)	23 (20.2)	-	-	0.001
	2-7 (n=16)	10 (62.5)			
	>7 (n=20)	17 (85.0)			
Fluconazole prophylaxis	Received (n=30)	3 (10.0)	0.26	0.17	0.004
	Not received (n=120)	47 (39.2)			

#Age (days) at time of clinic admission. *low birth weight, **very low birth weight, ***extremely low birth weight.

n: Number of patients in whom *Malassezia* species was identified, TPN: Total parenteral nutrition, SVB: Spontaneous vaginal birth

Table 2. *Malassezia* species (n=50) isolated in newborns according to various factors

		M. furfur (n=16)	*M. sympodialis* (n=13)	*M. restricta* (n=9)	*M. obtusa* (n=6)	*M. globosa* (n=6)
Sex	Male (n=88)	8	5	5	6	5
	Female (n=62)	8	8	4	-	1
TPN	Present (n=63)	12	7	-	3	2
	None (n=87)	4	6	9	3	4
Delivery type	Cesarean (n=123)	16	12	5	3	4
	SVB (n=27)	-	1	4	3	2
Gestational age, week	38-42 (n=49)	2	2	5	2	3
	<38 (n=101)	14	11	4	-	4
Birth weight, g	2,500-4,000 (n=12)	3	7	8	4	4
	1,500-2,500* (n=77)	7	6	1	-	2
	1,000-1,500** (n=39)	3	-	-	-	-
	<1,000*** (n=22)	3	-	-	-	-
Sampling day#	<2	14	9	-	-	-
	2-7	2	3	-	2	3
	>7	-	1	9	4	3

#Age (days) at date of clinic admission. *low birth weight, **very low birth weight, ***extremely low birth weight.

TPN: Total parenteral nutrition, SVB: Spontaneous vaginal birth

Table 3. MIC values of the antifungals for the 16 isolated *Malassezia furfur* strains

Antifungal (µg/ml)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64	Mean	MIC_{90}
Amphotericin B		2	3	5	5		1						0.39	0.5
Ketoconazole	4	2	2	4	3	1							0.25	0.5
Itraconazole	7	6		1		2							0.18	1
Voriconazole	1	4	6	5									0.14	0.25
Posaconazole	7	1	1	5	2								0.17	0.5
Fluconazole							1	3	3	2	6	1	20.4	32
Terbinafine						1	1	5	6	2	1		8.4	16

MIC: Minimum inhibitory concentration

Table 4. Reported colonizations and infections caused by *Malassezia furfur* in newborns admitted to the NICU at various geographical locations

Country	Year	Author(s)	Notable *M. furfur* presence
USA	1987	Powell et al.[40]	*M. furfur* colonization rate: 36.8%
Finland	1990	Ahtonen et al.[41]	After spending 20 days in the NICU, the *M. furfur* colonization rate was 70%
USA	1996	Shattuck et al.[42]	Skin surveillance cultures. Only *M. furfur*: 46%. *M. furfur* + *Candida* species: 18%
Argentina	2006	Guisiano et al.[31]	Catheter colonization rate: *M. furfur*, 2.2% and *M. sympodialis*, 2.2%
India	2014	Gupta et al.[17]	*Malassezia* species colonization rate during stay in NICU: 38%
Martinik	2016	Benjamin et al.[5]	*Malassezia* species colonization rate: 46%
Russia	2016	Rodchenko et al.[36]	Isolation rate (2012-2015): 5.9%
Russia	2018	Priputnevich et al.[37]	*M. furfur*-related NICU morbidity: 2.4%
Taiwan	2020	Chen et al.[4]	Catheter-related *M. furfur* colonization (n=19) or fungemia (n=1) was assessed. *M. furfur* colonization increased after prophylactic fluconazole use.
Iran	2021	Zomorodian et al.[10]	Colonization rates: *M. globosa*, 62.9%; *M. furfur*, 23.8%; *M. restricta*, 8.7%; *M. arunalokei*, 2.1%; and M. sympodialis, 2.1%
Italy	2022	Auriti et al.[27]	Infections were confirmed with fungal culture. *Malassezia* species colonization rate: 21.7%

NICU: Neonatal intensive care unit

itraconazole, voriconazole, posaconazole, and terbinafine were 0.5, 0.5, 1, 0.25, 0.5 and 16 µg/ml, respectively (Table 3). FLZ demonstrated a high MIC_{90} value (32 µg/ml) for *M. furfur*. One *Malessezia* strain was associated with a high MIC value (64 µg/ml) for FLZ in newborns with birth weights >2,500 g who did not receive FLZ prophylaxis.

Discussion

In this study, the rate of *Malassezia* species colonization in newborns admitted to our hospital's NICU was 33%. The presence and diversity of *Malassezia* species in newborns are highly variable and depend on the mothers with whom they are in direct contact and the environment[11,17,18,24]. Gupta et al.[17] reported that *Malassezia* species, which were not found at the time of birth, were isolated in 38% of the newborns who were hospitalized for 1 week in the NICU in Chandigarh, India. The colonization rate increased with the increase in duration of hospitalization, and the most frequently isolated strain (47 of the 48 *Malassezia* species) was *M. furfur*. However, in the study by Bernier et al.[12], *M. sympodialis* and *M. globosa* were predominantly isolated from a similar population in Bordeaux, France. In a study in Japan, Nagata et al.[24] demonstrated the presence of *Malassezia* species DNA that originated from the mother's vulva in the newborns. This emphasized the need of molecular methods to detect the presence of *Malassezia* species that cannot be isolated via culture. Nagata et al.[24] also reported that *M. globosa* and *M. restricta* are the common causative agents of neonatal infection in Japan, with *M. restricta* predominance in newborns aged >30 days. Furthermore, *Malassezia* skin colonization increases until the age of 6-12 months. In the study by Zomorodain et al.[18], *M. furfur* (88.06%) was the most isolated species in Shiraz, Iran, followed by *M. globosa* (10.48%), *M. obtusa* (0.73%), and *M. slooffiae* (0.73%). The species and proportions described by Zomorodain et al.[18] are the same

as those isolated in our study. Genetic analysis of *M. furfur* demonstrates that the environment significantly influences the genetic variation associated with related diseases, host ethnicity, and geographic origin. Based on microbiological culture data, *M. furfur* is isolated more frequently in the Southern Hemisphere than in the Northern Hemisphere. Although geographical and ethnic differences in adults reportedly affect the colonization of *Malassezia* species in relation to skin lipid content, there are no such ethnicity-related data in newborns[25,26].

Data regarding the colonization and infections caused by *Malassezia* sp. in the NICUs of different countries is lacking (Table 4). In a study of 541 newborns and infants in Italy who were followed up between 2018 and 2021, Auriti et al.[27] reported that the rate of invasive fungal infections caused by culture-confirmed *Malassezia* species was 21%. In a study of 57 infants with very low birth weight that was conducted by Benjamin et al.[5] in Martinique, fungal colonization was detected in 68% of the premature newborns, and 46% of these were caused by *Malassezia* species. In a prospective cohort study from India, Gupta et al.[17] reported that the rate of *Malassezia* species colonization was 38% in neonates and infants admitted to the pediatric and neonatal ICUs. Furthermore, colonization occurred in 3% of the newborns on admission to the NICU and in 52% of the newborns during hospitalization. Gupta et al.[17] also reported that 47 of the 48 isolates were *M. furfur*.

The skin flora, which begins to establish on the first day after birth, transforms into the adult-type flora during the first month of life[24]. In our study, although *Malassezia* species colonization was low during the first week of life, it increased with prolonged hospitalization in the NICU (Table 1). Similar studies have also reported an increase in *Malassezia* species colonization with prolonged NICU stays[10,11,28,29]. Very low birth weight, mechanical ventilation, intralipid emulsion therapy, immunosuppression, and prolonged hospitalization are predisposing risk factors for colonization, and breastfeeding is crucial for colonization[4,5,28]. The presented study revealed a higher frequency of *Malassezia* species in newborns aged ≥7 days than in those aged <7 days. Furthermore, *M. furfur* was more frequently isolated in neonates aged ≤2 days than in those aged >2 days. In the later stages of infancy, species other than *M. furfur* were the prevalent species (Table 2). In some studies, recurrent cultures with different clinical samples demonstrates a *M. furfur* colonization rate of >40% in infants hospitalized for >2 months[29,30]. Within the *Malassezia* genus, *M. furfur* is frequently isolated as the causative yeast of fungaemia[4,10,19,29]. *M. furfur* can also be isolated from 2.2% of NICU catheters[31]. In the study by Zhang et al.[32], all 36 premature infants with *M. furfur* fungemia had a central venous access and had received TPN. In non-premature neonates receiving intralipid emulsions via a central venous access, this percentage was 64.6%. Zhang et al.[32] also reported that 78.9% of the 86 premature neonates with *M. furfur* fungemia were <32 weeks in gestational age and had a very low birth weight, organ failure, and multiple organ hypoplasia.

The skin is permanently colonized with microorganisms at birth. According to the hygiene hypothesis, a deficiency recognized by the immune system early in life weakens the body's resistance to atopic diseases later in life[33]. Maternal *Malassezia* species colonize the skin of babies born via cesarean section more frequently than the skin of babies born naturally. This colonization interacts with the host immune system from infancy to childhood and suppresses the development of skin mycobiome dynamics and *Candida* colonization seen in vaginal births. These suppressions stabilize the immunity and cause poor immune recognition (imprinting), which affect the neonates later in life[33]. In this study, strains such as *M. furfur*, which are increasingly being reported as the causative agent of infection, were isolated in neonates born via cesarean section but not in those born via vaginal delivery.

Although prophylactic FLZ protocols have reduced the number of *Candida*-associated fungemia in the NICU, infections due to *Malassezia* species have become more common in recent years[4]. FLZ prophylaxis protocols, which have been used for several years in NICUs, do not increase the prevalence of FLZ-resistant *Candida* strains. However, there is a lack of available data regarding the resistance patterns of *Malassezia* species[34]. Furthermore, not using lipid-enriched mycological media in routine microbiological tests limits the data on *Malassezia* fungemia and colonization[9].

The lack of a standard method for testing the antifungal susceptibility of all *Malassezia* strains is a concerning issue[13,35]. Itraconazole and ketoconazole are widely considered the most effective antifungals against all *Malassezia* species, and FLZ, voriconazole, and amphotericin B have low antifungal activity[16]. Because antifungal susceptibilities vary greatly between genotypes, there is no general susceptibility profile. However, this has mostly been demonstrated in nonneonatal isolates[13,16,35]. In our study, although the MIC_{90} value of FLZ was high (Table 3), its presence in the group that did not receive FLZ prophylaxis indicated that the *Malassezia* species was a natively resistant strain. Thus, the *M. furfur* strains isolated in the neonates admitted to the NICU were FLZ-resistant. The study findings indicate that inoculating the samples of newborns admitted to the NICU with suspected fungemia in lipid-rich media and monitoring it is critical for early diagnosis. The increasing number of reports of *M. furfur* strains as the causative agent of fungemia requires special attention in neonates in the NICU[4,16,19].

Neonates who require lipid supplementation and intravenous nutrition are particularly susceptible to *Malassezia* infection. The aforementioned challenges in identifying neonatal *Malessezia* infection prevents the collection of accurate information on its global incidence. However, a one-year study exploring bloodstream infections caused by *Malassezia* and *Candida* in a surgical pediatric ward and NICU in the southern region of Italy revealed that the prevalence of *M. furfur*-related infections was 2.1% higher than that of *Candida*-related infections (1.4%)[19]. In a study in Russia in 2016, Rodchenko et al.[36] isolated etiological fungal agents from 4% of the 3,302 samples (e.g., blood, urine, and catheter) obtained from the NICU between May 2012 and December 2015. Approximately 67% of the isolated agents were *M. furfur*. The *M. furfur* colonization rate was 41% between 2015 and 2017 at the same center[37]. The treatment of *Malassezia* bloodstream infections includes removal of the catheter, short-term discontinuation of parenteral lipid nutrition, and intravenous antifungal therapy with liposomal amphotericin B[38].

Prophylactic administration of FLZ in neonates admitted to the NICU reportedly decreases fungemia. However, the presence of FLZ-resistant *M. furfur* strains makes amphotericin B treatment a priority. Oliveri et al.[39] reported the successful use of 4 mg/kg/day of liposomal amphotericin B in the treatment of a preterm patient with *M. furfur* fungemia. The drug was administered for 45 days without any side effects, TPN was discontinued, and the indwelling central venous catheter was removed. A Broviac catheter was inserted on the 30th day of treatment. *M. furfur* fungemia persisted for one month despite discontinuation of TPN and administration of high doses of amphotericin B. This case report highlights the importance of catheter exchange during the treatment process[39]. The significance of the duration of catheter retention in *M. furfur* fungemia was reported by Iatta et al.[19]. According to them, fungemia lasts for an average of five days when the catheter is retained for <9 days; this duration increases to 34 days when the catheter is retained for >17 days. In the study by Iatta et al.[19], the patients were treated with 2.5-5 mg/kg of liposomal amphotericin B. Of the six infants included in the report, four were preterm and one had a very low birth weight. Three of the patients with *M. furfur* fungemia received FLZ prophylaxis at a dose of 3 mg/kg/72 h. Liposomal amphotericin B was administered intravenously to all the patients at a dose of 5 mg/kg/d for varying durations based on their individual clinical conditions. *M. furfur* was isolated from the chest of all patients. It was also isolated from the surface of one patient's incubator. The report also mentioned contamination from another patient's bed cover[19]. However, although the MIC for FLZ is high against cutaneous strains, the FLZ susceptibility of *M. furfur* isolated from fungemia remains unclear[6].

Study Limitations

The current study has some limitations. The presence of *M. furfur* colonization could not be detected in the mothers of infants born via cesarean section who were admitted in the NICU. Furthermore, their genetic relationships could not be investigated. Considering that the geographic and sociodemographic differences in *Malassezia* colonization may affect the biological structure of the infant as well as the yeast distribution, we believe that similar studies should be conducted in different geographical locations.

Conclusion

In this study, it was possible to detect FLZ-resistant *M. furfur* during colonization screening. The fact that microbiologic cultures for *Malassezia* are not routinely performed masks the actual incidence of *Malassezia*-associated infections, especially in neonates being administered antifungals. Therefore, screening for *Malassezia* in NICUs and determining the presence of antifungal resistance will be useful for designing treatment protocols. More comprehensive studies are needed, especially due to the differences in geographical and local behavioral habits of the *Malessezia* species.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by Pamukkale University Non-interventional Medicine Ethics Committee (no: E-60116787-020, date: 22.12.2020).

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Concept: M.O., Ç.E., Design: M.O., Ö.M.A.Ö., H.E., Ç.E., Data Collection or Processing: M.O., Ç.E., Analysis or Interpretation: M.O., Ç.E., Literature Search: M.O., Ç.E., Writing: M.O., Ö.M.A.Ö., H.E., Ç.E.

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