IJN
Iranian Journal of Neonatology
http://ijn.mums.ac.ir

Open Access

Original Article

# Evaluation of the Fluconazole Prophylaxis against Fungal Colonization in the Preterm Neonates: A Double-blind Clinical Trial

**Sara Amini[1], Mehrdad Rezaei[1], Gholamreza Pouladfar[1,2], Fatemeh Ghasemi[2], Hadis Jafarian[2], Parisa Badiee[2*]**

1.Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran
2.Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

## ABSTRACT

***Background:*** Our study aimed to compare the twice-weekly and three-time weekly prophylactic fluconazole regimens among low birth weight premature neonates.
***Methods:*** Premature neonates (40 cases) were divided into two groups and received two and three-times-weekly fluconazole regimens for three weeks. Surveillance of fungal colonization was performed before treatment and twice weekly.
***Results:*** Fungal colonization occurred in 11 infants in group A and 4 infants in group B (55% vs. 20%, p=0.022). In both groups, the rectum was the most common site of colonization. *Candida glabrata, Candida orthopsilosis* and, *Candida albicans* were the most commonly isolated species in both groups. There were no significant differences between the two groups regarding birth weight, gestational age, and other baseline risk factors for fungal colonization.
***Conclusion:*** In this study, the three-times-weekly administration of prophylactic fluconazole appears to be more effective in preventing fungal colonization than the twice-weekly regimen among premature neonates.

***Keywords:*** *Candida*, Fluconazole, Neonates

## Introduction

Fungal infections, especially candidiasis, are one of the leading causes of death in neonates, particularly those with very low birth weight. The overall prevalence of invasive *Candida* (C) infections in infants is approximately 5-10 per 100,000 live infants (1-3). The incidence depends on the average gestational age and birth weight of the neonate. This risk is higher among very low birth weight premature infants (2, 4). The most common type of *Candida* species among neonates was reported as *C. albicans* (5, 6).

Fungal colonization in neonates mostly occurs on the skin, gastrointestinal, and respiratory tract. Transmission of neonatal *Candida* species is usually from the mother; however, transfers from healthcare staff or the hospital environment have been reported (7). Present therapies for fungal infections are not generally successful, and morbidity rates are high. Efforts can be focused on preventing invasive infections by disturbing the process of colonization and subsequently preventing the development of fungal infections (6). These findings suggest the need to prevent *Candida* infection in neonatal intensive care unit (ICU) patients (8).

Using fluconazole for preventing fungal

* *Corresponding author*: Parisa Badiee, Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Email: badieep@gmail.com

*Please cite this paper as:*
Amini S, Rezaei M, Pouladfar Gh, Ghasemi F, Jafarian H, Badiee P. Evaluation of the Fluconazole Prophylaxis against Fungal Colonization in the Preterm Neonates: A Double-blind Clinical Trial. Iranian Journal of Neonatology. 2024 Apr: 15(2). DOI: 10.22038/IJN.2024.72428.2403

infections in low birth weight infants in the first six weeks of life is effective and may be helpful (9, 10). Several studies have compared different administration schedules and fluconazole doses to recognize the best treatment choice (6, 11). However, it remains unknown which treatment dose and schedule are more efficient for controlling colonization and mortality in very low birth weight infants (6). Despite the prevention of *Candida* species colonization with fluconazole twice a week according to the neonatal protocol (12), our goal in this study was to compare the effectiveness of fluconazole prophylaxis twice a week with three times a week to prevent fungal colonization and invasive infections in premature neonates.

## Methods

This study was a cross-sectional study, and cases and controls were evaluated at the same time. The neonatal intensive care units (NICUs) of three teaching tertiary level care hospitals of Shiraz University of Medical Sciences in Shiraz, Iran (Namazi, Hafez, and Zeinabieh hospitals) were enrolled in this study. These hospitals are public and are known as centers in the south of Iran. Neonates with a birth weight less than 1500 gr and a birth date (maternal pregnancy week) less than 32 weeks within the first 24 hours of life were included in this study. Figure 1 shows the flowchart of the study populations. The exclusion criteria were neonates with a lack of parental consent and broad-spectrum intravenous antibiotics in the first 24 hours after delivery and infants with gross congenital malformations.

Sample procedures were explained to the parent before sampling. For six months (from 22 September 2020 to 21 March 2021), samples were collected from the throat, urine, and rectum of each case on the first day of birth, before starting prophylaxis with fluconazole, and twice weekly for three weeks after birth under sterile conditions. The neonates were studied in two groups, group A (21 neonates) received injectable fluconazole 6 mg/kg/dose twice a week and group B (22 neonates) received fluconazole three times a week with the same dose for three weeks (11, 13). The selection of neonates in each group was done one by one. The samples were immediately sent to the Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, and cultured on Sabouraud dextrose agar (Merck, Germany). The isolated *Candida* was re-cultured, purified, and analyzed using molecular methods. *Candida* species were amplified using ITS1 (5′TCCGTA GGTGAACCTGCG G-3′) and ITS4 (5′-TCCTCCGCT TATTGATATGC-3′) primers in a final volume of 50 µl according to Mirhendi et al. (14) *MspI* restriction enzyme was used to digest the PCR products. The amplified products and the digested fragments of the RFLP reaction were run on 1.5% and 2% agarose gels. Because *C. parapsilosis* was complex, the PCR products of these isolates were identified by sequencing and compared with the NCBI nucleotide database

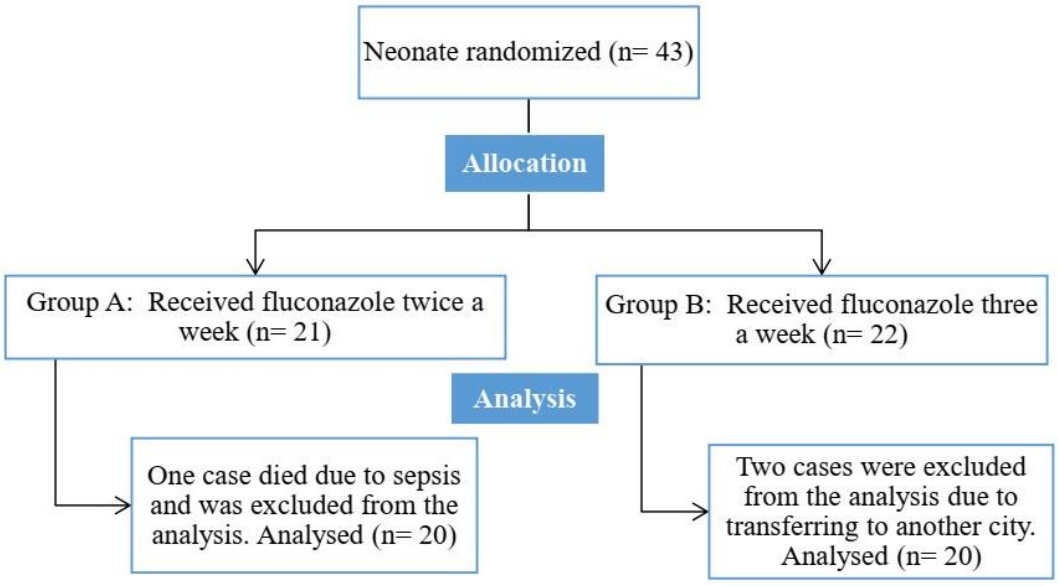


**Figure 1.** Flowchart of the study populations

(BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

In the first group, one of the neonates died due to sepsis before the end of the prophylaxis period, and in the second group, two neonates were excluded from the study because they were transferred to hospitals in another city. Finally, 40 neonates received a full course of prophylaxis. Information about gender, birth weight, C-reactive protein, blood culture, and the presence of the central line and respiratory tube was collected from patients' files. Both the participants (parents of neonates) and the personnel were blinded to the case and control groups.

The data obtained from this study were statistically analyzed using SPSS software version 22. Two Sample t-test statistical tests or its non-parametric equivalent, the Mann-Whitney test were used to investigate the relationship between continuous variables, while chi-square or Fisher's exact tests were used to evaluate discrete variables.

### *Ethical approval*

This study adhered to the guidelines of the Declaration of Helsinki. The ethics code of study was obtained from the Shiraz University of Medical Sciences ethical registration (IR.SUMS.MED.REC.1399.265) and the Iranian Registry of Clinical Trials registration code (IRCT20211006052685N1). Written inform Consent for publication obtained from parent or legal guardian.

## Results

In this study, 438 and 450 specimens, including rectal swabs, throat, and urine, were examined for three consecutive weeks after the birth of groups A and B, respectively. Forty neonates who received a full course of prophylaxis were included in this study (Figure 1). There was no significant difference between the mean gestational age and weight in the two groups (P-value> 0.05) (Table 1). The incidence of fungal colonization in premature neonates studied with fluconazole administration three times a week (group B) compared to twice a week (group A) is different (Table 2), and prevention of fungal colonization using fluconazole three times a week is more successful (P-value =0.02). Fifty-five *Candida* species were isolated from neonates (in some cases during the study, more than one species was isolated). Using the PCR-RFLP method (Figure 2) the isolated type of *Candida* species in group A included *C. glabrata*, *C. parapsilosis*, *C. albicans*, *C. orthopsilosis* and, *C. famata*. In group B, the isolated species were *C. parapsilosis, C. orthopsilosis,* and *C. albicans*. The most frequent site of colonization in both groups was the rectum (Figure 3). There was no significant difference in the culture results between hospitals in terms of culture tests. Twenty-two isolates were sequenced for precise identification and compared with the NCBI nucleotide Bank. Identified isolates were deposited in GenBank, including eight isolates of *C. parapsilosis* (accession numbers: OK298402-9),

**Table 1**. Baseline characteristics of neonates' participants in the study

| Variables | | Received fluconazole two times a week (Number = 20) | Received fluconazole three times a week (Number = 20) | P-value |
|---|---|---|---|---|
| Mean of gestational age (week) | | Mean: 29.5 (SD: 1.98) | Mean: 29.3 (SD: 1.26) | 0.70 |
| Birth weight (gram) | | Mean: 1169.15 (SD:232.81) | Mean: 1187.0 (SD:201.78) | 0.79 |
| Sex | girl | 11 (55%) | 12 (60%) | 0.75[a] |
| | boy | 9 (45%) | 8 (40%) | |
| Central line | Yes | 17 (85%) | 19 (95%) | 0.29[b] |
| | No | 3 (15.1%) | 1 (5.2%) | |
| Respiratory tube | Yes | 7 (35%) | 6 (30%) | 0.74 [a] |
| | No | 13 (65%) | 14 (70%) | |
| C-Reactive Protein | Positive | 6 (30) | 6 (30) | 1.0 [a] |
| | Negative | 14 (70) | 14 (70) | |
| Blood culture | Positive | 3 (15%) | 2 (10%) | 0.63[b] |
| | Negative | 17 (85%) | 18 (90%) | |

[a] Chi-squared test was performed
[b] Fisher's exact test was performed

**Table 2**. Comparison of neonates with fungal colonization by fluconazole groups two and three times a week

| Fluconazole regimen | Number (percent) | P-value |
|---|---|---|
| fluconazole twice a week (group A) | Positive 11 (55)<br>Negative 9 (45) | 0.02 |
| fluconazole three times a week (group B) | Positive 4 (20)<br>Negative 16 (80) | |

Significant at 0.05 level. Chi-square test performed

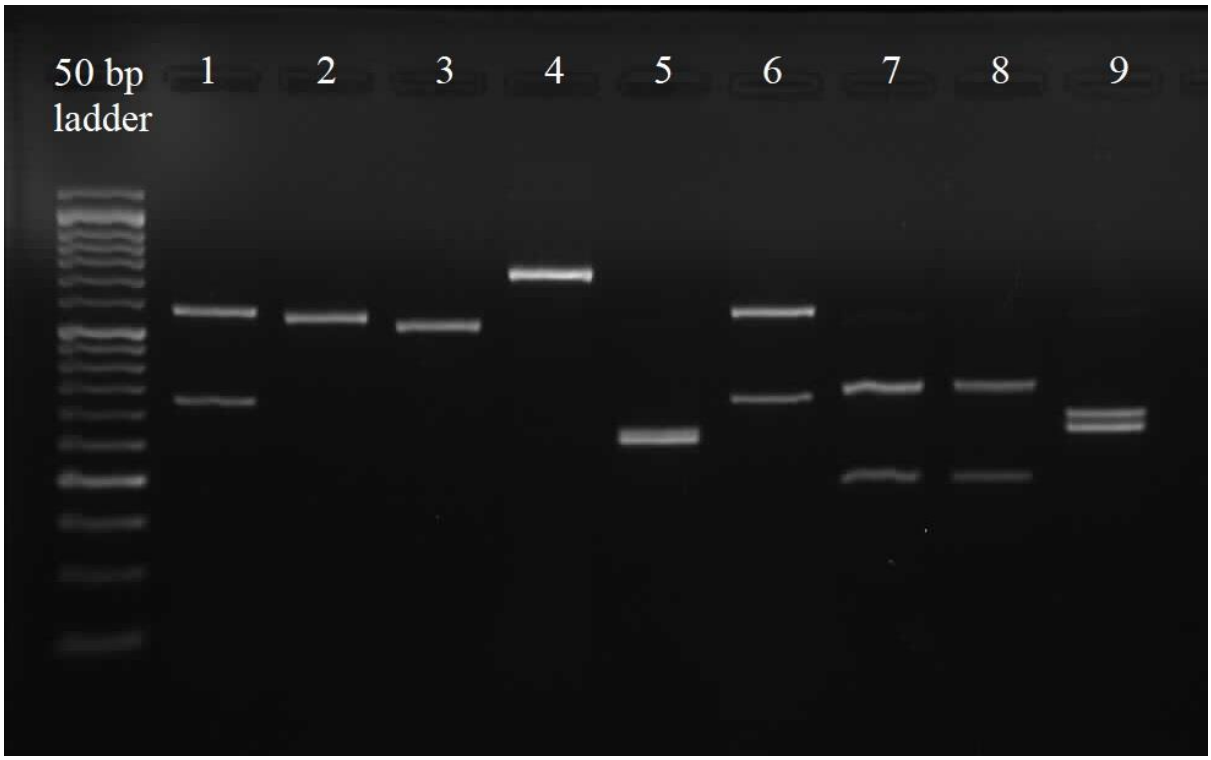


**Figure 2.** PCR product of isolated yeasts after digestion by *MspI* restriction enzyme on 2% agarose gel. Lanes 1 – 9 are *Candida glabrata, Candida parapsilosis, Candida parapsilosis, Candida famata, candida krusei, Candida glabrata, Candida tropicalis* (ATCC 750), *Candida tropicalis* (ATCC 750) and *Candida albicans*, respectively.

twelve isolates of *C. orthopsilosis* (accession numbers: OK298481-8 and OK310780-3), and two isolates of *C. albicans* (accession numbers: OK618520 and OK618521).

In terms of the blood culture results in group A, 3 neonates (15%) tested positive, and 17

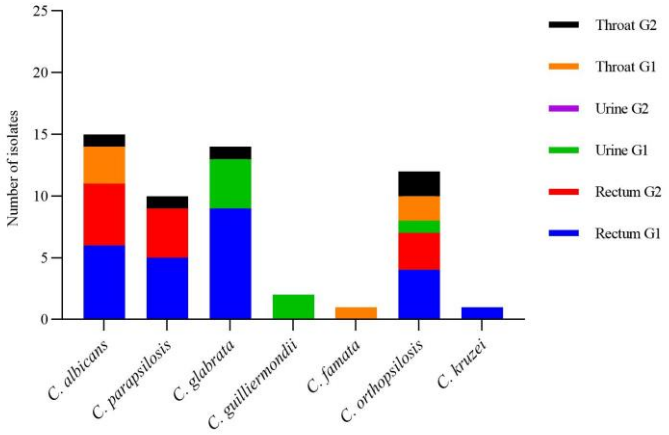


**Figure 3.** Site and type of *Candida* colonization in both groups

neonates (85%) had negative results. In group B, 2 neonates (10%) tested positive blood culture results, and 18 (90%) had negative results. There was no significant difference between the two groups in terms of blood culture tests. None of the neonates presented side effects of fluconazole.

## Discussion

In this study, we compared the effect of fluconazole prophylaxis at two different doses on premature neonates and observed that administering it three times a week was more effective in preventing fungal colonization. *Candida* colonization is considered a major complication in the preterm neonate (15-17). Candidiasis is the most concerning fungal infection among very low and extremely low birth weight neonates in NICU wards (1, 18). Studies have focused on the effect of fluconazole on the prophylaxis of *Candida* infection in infants, reducing mortality and complications from *Candida* infection, and reducing the length of hospital stay (6, 9, 10, 15, 17, 19, 20). Zhang et al. reported, "The incidence of nosocomial fungal infection was significantly lower in the prophylaxis group than in the rescue group (15.9 vs. 45.8%, P < 0.001)" (5). In the literature, different methods of prescribing fluconazole prophylaxis in high-risk neonates have been compared and the use of fluconazole twice a week has been recommended (11, 13, 15).

In previous studies, the effect of fluconazole on the prophylaxis of *Candida* infection in neonates weighing less than 1000 gr was evaluated, and the reported incidence of fungal infection was lower in patients receiving fluconazole twice a week than in patients receiving a placebo (9, 17). However, because of the lack of data concerns about fluconazole side effects and the development of fluconazole resistance in *Candida* species, prevention in high-risk infants are still controversial. In addition, clinical observations in the studied hospitals showed that despite the lower incidence of invasive fungal infections in premature neonates who receive fluconazole twice a week, colonization and invasive fungal infections still occur in these neonates (5, 21). In the present study, in group A, 55% of neonates were positive for *Candida* species colonization, and in group B, 20 % were positive, which showed a significant difference between the two groups. According to Saiman et al., the prevalence of *Candida* colonization in NICU patients was 23% (486/2157 infants). In 2019, (22) Rezaei et al reported that 45.7% (48 cases) of 105 newborns were colonized with *Candida* species. (23). A significant difference in fungal colonization was reported by Manzoni et al. between the two groups of neonates, one group receiving fluconazole and one did not receive it (10).

In this study, the most common types of fungus grown in the samples taken from groups A and B were *C. glabrata* and *C. parapsilosis*, respectively. The highest site of colonization in both groups was the rectum. Ali et al. reported that *Candida* colonization was detected in 12.8% of very low birth weight neonates in the NICU, and the initial site of colonization was the anus (88.8%), followed by an oral cavity (66.6%) and the umbilicus (55%) (24). According to a study by Kaufman et al., the most common site of colonization was the skin and then the rectum, and the most common type of fungus was *C. parapsilosis*, followed by *C. glabrata*. The colonization of *C. albicans* was reduced by receiving fluconazole (9). The etiologic agents of *Candida* colonization in neonates admitted to the NICU were reported as 299 cases of *C. albicans* (14%), 151 cases of *C. parapsilosis* (7%), and 74 cases of other *Candida* species (3%) (22). The type of etiologic agents is related to the management of neonates in ICU wards with antifungal agents and rates of resistance to fluconazole in each region.

Prescribing antifungal agents is very important due to reports of resistance in different *Candida* species to antifungal agents in the literature (4, 25). The limitation of our study was the relatively small study population. We recommend conducting the study with a larger population to better reveal the relationship between the use of fluconazole as prophylaxis in neonates.

## Conclusion

In this study, the three-times-weekly administration of prophylactic fluconazole appeared to be more effective in preventing fungal colonization compared with the twice-weekly regimen among premature neonates weighing less than 1500 g and gestational age under 32 weeks. Moreover, the rectum was the most common site of colonization, and non-*albicans Candida* species were the most commonly isolated species.

## Acknowledgments

It is necessary to thank all the staff and those in the three hospitals of Namazi, Hafez, and Zeinabieh hospitals for all their cooperation in this project. Shiraz University of Medical Sciences

provided the funds for this study (No. 19085-01-01-97).

## Conflicts of interest

The authors declare that there is no conflict of interest.

## References


1. Benedict K, Roy M, Kabbani S, Anderson EJ, Farley MM, Harb S, et al. Neonatal and pediatric candidemia: results from population-based active laboratory surveillance in four US locations, 2009–2015. J Pediatric Infect Dis Soc. 2018;7(3):e78-e85.
2. Lausch KR, Schultz Dungu KH, Callesen MT, Schroder H, Rosthoj S, Poulsen A, et al. Pediatric Candidemia Epidemiology and Morbidities: A Nationwide Cohort. Pediatr Infect Dis J. 2019;38(5):464-469.
3. Hornik CD, Bondi DS, Greene NM, Cober MP, John B. Review of Fluconazole Treatment and Prophylaxis for Invasive Candidiasis in Neonates. J Pediatr Pharmacol Ther. 2021;26(2):115-122.
4. Shamsizadeh A, Nikfar R, Mombini M, Keikhaei B, Jafarian H, Badiee P. The Relative Frequency and Susceptibility Patterns of Candida Species Isolated from Blood and Urine of Children with Malignancy. Arch Pediatr Infect Dis. 2018;6(2).
5. Zhang D, Xie D, He N, Wang X, Dong W, Lei X. Prophylactic use of fluconazole in very premature infants. Front Pediatr. 2021;9:726769.
6. Anaraki MR, Nouri-Vaskeh M, Oskoei SA. Fluconazole prophylaxis against invasive candidiasis in very low and extremely low birth weight preterm neonates: a systematic review and meta-analysis. Clin Exp Pediatr. 2021;64(4):172-179.
7. Mesini A, Saffioti C, Mariani M, Florio A, Medici C, Moscatelli A, et al. First Case of Candida auris Colonization in a Preterm, Extremely Low-Birth-Weight Newborn after Vaginal Delivery. J Fungi (Basel). 2021;7(8):649.
8. Agarwal RR, Agarwal RL, Chen X, Lua JL, Ang JY. Epidemiology of invasive fungal infections at two tertiary care neonatal intensive care units over a 12-year period (2000-2011). Glob Pediatr Health. 2017;4:2333794X17696684.
9. Kaufman D, Boyle R, Hazen KC, Patrie JT, Robinson M, Donowitz LG. Fluconazole prophylaxis against fungal colonization and infection in preterm infants. N Engl J Med. 2001;345(23):1660-1666.
10. Manzoni P, Arisio R, Mostert M, Leonessa M, Farina D, Latino MA, et al. Prophylactic fluconazole is effective in preventing fungal colonization and fungal systemic infections in preterm neonates: a single-center, 6-year, retrospective cohort study. Pediatrics. 2006;117(1):e22-32.
11. Leonart LP, Tonin FS, Ferreira VL, Tavares da Silva Penteado S, de Araujo Motta F, Pontarolo R. Fluconazole Doses Used for Prophylaxis of Invasive Fungal Infection in Neonatal Intensive Care Units: A Network Meta-Analysis. J Pediatr. 2017;185:129-135.e6.
12. Schoeni MH, Martin RJ, Avroy A. Fanaroff and Michele C. Walsh, Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant—10th Edition: Saunders, an imprint of Elsevier. Inc., 2015, ISBN: 978-1-4557-5617-9. Eur J Pediatr. 2015; 174:1699-700.
13. Hope WW, Castagnola E, Groll AH, Roilides E, Akova M, Arendrup MC, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: prevention and management of invasive infections in neonates and children caused by Candida spp. Clin Microbiol Infect. 2012;18 Suppl 7:38-52.
14. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. Nihon Ishinkin Gakkai Zasshi. 2006;47(3):225-229.
15. Ericson JE, Kaufman DA, Kicklighter SD, Bhatia J, Testoni D, Gao J, et al. Fluconazole prophylaxis for the prevention of candidiasis in premature infants: A meta-analysis using patient-level data. Clin Infect Dis. 2016;**63**:604-610.
16. Hsu JF, Lai MY, Lee CW, Chu SM, Wu IH, Huang HR, et al. Comparison of the incidence, clinical features and outcomes of invasive candidiasis in children and neonates. BMC Infect Dis. 2018;18(1):194.
17. Manzoni P, Stolfi I, Pugni L, Decembrino L, Magnani C, Vetrano G, et al. A multicenter, randomized trial of prophylactic fluconazole in preterm neonates. N Engl J Med. 2007;356:2483-2495.
18. Kaufman DA, Gurka MJ, Hazen KC, Boyle R, Robinson M, Grossman LB. Patterns of fungal colonization in preterm infants weighing less than 1000 grams at birth. J Pediatric Infect Dis Soc. 2006;25(8):733-737.
19. Rios J, Camargos PAM, Correa LP, Romanelli RMC. Fluconazole prophylaxis in preterm infants: a systematic review. Braz J Infect Dis. 2017;21(3):333-338.
20. Weitkamp JH, Ozdas A, LaFleur B, Potts AL. Fluconazole prophylaxis for prevention of invasive fungal infections in targeted highest risk preterm infants limits drug exposure. J Perinatol. 2008;28(6):405-411.
21. Turner K, Manzoni P, Benjamin DK, Cohen-Wolkowiez M, Smith PB, Laughon MM. Fluconazole pharmacokinetics and safety in premature infants. Curr Med Chem. 2012;19(27):4617-4620.
22. Saiman L, Ludington E, Dawson JD, Patterson JE, Rangel-Frausto S, Wiblin RT, et al. Risk factors for Candida species colonization of neonatal intensive care unit patients. Pediatr Infect Dis J. 2001;20(12):1119-1124.
23. Rezaei M, Moghtaderi M, Badiee P, Zahadatpoor Z, Pooladfar G. Fungal colonization among iranian infants hospitalized in the neonatal intensive care unit: occurrence rate, risk factors and health outcome. Int J Pediatr. 2019;7(7):9719-9728.

24. Ali GY, Algohary EHSS, Rashed KA, Almoghanum M, Khalifa AA. Prevalence of Candida colonization in preterm newborns and VLBW in neonatal intensive care unit: role of maternal colonization as a risk factor in transmission of disease. J Matern Fetal Neonatal Med. 2012;25(6):789-795.

25. Badiee P, Badali H, Diba K, Jafarian H, Mohammadi R, Mirhendi H, et al. Multicenter identification and antifungal susceptibility patterns of candida species isolated from clinical samples. Jundishapur J Microbiol. 2017;10(12):e56117.