Antifungal susceptibility patterns of colonized Candida species isolates from immunocompromised pediatric patients in five university hospitals

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ABSTRACT

Background and Objectives: Colonization of Candida species is common in pediatric patients admitted to hematology-oncology wards. The aim of this study was to identify colonized Candida species and their susceptibility patterns in hematology pediatric patients.

Materials and Methods: Samples were collected from mouth, nose, urine and stool of the patients admitted to five university hospitals and cultured on sabouraud dextrose agar. The isolates were identified by API 20 C AUX system and their susceptibility patterns were evaluated by CLSI M27-A3 and S4.

Results: From 650 patients, 320 (49.2%) were colonized with 387 Candida species. Candida albicans was the most prevalent isolated species, followed by Candida glabrata, Candida tropicalis, Candida famata, Candida kefyr and Candida krusei. The epidemiological cut off value (ECV) for all Candida species to amphotericin B was ≤0.25 μg except C. krusei (4 μg). The resistance rate to fluconazole in this study in C. albicans was 4.9% with ECV 8 μg/ml, followed by C. tropicalis 8.8% with ECV 0.5 μg/ml. Voriconazole and posaconazole were effective antifungal agents for all Candida isolates. The ECV of C. albicans, Candida parapsilosis, C. tropicalis, C. glabrata and C. krusei for itraconazole were 0.5, 0.25, 0.5, 1 and 2 μg, respectively. The resistant and intermediate rates of Candida species to caspofungin in this study were 2.9%, 5.9%, 18.8%, 47.9%, 0.0% and 16.7% in C. tropicalis, C. glabrata and C. parapsilosis respectively.

Conclusion: C. albicans was the most prevalent species in pediatric colonized patients. New azole agents like voriconazole and posaconazole are effective against non-albicans Candida species. Increase in intermediate species is alarming to future emerging resistant species.

Keywords: Candida species, Colonized, Amphotericin B, Voriconazole, Posaconazole, Itraconazole
INTRODUCTION

*Candida* species are the main cause of superficial to systemic fungal infection in humans and the major source of infection in health care centers (1). Systemic infections are common in immunocompromised pediatrics individuals including patients in hematology-oncology wards (1, 2). According to Center for Diseases Control and Prevention (CDC), *Candida* species is ranked fifth among hospital-acquired pathogens and forth among blood stream infection pathogens (3). The colonized patients are most susceptible to infection. The rates of *Candida* colonization were reported 48.8%, and 78.8% in pediatric hematologic patients (4, 5).

*C. albicans* is the main pathogenic agent of systemic infections, however, during the recent years, the rate of non-*albicans* *Candida* species has increased in many reports (2, 5, 6). Improvement in diagnostic technical methods has led to diagnosis of other *Candida* species. And also, *C. albicans* is susceptible to most antifungal agents and during the prophylaxis is cleaned from the patient's body, but non-*albicans* *Candida* species like *C. glabrata* and *C. krusei* are resistant and more emerging in the infected patients.

Caggiano et al. reported "surveillance cultures are useful to monitor the *Candida* colonization in ICU patients" (7, 8). Colonization with *Candida* species is recognized as a risk factor for systemic candidiasis in immunocompromised patients (8). The susceptibility of *Candida* species varies, depending on certain species responsible for infection, geographic region, patient population and health care management in each region. Limited studies have investigated the rate of colonization and susceptibility patterns of *Candida* species isolated from colonized children. The aim of this study was to identify *Candida* species isolated from colonized hematologic pediatric patients and investigate their susceptibility patterns to seven antifungal agents in five university hospital centers by Clinical and Laboratory Standards Institute (CLSI).

MATERIALS AND METHODS

Colonizing isolate was defined as *Candida* species isolated from the body site of patients without any signs and symptoms of infection.

Sample collection. The present study was conducted from 2014 to 2015 in order to investigate the fungal colonization from immunocompromised children admitted to five university hospitals in Iran (Shiraz, Kerman, Yasouj, Ahvaz and Sannandaj). Totally, 1950 samples were collected from mouth, nose, urine and anus. Samples were cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) and transferred to Prof. Alborzi Clinical Microbiology Research Center for further examination. To evaluate the purity of isolates, the samples were cultured on potato dextrose agar (OXOID LTD, Basind stoke, Hampshire, England) twice at 35°C for 48h. The isolates were identified by carbohydrate assimilation reactions on API 20 C AUX system (bioMerieux, Swiss), according to the manufacturer's instructions.

Antifungal susceptibility testing. The susceptibility patterns of the isolates against amphotericin B, fluconazole, ketoconazole, voriconazole, itraconazole, caspofungin and posaconazole (GmbH-Steinheim-Sigma-AldrichWie) were investigated using broth micro dilution assay, according to CLSI M27-A3 and S4 guidelines (9, 10). *C. parapsilosis* ATCC22019 and *C. krusei* ATCC6258 were considered as standard strains. The final concentrations of amphotericin B, itraconazole, posaconazole and voriconazole were ranged from 0.032 to 16 μg/ml and for fluconazole and caspofungin from 0.125 to 64 μg/ml and 0.016 to 8 μg/ml, respectively. In each series, one negative control without any yeast suspension and one positive control without any drugs were considered. The plates were sealed and incubated for 24 and 48h at 35°C and visual minimum inhibitory concentration (MIC) end points were determined. The recommended end-point for azole and caspofungin are the lowest drug concentration with a prominent decrease in turbidity (inhibitory concentration that gives 50% growth reduction), while for amphotericin B, MIC was the drug concentration showing a complete inhibition of growth. According to CLSI M27-A3 and S4, there is not any breakpoint for posaconazole and ketoconazole (9, 10).

Statistical analysis. Statistical analysis was performed using WHO NET (version 5.6). Epidemiological cutoff value (ECV), Wild-type (WT) and non-WT strain, MIC50 and MIC90 value and Geometric Mean (GM) were reported.

Ethical considerations. The ethics committee of
Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences reviewed and approved the study.

RESULTS

From 650 pediatric patients, 1950 samples were cultured and 320/650 (49.2%) were colonized with *Candida* species in different parts of their bodies and 387 *Candida* species isolated. The most prevalent backgrounds of patients were acute lymphoblastic leukemia, followed by lymphoma, and acute myelocytic leukemia (Table 1). All immunocompromised patients entered in this study had history of admission in the hospital and use of fluconazole for treatment or prophylaxis. *C. albicans* (223, 57.6%) was the most prevalent isolated species, followed by *C. glabrata* 48 (12.4%), *C. tropicalis* 34 (8.7%), *C. famata* 23 (5.9%), *C. kefir* 18 (4.6%), *C. kuresi* 13 (3.3%), *C. parapsilosis* 12 (3.1%), *C. dublinsiens* 10 (2.5%), *C. guilliermondii* 3 (0.7%), *C. lusitaniae* 2 (0.5%) and *C. intermedia* 1 (0.25%) (Fig. 1). Distribution of *Candida* species isolated from each university hospital was shown in Table 2. According to Table 3, the sen-

![Fig. 1. Distributions of *Candida* species isolated from pediatric patients.](http://ijm.tums.ac.ir)

*Others: *C. parapsilosis, C. guilliermondii, C. lusitaniae, C. intermedia

| Table 1. Distributions of background illness of immunocompromised patients in five university hospitals in Iran |
|-------------------------------------------------|
| **Background illness** | **City** | Kerman | Shiraz | Yasouj | Sannandaj | Ahvaz |
| Acute lymphoblastic leukemia | 28 | 58 | 26 | 41 | 20 |
| Lymphoma | 16 | 15 | 16 | 13 | 7 |
| Acute myeloid leukemia | 8 | 22 | 3 | 7 | 9 |
| *Others* | 27 | 21 | 12 | 22 | 16 |
| Total | 79 | 116 | 57 | 83 | 52 |

*Others: Hodgkin’s lymphoma, Aplastic anemia, Burkitt lymphoma, Yolk sac cancer, Megaloblastic anemia, Sarcoma

| Table 2. Distributions of *Candida* species isolated from five university hospitals in the selected cities |
|-------------------------------------------------|
| **Species** | **No. of isolates** | **City** | Kerman | Shiraz | Yasouj | Sannandaj | Ahvaz |
| *C. albicans* | 223 (57.6%) | 41 | 78 | 26 | 50 | 28 |
| *C. glabrata* | 48 (12.4%) | 8 | 13 | 12 | 14 | 1 |
| *C. tropicalis* | 34 (8.7%) | 7 | 4 | 12 | 3 | 8 |
| *C. famata* | 23 (5.9%) | 11 | 3 | 2 | 3 | 4 |
| *C. kefir* | 18 (4.6%) | 4 | 3 | ---- | 8 | 3 |
| *C. krusei* | 13 (3.3%) | 5 | 6 | ---- | 2 | ---- |
| *C. parapsilosis* | 12 (3.1%) | 1 | 4 | 1 | 1 | 5 |
| Others | 16 (4.1%) | 2 | 5 | 4 | 2 | 3 |
| Total | 387 | 79 | 116 | 57 | 83 | 52 |

*Others: *C. parapsilosis, C. guilliermondii, C. lusitaniae, C. intermedia
sitivity rates for \textit{C. albicans}, the most frequently isolated species, were 99.1\% to amphotericin B, 96.9\% to caspofungin with 2.7\% intermediate dose, 91\% to voriconazole with 3.6\% intermediate dose, 90.6\% to fluconazole with 4.5\% intermediate dose, 73.1\% to itraconazole with 24.2\% intermediate dose. MIC90 values for posaconazole and ketoconazole were 0.032 μg/ml and 0.125 μg/ml with GM 0.031 and 0.032, respectively. ECV in μg/ml and 0.125 μg/ml with GM 0.031 and 0.032, respectively. Few non-WT types’ \textit{C. albicans} was isolated from the patients. According to Table 4, ECV for all \textit{Candida} species to amphotericin B was ≤0.25 μg except \textit{C. krusei} (4μg). The ECVs and WT rates of \textit{C. albicans}, \textit{C. parapsilosis}, \textit{C. tropicalis}, \textit{C. glabrata} and \textit{C. krusei} for itraconazole were 0.5μg/ml, 96%; 0.25μg/ml, 100%; 0.5 μg/ml, 98%; 1μg/ml, 96%, 2μg/ml and 92% respectively. Other \textit{Candida} species (\textit{C. kefyr}, \textit{C. guilliermondii}, \textit{C. isitaniae} and \textit{C. intermedia}) were sensitive to antifungal agents and resistant rate to itraconazole in \textit{C. famata} was 4.3\%.

\section*{DISCUSSION}

For the management of systemic candidiasis in immunocompromised patients, early diagnosis and empirical antifungal therapies are in focus. Leon et al. reported multifocal colonization (OR=3.04, 95\% CI, 1.45-6.39) was predictive of proven \textit{Candida} infection and would benefit from early antifungal therapy (11). As the colonized \textit{Candida} may transfer to pathogen due to change in patients’ immune system, knowl-

Table 3. Antifungal susceptibility patterns of \textit{Candida} species isolated from pediatric patients by CLSI breakpoint.

| Species               | Antifungal agents | Range (μg/ml) | %R | %I | %S | MIC50 | MIC90 | Geom. Mean |
|-----------------------|-------------------|---------------|----|----|----|-------|-------|------------|
| \textit{C. albicans}  | Amphotericin B    | 0.016-32      | 0.9| 0  | 99.1| 0.032 | 0.25  | 0.039      |
|                       | Caspofungin       | 0.016-64      | 0.4| 2.7| 96.9| 0.016 | 0.25  | 0.041      |
|                       | Voriconazole      | 0.016-16      | 5.4| 3.6| 91  | 0.016 | 0.064 | 0.035      |
|                       | Fluconazole       | 0.016-64      | 4.9| 4.5| 90.6| 0.125 | 2     | 0.254      |
|                       | Posaconazole      | 0.016-16      | ----|----|----| 0.016 | 0.032 | 0.031      |
|                       | Itraconazole      | 0.016-16      | 2.7 | 24.2| 73.1| 0.032 | 0.125 | 0.049      |
|                       | Ketoconazole      | 0.016-16      | ----|----|----| 0.016 | 0.125 | 0.032      |
|                       | Amphotericin B    | 0.016-0.5     | 0  | 0  | 100 | 0.032 | 0.064 | 0.31       |
|                       | Caspofungin       | 0.016-0.5     | 18.8|47.9| 33.3| 0.125 | 0.5   | 0.113      |
|                       | Voriconazole      | 0.016-0.5     | 0  | 0  | 100 | 0.032 | 0.025 | 0.05       |
|                       | Fluconazole       | 0.064-16      | 0  | 0  | 100 | 1     | 4    | 0.042      |
|                       | Posaconazole      | 0.016-16      | ----|----|----| 0.064 | 0.5  | 0.082      |
|                       | Itraconazole      | 0.032-16      | 14.6|72.9| 12.5| 0.25  | 1    | 0.233      |
|                       | Ketoconazole      | 0.016-16      | ----|----|----| 0.032 | 0.125 | 0.037      |
|                       | Amphotericin B    | 0.016-0.5     | 0  | 0  | 100 | 0.016 | 0.125 | 0.033      |
|                       | Caspofungin       | 0.016-4       | 2.9 | 5.9| 91.2| 0.032 | 0.25  | 0.046      |
|                       | Voriconazole      | 0.016-16      | 8.8 | 5.9| 85.3| 0.016 | 0.125 | 0.033      |
|                       | Fluconazole       | 0.064-64      | 8.8 | 5.9| 85.3| 0.125 | 4    | 0.032      |
|                       | Posaconazole      | 0.016-16      | ----|----|----| 0.016 | 0.25  | 0.035      |
|                       | Itraconazole      | 0.016-16      | 2.9 | 38.2| 58.8| 0.064 | 0.5   | 0.078      |
|                       | Ketoconazole      | 0.016-16      | ----|----|----| 0.016 | 0.25  | 0.029      |
|                       | Amphotericin B    | 0.016-1       | 0  | 0  | 100 | 0.032 | 0.25  | 0.037      |
|                       | Caspofungin       | 0.016-0.25    | 0  | 0  | 100 | 0.016 | 0.25  | 0.035      |
|                       | Voriconazole      | 0.016-1       | 0  | 0  | 100 | 0.016 | 0.125 | 0.034      |
|                       | Fluconazole       | 0.032-8      | 0  | 0  | 100 | 0.125 | 4    | 0.268      |
|                       | Posaconazole      | 0.016-0.5     | ----|----|----| 0.016 | 0.064 | 0.031      |
|                       | Itraconazole      | 0.016-1       | 4.3 | 21.7| 73.9| 0.064 | 5    | 0.064      |
|                       | Ketoconazole      | 0.016-0.5     | ----|----|----| 0.016 | 0.125 | 0.03       |
There is no breakpoint for Posaconazole and Ketoconazole, only MIC was reported. R: Resistant, I: Intermediate, S: Susceptible, MIC: Minimum inhibitory concentration.

Candida (Table 2). In our study, 43.1% of all isolates from Shiraz, Yasouj and Sannandaj but prevalent species in all cities. C. glabrata was the second isolates from C. tropicalis and were the second isolates from C. kefyr and C. krusei (Table 2). In our study, 43.1% of all Candida isolates was non albicans species, while the rates in other studies were reported 48.8% (13), 45% (15) and 21.8% (16). In the study conducted by Wisplinghoff et al. C. parapsilosis (17.4%), C. glabrata (16.7%) and C. tropicalis (10.2%) were responsible for bloodstream infections (18). The sensitivity rates of 178 C. albicans isolated from immunocompromised patients were reported 93%, 95.4%, 93% and 97.7% for amphotericin B, fluconazole, itraconazole, and voriconazole, respectively (17). In another study by Moran et al. "Children with non-albicans bloodstream infections were approximately twice as likely to die as children with C. albicans bloodstream infections (35.2% versus 18.2%; P= 0.03)"(19). The mortality

| Species         | Antifungal agents | Rang (μg/ml) | %R | %I | %S | MIC 50 | MIC 90 | Geom. Mean |
|-----------------|-------------------|-------------|----|----|----|--------|--------|-----------|
| C. krusei       | Amphotericin B    | 0.016-1     | 0  | 0  | 100| 0.016  | 0.064  | 0.03      |
|                 | Caspofungin       | 0.016-2     | 0  | 0  | 100| 0.016  | 0.025  | 0.031     |
|                 | Voriconazole      | 0.016-0.125 | 0  | 0  | 100| 0.016  | 0.032  | 0.021     |
|                 | Fluconazole       | 0.064-2     | 0  | 0  | 100| 0.125  | 1       | 0.185     |
|                 | Posaconazole      | 0.016-0.125 | ---| ---| ---| 0.016  | 0.032  | 0.021     |
|                 | Itraconazole      | 0.016-0.25  | 0  | 22.2| 77.8| 0.032  | 0.125  | 0.037     |
|                 | Ketoconazole      | 0.016-0.16  | ---| ---| ---| 0.016  | 0.064  | 0.021     |
|                 | Amphotericin B    | 0.032-4     | 38.5| 0  | 61.5| 0.025  | 4       | 0.386     |
|                 | Caspofungin       | 0.016-0.5   | 0  | 7.7 | 92.3| 0.125  | 0.25   | 0.092     |
|                 | Voriconazole      | 0.032-16    | 7.7 | 0  | 92.3| 0.25   | 0.5    | 0.238     |
|                 | Fluconazole       | 0.25-64     | 0  | 0  | ---| 4      | 66     | 6.817     |
|                 | Posaconazole      | 0.016-16    | ---| ---| ---| 0.25   | 0.5    | 0.214     |
|                 | Itraconazole      | 0.064-16    | 15.4| 69.2| 15.4| 0.25   | 2      | 0.346     |
|                 | Ketoconazole      | 0.032-16    | ---| ---| ---| 0.125  | 4      | 0.33      |
|                 | Amphotericin B    | 0.016-0.25  | 0  | 0  | 100| 0.016  | 0.032  | 0.027     |
|                 | Caspofungin       | 0.016-4     | 0  | 16.7| 83.3| 0.5    | 4      | 0.282     |
|                 | Voriconazole      | 0.016-0.25  | 0  | 8.3 | 91.7| 0.016  | 0.032  | 0.025     |
|                 | Fluconazole       | 0.064-2     | 0  | 0  | 100| 0.25   | 2      | 0.298     |
|                 | Posaconazole      | 0.016-0.5   | ---| ---| ---| 0.032  | 0.032  | 0.03      |
|                 | Itraconazole      | 0.016-0.25  | 0  | 41.7| 58.3| 0.064  | 0.125  | 0.06      |
|                 | Ketoconazole      | 0.016-0.032 | ---| ---| ---| 0.016  | 0.032  | 0.02      |
|                 | Amphotericin B    | 0.016-1     | 0  | 0  | 100| 0.032  | 0.064  | 0.032     |
|                 | Caspofungin       | 0.016-1     | 0  | 0  | 100| 0.032  | 0.064  | 0.040     |
|                 | Voriconazole      | 0.016-0.125 | 0  | 0  | 100| 0.032  | 0.032  | 0.026     |
|                 | Fluconazole       | 0.064-8     | 0  | 0  | 100| 0.25   | 0.5    | 0.227     |
|                 | Posaconazole      | 0.016-0.064 | ---| ---| ---| 0.016  | 0.032  | 0.023     |
|                 | Itraconazole      | 0.016-0.25  | 0  | 23.1| 76.9| 0.032  | 0.25   | 0.042     |
|                 | Ketoconazole      | 0.016-0.064 | ---| ---| ---| 0.016  | 0.032  | 0.022     |

R: Resistant, I: Intermediate, S: Susceptible, MIC: Minimum inhibitory concentration.
There is no breakpoint for Posaconazole and Ketoconazole, only MIC was reported.
### Table 4. CLSI Clinical breakpoints and epidemiological cut off values for common Candida species

| Antifungal            | Organism          | S   | SDD | I   | R   | ECV | WT   | NWT   |
|-----------------------|-------------------|-----|-----|-----|-----|-----|------|-------|
| **Amphotericin**      |                   |     |     |     |     |     |      |       |
| C. albicans           | 1≤                |     |     | 1≤  | 0.25| ≥0.25| >0.25| <0.25 |
| C. parapsilosis       | 1≤                |     |     | 1≤  | 0.25| ≥0.25| >0.25| <0.25 |
| C. tropicalis         | 1≤                |     |     | 1≤  | 0.25| ≥0.25| >0.25| <0.25 |
| C. glabrata           | 1≤                |     |     | 1≤  | 0.064| ≥0.064| >0.064| <0.064|
| C. krusei             | 1≤                |     |     | 1≤  | 4   | ≥4   | >4   | <4    |
| Others                | 1≤                |     |     | 1≤  | 0.064| ≥0.064| >0.064| <0.064|
| C. albicans           | 0.25≥             |     |     | 0.5 | 1≤  | 0.25| ≥0.25| >0.25 |
| C. parapsilosis       | 2≥                |     |     | 4   | ≥4  | ≥4 (100%)| >4 (0%)| <4 (0)%|
| C. tropicalis         | 0.25≥             |     |     | 0.5 | 1≤  | 0.5 | ≥0.5 (98%)| >0.5 (2%)|
| C. glabrata           | 0.125≥            |     |     | 0.25| 0.5≤| 0.5 | ≥0.5 (98%)| >0.5 (2%)|
| C. krusei             | 0.25≥             |     |     | 0.5 | 1≤  | 0.5 | ≥0.5 (100%)| >0.5 (0%)|
| Others                | 2≥                |     | 4   | 8≤  | 0.064| ≥0.064| >0.064| <0.064|
| C. albicans           | 0.12≥             |     |     | 0.25-0.5| 1≤ | 0.064| ≥0.064| >0.064| <0.064|
| C. parapsilosis       | 0.12≥             |     |     | 0.25-0.5| 1≤ | 0.032| ≥0.032| >0.032| <0.032|
| C. tropicalis         | 0.125≥            |     |     | 0.25-0.5| 1≤ | 0.125| ≥0.125| >0.125| <0.125|
| C. glabrata           | ECV 0.5 µɡ⁄ml WT: MIC ≤ ECV, non-WT | 0.25| ≥0.25(96%)| ≥0.25| >0.25(4%)|
|                       |                   |     |      |     |      |      |      |       |
| **Amphotericin**      |                   |     |     |     |     |     |      |       |
|                       |                   |     |     |     |     |      |      |       |
| **Camoufla**          |                   |     |     |     |     |     |      |       |
| C. krusei             | 0.5≥              |     |     | 1   | 2≤  | 0.5 | ≥0.5 (93%)| >0.5 (7%)|
| Others                | 1≤                |     |     |     |     |     |      |       |
| C. albicans           | 2≥                |     |     | 4   | 8≤  | 8   | ≥8 (94%)| >8 (6%)|
| C. parapsilosis       | 2≥                |     |     | 4   | 8≤  | 0.5 | ≥0.5 (84%)| >0.5 (16%)|
| C. tropicalis         | 2≥                |     |     | 4   | 8≤  | 4   | ≥4 (92%)| >4 (8%)|
| C. glabrata           | 32≥               |     |     | 64≤ | 4   | ≥4 (95%)| >4 (5%)|
|                       |                   |     |     |     |     |      |      |       |
|                       | C. krusei         | 64   | ≥64 (100%)| ≥64| >64 (0%)|
| Others                | 1≤                |     |     |     |     |     |      |       |
| C. albicans           | 0.12≥             |     |     | 0.25-0.5| 1≤ | 0.5 | ≥0.5 (96%)| >0.5 (5%)|
| C. parapsilosis       | 0.12≥             |     |     | 0.25-0.5| 1≤ | 0.5 | ≥0.5 (98%)| >0.5 (2%)|
| C. tropicalis         | 0.12≥             |     |     | 0.25-0.5| 1≤ | 1    | ≥1 (96%)| >1 (4%)|
| C. krusei             | 0.12≥             |     |     | 0.25-0.5| 1≤ | 2    | ≥2 (92%)| >2 (8%)|
| Others                | 0.12≥             |     |     | 0.25-0.5| 1≤ | 0.032| ≥0.032| >0.032|
| C. albicans           | 0.25              |     |     |     |     | ≥0.25(95%)| >0.25(5%)|
| C. parapsilosis       | 0.032             |     |     |     |     | ≥0.032(92%)| >0.032(8%)|
| C. tropicalis         | There is no breakpoint, only MIC was reported. | 1   | ≥1(98%)| ≥1(2%)|
| C. glabrata           | 1≤                |     |     | 1≤  | 0.5 | ≥0.5(93%)| >0.5(7%)|
| C. krusei             | 0.5               |     |     |     |     | ≥0.5(93%)| >0.5(7%)|
| Others                | 0.064             |     |     |     |     | ≥0.064(100%)| >0.064(0%)|
| C. albicans           | 0.25              |     |     |     |     | ≥0.25(95%)| >0.25(5%)|
| C. parapsilosis       | 0.032             |     |     |     |     | ≥0.032(100%)| >0.032(0%)|
| C. tropicalis         | There is no breakpoint, only MIC was reported. | 0.5 | ≥0.5(97%)| ≥0.5(3%)|
| C. glabrata           | 0.125             |     |     | 0.125| 0.064| ≥0.125(96%)| >0.125(4%)|
| C. krusei             | 16                |     |     | ≥16(100%)| ≥16(0%)|
| Others                | 0.032             |     |     |     |     | ≥0.32(93%)| >0.32(7%)|

S: Susceptible, SSD: Susceptible dose dependent, I: Intermediate, R: Resistant, ECV: Epidemiological cut off value; WT: Wild type, NWT: Non-wild type
rates of non albicans Candida bloodstream infection in children were reported 29.7%, 41.7% and 57.1% for C. parapsilosis, C. tropicalis and C. glabrata, respectively (19). Distributions of Candida species are different according to region and patient’s populations. Therefore, identification of Candida species isolated from pediatric patients is valuable in each region.

Amphotericin B is a common antifungal agent recommended for fungal infection therapy but its use has some limitations due to the risk of toxicity. In the present study, most of Candida species isolates were susceptible to amphotericin B except C. albicans and C. krusei with resistance rates of 0.9% and 38.5%, respectively.

In the Candida species isolated from immunocompromised patients the resistance rates to amphotericin B were reported 7% (12/172) in C. albicans, 10% (6/62) in C. krusei, 15% (6/40) in C. glabrata, 22.3% (4/18) in C. parapsilosis and 33.3% (2/6) in C. tropicalis (12). While these rates in colonized pediatric patients were reported 3.4% (4/117), 27.7% (5/18) and 7.1% (1/14) in C. albicans, C. krusei and C. glabrata, respectively (13).

Fluconazole is a triazole agent that is the most prescribed antifungal agents for the treatment of Candida infections. Other azoles antifungal agents include voriconazole, posaconazole and itraconazole. The resistance rate to fluconazole in this study in C. albicans was 4.9% with MIC90 value 2 μg/ml and ECV 0.5 μg/ml, followed by C. tropicalis 8.8%, MIC90 value 4 μg/ml and ECV 0.5 μg/ml. The resistance rates in C. albicans to fluconazole were reported 12% (14/117) in colonizing isolates in neutropenic patients, 9.3% (16/160); and 81% (43/53) in infecting isolates (12-14). In Wisplinghoff et al. report 100% of C. glabrata, 4.9% of C. tropicalis, 2.9% of C. parapsilosis and 0.8% of C. albicans were not susceptible to fluconazole (18). The resistance rate of C. glabrata to fluconazole was reported 36% with 64% susceptible dose dependent (20). The acquired resistance to fluconazole (29.4%; P<0.05) is reported in C. glabrata isolates from colonized oral cavity in patients exposed to azoles (21). The increase resistance rate of Candida species to fluconazole maybe due to the frequent use of its medication. Voriconazole is an active azole antifungal agent against Candida species. In the present study, its susceptibility rate in C. glabrata and C. krusei, as the resistant Candida species, were 100%, (MIC50: 0.032 μg and MIC90: 0.016μg), and 92.3% (MIC50: 0.25 and MIC90: 0.5), respectively. The non-susceptible rates of Candida species to voriconazole were reported 9.8% of C. tropicalis, 7.6% of C. parapsilosis, 5.0% of C. krusei and 0.6% of C. albicans (18). In study done by pfaler et al., only C. krusei was resistant to voriconazole and other Candida species were susceptible to it (22). There is no breakpoint for posaconazole and ketoconazole, according to CSM M27 S4(10). Posaconazole is the newest triazole antifungal and very expensive in our region. All Candida species had MIC value between 0.032 and 0.5 μg/ml. Candida glabrata MIC90 values for posaconazole and ketoconazole were 0.5 μg/ml and 0.125 μg/ml with GM 0.082 μg/ml and 0.037 μg/ml, respectively. MIC90 value and GM for C. tropicalis to posaconazole and ketoconazole were 0.25 μg/ml and 0.25 μg/ml; and 0.035 μg/ml and 0.029 μg/ml, respectively. The MIC values for posaconazole were reported 0.016 μg/ml, 0.25 μg/ml, 0.125 μg/ml and 0.5 μg/ml in C. albicans, C. tropicalis, C. parapsilosis and C. glabrata, respectively (23). Posaconazole and voriconazole are used limitedly and are effective on Candida species isolates from the patients.

The sensitivity rates of C. glabrata and C. krusei to itraconazole were 12.5% (with 72.9% intermediate dose) and 15.4% (with intermediate dose 69.2%), respectively. The resistance rates to itraconazole in colonized species were reported 28% (36/117), 30% (6/18) and 50% (7/14) in C. albicans, C. krusei and C. glabrata, respectively (13). These rates were reported 86% (80/93), 59.5% (25/42) and 7.7% (2/26) in C. albicans, C. glabrata and C. parapsilosis, respectively (24). The increase in intermediate rates is alarming for future resistant strains. Ketoconazole is mostly used as a topical due to its side effects for humans. In the present study, the MIC90 values for all Candida species to ketoconazole were ≤0.25μg/ml except C. krusei which was 4 μg/ml. The resistance rates for this drug in C. glabrata and C. albicans were reported 33.3% (14/42) and 17.2% (16/93) respectively (24).

Of the echinocandin antifungal agents, caspofungin is more prescribed in our region. The resistant and intermediate rates of Candida species to caspofungin in this study were 0.4% and 2.7%; 2.9% and 5.9%; 18.8% and 47.9%; and 0.0% and 16.7% in C. albicans, C. tropicalis, C. glabrata and C. parapsilosis, respectively. Other Candida species were susceptible to caspofungin. C. krusei has intrinsic resistance to
fluconazole and has been shown the highest sensitivity to caspofungin and voriconazole (S=93.3%). In Korean patient, none of the Candida species was resistant to caspofungin (25).

CONCLUSION

Colonizing Candida species may be present as reservoir for future systemic candidiasis. In the present study, 49.2% of pediatric patients with hematologic disorders were colonized with Candida species. C. albicans was the most prevalent species in pediatric colonized patients. New azole agents like voriconazole and posaconazole are effective to non-albicans Candida species. Increase in intermediate species is alarming to future emerging resistant species. The information about distribution and susceptibility patterns of species can be useful to appropriate treatment in hemato-topietic pediatric patients at the duration of infection when sampling is impossible.

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