Identification of Candida Species and Antifungal Susceptibility in Cancer Patients with Oral Lesions in Ahvaz, Southern West of Iran

Abstract

**Background:** Oral candidiasis is a common disease in cancer patients subject to chemotherapy. The aim of this study was to evaluate the risk factors of rising oral candidiasis incidence and to identify the Candida species isolated from oral lesions of cancer patients and their antifungal sensitivity. **Materials and Methods:** A total of 645 patients with cancer were examined. Several Candida species were isolated from specimens and identified by morphological and molecular methods. The susceptibility of isolates to amphotericin B, fluconazole, and nystatin was also investigated. **Results:** A total of 74 isolates of Candida were recovered from oral cavity of 61 cancer patients with oral candidiasis. The isolates included Candida albicans (n = 56; 75.5%), Candida glabrata (n = 4; 5.4%), Candida krusei (n = 5; 7%), Candida tropicalis (n = 7; 9.4%), and Candida kefyr (n = 2; 2.7%). A total (n = 72; 98.6%) of isolates were susceptible to nystatin, (n = 58; 78.4%) of them were susceptible to fluconazole, and (n = 8; 10.8%) of susceptible dose-dependent isolates were specified, (n = 46; 62.16%) of isolates were susceptible to amphotericin B. **Conclusion:** Finally, in addition to emphasis on topical nystatin application in the first stage of oral candidiasis in these patients, using alternative systemic drugs such as fluconazole and amphotericin B can be considered for the resistant candida isolates to nystatin.

**Keywords:** Amphotericin B, Candida, chemotherapy, fluconazole, nystatin, oral candidiasis

Introduction

*Candida albicans,* as the most important commensal opportunistic yeast, is usually present in mouth, digestive system, and urinary tract; however, it can cause infection when the host is vulnerable or immunocompromised. The infection can be superficial or on mucous membranes, which may infiltrate into blood and cause internal organ infection.[1-4] *Candida* species have been identified as human pathogens, including *C. albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Candida kefyr*, *Candida lusitaniae*, *Candida dubliniensis*, and *Candida guilliermondii.*[5-6] Oral candidiasis is common in patients undergoing chemotherapy. Over the years, the prevalence of oral candidiasis in chemotherapy patients has been reported to be 7.2%–52%, which is affected by various factors such as therapeutic intervention, type of cancer, and disease stage.[7-10] *Candida* species are opportunist pathogens and their pathogenicity is influenced by the inherent potential of the organism as well as host factors.[11,12] The main factors of aggressive candidiasis in a host are long-term hospitalization in intensive care unit and administration of broad-spectrum antibiotics or immunosuppressive drugs.[13,14]

Several studies around the world show that colonization rate and infection in mouth vary among different groups of patients under chemotherapy, and most studies have been conducted on a specific population of patients, for example, those with head and neck cancer.[7,15] There have been few studies on the incidence of oral candidiasis in different groups of cancer patients to compare its prevalence among these groups. Increasing resistance of *Candida* species (especially non-*Albicans* ones) to antifungal agents such as fluconazole and amphotericin B have been reported in some centers.[16,17] Widespread use of these drugs for prophylaxis of fungal diseases in cancer patients with neutropenia is the reason for such resistance.[18] In general, early diagnosis and identification

How to cite this article: Mahernonnaghsh M, Fatahinia M, Dehghan P, Teimoori A. Identification of Candida species and antifungal susceptibility in cancer patients with oral lesions in Ahvaz, southern west of Iran. Adv Biomed Res 2020;9:50.
of fungal pathogens for targeted antifungal therapy is essential among these patients.[19,20] On the other hand, nystatin in the form of pastille is used to treat or prevent oral candidiasis lesions. In one study, nystatin pastille proved more effective than fluconazole in the treatment of oral candidiasis.[21] Fluconazole is an antifungal drug administered orally or intravenously to treat various fungal diseases. Moreover, it is used to prevent infection in patients immunocompromised due to chemotherapy drugs (e.g., neutropenic patients), those with organ transplants, and premature neonates.[22] Amphotericin B is considered as the treatment of choice for systemic fungal infections; however, resistance to amphotericin B has been reported in some species such as C. lusitaniae, C. guilliermondii, and C. kefyr.[16,23] Resistance to antifungal drugs has a major impact on the development of fungal infections and is significantly associated with increasing dissatisfaction with treatment, mortality, and prolonged hospital stay. This research was conducted at Jundishapur Alvah University of Medical Sciences in Baqee, Golestan, and Shafa hospitals with the aim of evaluating the frequency of oral candidiasis in a variety of patients undergoing chemotherapy to evaluate the risk factors associated with oral candidiasis among patients with various types of malignancies. Another goal of this research was to investigate antifungal susceptibility patterns of different Candida isolates against three antifungal agents of fluconazole, amphotericin B, and nystatin that are commonly used in these patients in Iran.

Materials and Methods

Isolation of samples

This study, which was approved by Ethics Committee of Jundishapur University of Medical Sciences (code number IR.AJUMS.REC.1397.699), was conducted on patients with solid tumors and hematologic malignancies who needed daily care. Patients with immunodeficiency, mental retardation, and those receiving antifungal agents over the past 4 weeks were excluded from the study. Of 645 patients with cancer, samples were taken from patients with signs and symptoms such as inflammation/ mucositis and/or presence of white plaques and feeling changes in their taste, leading to the detection of 61 oral candidiasis cases.[24-26] Specifications of patients including age, sex, chronic diseases, type of cancer, surgery, chemotherapy, radiotherapy, and oral dryness were determined. Sampling was done using two sterile swabs placed in tubes containing 0.5 ml sterilized distilled water, which were used to prepare direct smears and culture on CHROMagar Candida medium (CHROMagar Company, France). The culture medium was incubated at 35°C for 48 h. The sample was considered positive if there were growth of ≥10 colony-forming units (CFUs).[26-28]

Identification of samples using morphological methods

Primary diagnosis was based on the color of colony on CHROMagar Candida medium. Chlamydoconidia formation on corn Meal Agar-Tween 80 medium (Merck, Germany) and incubation at 25°C for 3 days distinguished Candida albicans from non-Albicans species.

Identification of isolates using molecular methods

After isolation, the samples were cultured on Sabouraud Dextrose Agar (SDA) medium (Merck, Germany) and the species were detected by polymerase chain reaction (PCR)-restriction fragment length polymorphism using MSPI enzyme for all isolates.[28] Then, to isolate C. albicans from Candida dublinensis, Duplex PCR was run directly on DNA extracted using two pairs of primers targeting ITS-1 and ITS-2 regions. The primer sequences were as follows.[29]

- CAL F: 5´ TGGTAAGGGCGGATCGCTT 3´
- CAL R: 5´ GGCTAAAGTTGAGATATAC 3´
- CDU F: 5´ AAACCTGTGACGAATTTT 3´
- CDU R: 5´ AAAGTTTGAAGAATAAATGCG 3´.

Drug susceptibility

Drug susceptibility test was performed using microdilution according to CLSI guidelines. Minimal inhibitory concentrations (MICs) for fluconazole and nystatin were determined as the lowest drug concentration significantly reducing the growth of organisms compared with positive control well, which was identified as MIC well. Clinical breakpoints for fluconazole and amphotericin B were determined based on CLSI M27-S3 and M27-A4 guidelines.[30,31] To determine MIC values for fluconazole, the drug was diluted in 0.125–64 range, so that ≤8 values were susceptible, 16–32 susceptible dose-dependent (SDD), and ≥64 resistant. For amphotericin B, drug dilution ranged 0.003–2, and ≥2 values were considered as resistant.[30] MIC for topical nystatin was determined based on CLSI M27-A2 instructions.[32] For nystatin, 0.003–2 µg/ml dilution of the drug was prepared, with ≤2 and >2 values showing susceptibility and resistance, respectively.[33]

Briefly, all Candida isolates were cultured on SDA medium. After incubation of isolates for 24 h at 30°C, a suspension of 0.5 McFarland standard was prepared from colonies, which was diluted with RPMI-1640 medium buffered with (3-(N-morpholino) propanesulfonic acid) to pH 7.0–7.2 at a rate of 1:100 CFU/ml. One hundred microliter of the mentioned dilution range of each drug was added to the wells. One hundred microliter of diluted yeast suspension was then added to each well, and two wells were used as a positive and negative control. Microplates were incubated at 35°C for 24–48 h, and the opacity of solution was assessed with the naked eye.[34,35]
Statistical analysis

Mann–Whitney nonparametric test in SPSS V23 (IBM, Armonk, NY, USA) with error rate of $P < 0.05$ was used to analyze the effects of variables of age, radiotherapy in addition to chemotherapy, surgery, and dryness of oral mucosa.

Results

Patients and organisms

Samples were taken from 286 patients (51.4% females and 48.9% males) with various malignancies, among whom 61 were diagnosed with oral candidiasis (57.4% females and 42.6% males) with a mean age of 26–87 years. The incidence of oral candidiasis was 14.8% higher in women than in men. Oral candidiasis was detected in types of cancers [Table 1].

Table 1: Characteristics of 61 cancer patients with oral candidiasis caused by Candida species

| Parameter          | Results       |
|--------------------|---------------|
| Median age, year (range) | 59 (26-87)    |
| Male sex, n (%)    | 26 (42.6)     |
| Female sex, n (%)  | 35 (57.4)     |
| Malignancy, n (%)  |               |
| Leukemia           | 13 (21.5)     |
| Lung               | 10 (16.4)     |
| Colon              | 10 (16.4)     |
| Breast             | 9 (14.7)      |
| Liver              | 4 (6.5)       |
| Lymphoma           | 4 (6.5)       |
| RCC                | 4 (6.5)       |
| Uterine            | 3 (5)         |
| Gastric            | 2 (3.3)       |
| Throat             | 1 (1.6)       |
| Bladder            | 1 (1.6)       |

RCC: Renal cell carcinoma

Out of these patients with 11 types of cancer, 74 different isolates of *Candida* were isolated, including 75.5% *Candida albicans* and 24.5% non-albicans species, namely *C. albicans* ($n = 56$), *glabrata* ($n = 4$), *C. krusei* ($n = 5$), *C. tropicalis* ($n = 7$) and *C. kefyr* ($n = 2$) [Table 2].

Risk factors

Oral candidiasis in patients undergoing chemotherapy and radiation therapy was 67.21% versus 32.7% in those with chemotherapy alone. Considering error rate of $P < 0.05$, the difference was statistically significant ($P = 0.045$). Furthermore, 68.85% of patients with oral candidiasis were diagnosed with dryness of mouth, which was significant in comparison with their patients having normal saliva ($P = 0.014$). In cancer patients with oral candidiasis, the effect of ≥60 years of age variable was 62.29%. On the other hand, more than half of the patients (63.93%) underwent surgery, for whom nonparametric analysis to determine the effect of age and surgery in the incidence of oral candidiasis was $P = 0.042$ and $P = 0.036$, respectively, indicating the significance of these values [Table 3].

Antifungal susceptibility

In this study, three antifungal agents of fluconazole, amphotericin B, and nystatin were used for 74 isolates of *Candida*.

After 24 h, 21.6% of isolates were resistant to fluconazole (MIC ≥64 µg/ml), including 20.3% and 1.4% of *C. albicans* and *C. kefyr* species, respectively. Ten and eighty percent of isolates were SDD (MIC = 16–23 µg/ml), 6.7%, 1.4%, and 2.7% of which were *C. albicans*, *C. tropicalis*, and *C. krusei*, respectively, and the remaining isolates (67.6%) were susceptible (MIC ≤8 µg/ml).

After incubation for 24–48 h, 37.8% of isolates were resistant to amphotericin B (≥2), including 36.4% and 1.4% of *C. albicans* and *C. kefyr* species, respectively, Table 1: Characteristics of 61 cancer patients with oral candidiasis caused by Candida species

| Cancer type | Candida albicans, n (%) | Candida glabrata, n (%) | Candida kefyr, n (%) | Candida krusei, n (%) | Candida tropicalis, n (%) | Total, n (%) |
|-------------|-------------------------|-------------------------|----------------------|-----------------------|--------------------------|--------------|
| Leukemia    | 12 (16.2)               | 1 (1.4)                 | 1 (1.4)              | -                     | 1 (1.4)                  | 15 (20.4)    |
| Lung        | 9 (12.1)                | 1 (1.4)                 | -                    | 1 (1.4)               | 1 (1.4)                  | 12 (16.2)    |
| Colon       | 10 (13.5)               | -                       | -                    | 1 (1.4)               | -                        | 11 (14.9)    |
| Breast      | 8 (10.8)                | -                       | -                    | 1 (1.4)               | -                        | 9 (12.1)     |
| Liver       | 3 (4)                   | 2 (2.7)                 | -                    | -                     | 2 (2.7)                  | 7 (9.4)      |
| Lymphoma    | 3 (4)                   | -                       | -                    | 1 (1.4)               | 2 (2.7)                  | 6 (8.1)      |
| RCC         | 4 (5.4)                 | -                       | -                    | -                     | 1 (1.4)                  | 5 (6.8)      |
| Uterine     | 4 (5.4)                 | -                       | -                    | -                     | -                        | 4 (5.4)      |
| Gastric     | 2 (2.7)                 | -                       | -                    | 1 (1.4)               | -                        | 3 (4)        |
| Throat      | 1 (1.4)                 | -                       | -                    | -                     | -                        | 1 (1.4)      |
| Bladder     | -                       | -                       | 1 (1.4)              | -                     | -                        | 1 (1.4)      |
| Total, n (%)| 56 (75.5)               | 4 (5.4)                 | 2 (2.7)              | 5 (7)                 | 7 (9.4)                  | 74 (100)     |

RCC: Renal cell carcinoma
and the remaining isolates (62.2%) were susceptible to fluconazole (<2 µg/ml).

After 24 h, 2.8% of isolates were resistant to nystatin (MIC >2 µg/ml), which was observed in only two isolates: One C. albicans and one C. krusei. The rest of the isolates (97.2%) were susceptible (MIC ≤2 µg/ml). MIC ranges, MIC50, MIC90, and geometric mean MIC were calculated for the three drugs [Table 4].

In this study, C. albicans isolates showed the highest resistance to amphotericin B (48.22%), followed by fluconazole and nystatin (26.79% and 1.35%, respectively), while the resistance of isolated non-Albicans species to fluconazole and amphotericin B was 5.55% and 5.56%, respectively, followed by nystatin (1.35%).

Dose-dependent species susceptible to fluconazole among C. albicans and non-Albicans isolates were 8.92% and 16.67%, respectively. The latter included C. tropicalis and C. krusei [Figure 1].

**Discussion**

Oral candidiasis is a common fungal infection in cancer patients and is currently recognized as the most prevalent fungal disease in humans.[36] Chemotherapy and radiotherapy in cancer patients can impair immune system cells and lead to neutropenia, resulting in colonization of Candida species in mucosal tissue, including oral cavity, which can pass into the blood and cause invasive candidiasis. Therefore, these patients are at high risk of invasive candidiasis.[27,37] The aim of this study was to investigate the frequency of various Candida species in different types of cancers and to evaluate the risk factors of increasing incidence of oral candidiasis in these patients. Antifungal sensitivity of the isolated species was investigated to achieve this goal. Our results showed oral candidiasis in 61 (21.3%) patients (57.4% women and 42.6% men) which was consistent with other studies. Schelenz et al. reported 18.9% rate of oral candidiasis in patients with cancer.[27] Bashir et al. reported 30% incidence of this disease among cancer patients.[19] In addition, Zolnner-Schwetz et al. believed that the colonization of Candida species in the digestive

### Table 3: Analysis of risk factors for oral candidiasis caused by Candida species

| Risk factors                  | n (%)   | P    |
|-------------------------------|---------|------|
| Chemotherapy and radiotherapy | 41 (67.21) | 0.045 |
| Dry mouth                     | 42 (68.85) | 0.014 |
| Age ≥60 years old             | 38 (62.29) | 0.042 |
| Surgery                       | 39 (63.93) | 0.036 |

### Table 4: In vitro antifungal susceptibilities of 74 clinical isolates against fluconazole, amphotericin B, and nystatin

| Antifungal agents | MIC ranges | Minimum inhibitory concentration (µg/mL) | Geometric mean |
|-------------------|------------|------------------------------------------|----------------|
| **Candida albicans (n=56)** |            |                                          |                |
| Fluconazole       | 64-0.06    | 0.5                                       | 19.73345       |
| Amphotericin B    | 2-0.015    | 1                                         | 1.19563        |
| Nystatin          | 4-1        | 1                                         | 1.26786        |
| **Candida glabrata (n=4)** |            |                                          |                |
| Fluconazole       | 4-0.12     | 4                                         | 3.03000        |
| Amphotericin B    | 1-0.07     | 0.5                                       | 0.50175        |
| Nystatin          | 2-1        | 2                                         | 1.75000        |
| **Candida tropicalis (n=7)** |            |                                          |                |
| Fluconazole       | 16-0.06    | 0.5                                       | 3.53429        |
| Amphotericin B    | 1-0.015    | 0.12                                      | 0.31571        |
| Nystatin          | 2-1        | 1                                         | 1.14286        |
| **Candida krusei (n=5)** |            |                                          |                |
| Fluconazole       | 64-0.25    | 8                                         | 12.0500        |
| Amphotericin B    | 1-0.03     | 1                                         | 0.80600        |
| Nystatin          | 4-1        | 2                                         | 2.20000        |
| **Candida kefyr (n=2)** |            |                                          |                |
| Fluconazole       | 64-2       |                                           |                |
| Amphotericin B    | 2-0.25     |                                           |                |
| Nystatin          | 1          |                                           |                |
| All isolated yeasts (n=74) |            |                                          |                |
| Fluconazole       | 64-0.06    | 1                                         | 16.43153       |
| Amphotericin B    | 2-0.015    | 1                                         | 1.04750        |
| Nystatin          | 4-1        | 1                                         | 1.3378         |

aMIC which inhibits 50% of Candida species isolates in test, bMIC which inhibits 90% of Candida species isolates in test, cGeometric mean MIC. MIC: Minimal inhibitory concentration
of oral candidiasis in patients receiving radiotherapy along with chemotherapy. Other risk factors investigated in this research were ≥60-year-old patients as well as those who underwent surgery, both of which significantly contributed to the incidence of oral candidiasis. In a research conducted on cancer patients and healthy subjects with oral candidiasis to evaluate the risk factors for candidiasis, it was found that ≥60-year-old cancer patients accounted for a significant share of patients with oral candidiasis. They also identified surgery as one of the risk factors for colonization of Candida species. In this study, the highest frequency of oral candidiasis was observed in patients with leukemia as well as lung and colon cancers, and C. albicans was the most frequent causative agent of candidiasis in the mouth of patients with different types of cancers (75.5%). Many investigations have shown that C. albicans is the most typical cause of colonization as well as oral candidiasis among cancer patients. Although C. albicans is recognized as the most frequent cause of colonization and candidiasis in patients, the increase in non-Albicans species such as C. glabrata, C. krusei, C. parapsilosis, and C. tropicalis has been reported by several researchers in recent decades. In Betts et al. study, the most common species of non-Albicans candida were C. tropicalis (37%) and C. krusei (11%). In the present study, the most prevalent non-Albicans species were C. tropicalis (9.4%), followed by C. krusei (7%), C. glabrata (5.4%), and C. kefyr (2.7%). Fluconazole is a triazole antifungal agent that is used as the first line of systemic therapy in patients undergoing chemotherapy. On the other hand, fluconazole and amphotericin B are widely used for prophylaxis of fungal infections in neutropenic patients with malignancies. Infectious Diseases Society of America also recommends the use of nystatin in the form of suspension or pastille for the treatment of primary oral candidiasis and mentions easy access and convenience of this drug. Reports have shown that the susceptibility of C. albicans and non-Albicans species such as C. tropicalis, C. krusei, and C. glabrata to fluconazole and amphotericin B has gradually decreased in the past decades. In the present study, general resistance of C. albicans species to amphotericin B and fluconazole was detected, and only one C. kefyr isolate was found to be resistant to both drugs. In a research on patients undergoing chemotherapy, the resistance of albicans species to fluconazole was reported to be 47.2%, and the highest level of resistance was observed in C. albicans. Increasing resistance of C. albicans species to fluconazole has been reported in several researches. On the other hand, in an investigation conducted by Haddadi and colleagues, resistance to amphotericin B was reported in C. albicans, C. krusei, and C. glabrata species. Therefore, Our results on C. albicans isolates were consistent with these studies, while C. tropicalis, C. krusei, and C. glabrata species isolated from patients were 94.4% and 77.78% susceptible to amphotericin B and
fluconazole, respectively. About 16.67% of dose-dependent species susceptible to fluconazole were *C. albicans*, *C. krusei*, and *C. kefyr*.

Overall, topical nystatin used in the form of suspension by patients had the best effect (98.65%) on *C. albicans* and non-*Albicans* species of *Candida in vitro*. Fluconazole and amphotericin B respectively had a stronger effect on *C. albicans* isolates while non-*Albicans* species were susceptible to amphotericin B and fluconazole, respectively. Consequently, the colonization of *Candida* species, which leads to fungal infections in hospitalized patients with various malignancies, is of high importance. In addition, multiple risk factors contribute to this problem in this vulnerable group. On the other hand, unchecked use of fluconazole as the first line of treatment in *C. albicans* species has led to the resistance of these species to amphotericin B. Given that cell membrane ergosterol in *C. albicans* isolates is the target of treatment by azole and polyene drugs, long-term treatment with fluconazole induces mutation in one or more alleles and eventual mutation in a number of genes, resulting in impaired synthesis and increasing the resistance of daughter cells. The change in ergosterol structure among refractory *C. albicans* species can also account for the resistance of these species to amphotericin B. Based on the results of this study, it seems that among the three antifungal drugs used for the treatment of candidiasis over decades, application of topical nystatin is advisable in the first stage of treatment and even for the prevention of high-risk patients because most *Candida* isolates were susceptible to this drug, while the increasing resistance to fluconazole and amphotericin B demands novel antifungal drugs with a different function. Moreover, monitoring the epidemiological trend and assessment of drug susceptibility in various *Candida* species is suggested to achieve optimal drug response.

**Conclusion**

Data were shown that *C. albicans* is the most commonly identified species in oral candidiasis. Chemotherapy, radiotherapy, surgery and ≥60-year-old patients significantly contributed to the incidence of oral candidiasis. Based on the results of this study, among the three antifungal drugs for the treatment of oral candidiasis, application of topical nystatin is advisable in the first stage of treatment and even for the prevention of high-risk patients because most *Candida* isolates were susceptible to this drug. In cases *Candida* species resistant to nystatin, using of systemic drugs fluconazole and amphotericin B is recommended.

**Acknowledgments**

The authors would like to thank and gratefully acknowledges the staff of Baqae, Golestan, and Shafa hospitals who helped make this project.

**Financial support and sponsorship**

This study was supported by a grant (No: OG-9733) from Infectious and Tropical Diseases Research Center, Health Research Institute Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Jackson BE, Wilemhus KR, Hube B. The role of secreted aspartyl proteinases in *Candida albicans* keratitis. Invest Ophthalmol Vis Sci 2007;48:3559-65.
2. Archak JM, Fries BC. *Candida* infections of the genitourinary tract. Clin Microbiol Rev 2010;23:253-73.
3. Rosenbach A, Dignard D, Pierce JV, Whately M, Kumamoto CA. Adaptations of *Candida albicans* for growth in the mammalian intestinal tract. Eukaryot Cell 2010;9:1075-86.
4. López-Martínez R. Candidosis, a new challenge. Clin Dermatol 2010;28:178-84.
5. Odds FC. *Candida* and Candidosis: A Review and Bibliography. UK: Bailliere Tindall; 1988.
6. Edwards JE Jr. *Candida* species. In: Mendel GL, Bennett JE, Dolin R, editors. Principles and Practice of Infectious Diseases. New York: Elsevier, Churchill Livingstone; 2010.
7. Jham BC, França EC, Oliveira RR, Santos VR, Kowalski LP, da Silva Freire AR. *Candida* oral colonization and infection in Brazilian patients undergoing head and neck radiotherapy: A pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:355-8.
8. Jham BC, Reis PM, Miranda EL, Lopes RC, Carvalho AL, Schepet MA, et al. Oral health status of 207 head and neck cancer patients before, during and after radiotherapy. Clin Oral Investig 2008;12:19-24.
9. Davies AN, Brailsford SR, Beighton D. Oral candidosis in patients with advanced cancer. Oral Oncol 2006;42:698-702.
10. Davies AN, Brailsford SR, Beighton D, Shorthose K, Stevens VC. Oral candidosis in community-based patients with advanced cancer. J Pain Symptom Manage 2008;35:508-14.
11. Samaranayake LP, MacFarlane TW, Lamey PJ, Ferguson MM. A comparison of oral rinse and imprint sampling techniques for the detection of yeast, coliform and *Staphylococcus aureus* carriage in the oral cavity. J Oral Pathol 1986;15:386-8.
12. Fatahnia M, Poormohamadi F, Zarei Mahmoudabadi A. Comparative study of esterase and hemolytic important candida activities in clinically species, isolated from oral cavity of diabetic and non-diabetic individuals. Jundishapur J Microbiol 2015; 8:e20893.
13. Kontoyiannis DP, Mantadakis E, Samonis G. Systemic mycoses in the immunocompromised host: An update in antifungal therapy. J Hosp Infect 2003;53:243-58.
14. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev 2011; 24:141-73.
15. Jahanshiri Z, Manifar S, Moosa H, Asghari-Paskiabi F, Mahmoodzadeh H, Shams-Ghaftarokhi M, et al. Oropharyngeal candidiasis in head and neck cancer patients in Iran: Species identification, antifungal susceptibility and pathogenic characterization. J Mycol Med 2018;28:361-6.
16. Amran F, Aziz MN, Ibrahim HM, Atiqah NH, Parameswari S, Hafiza MR, et al. *In vitro* antifungal susceptibilities of *Candida*.
isolates from patients with invasive candidiasis in Kuala Lumpur Hospital, Malaysia. J Med Microbiol 2011; 60:1312-6.

17. Colombo AL, Da Matta D, De Almeida LP, Rosas R. Fluconazole susceptibility of Brazilian Candida isolates assessed by a disk diffusion method. Braz J Infect Dis 2002; 6:118-23.

18. Robenshtok E, Gafter-Gvili A, Goldberg E, Weinberger M, Yeshurun M, Leibovici L, et al. Antifungal prophylaxis in cancer patients after chemotherapy or hematopoietic stem-cell transplantation: Systematic review and meta-analysis. J Clin Oncol 2007; 25:5471-89.

19. Bashir H, Ahmad J. Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital. Int J 2014; 2:541-50.

20. Vazin A, Davarpanah MA, Ghalesoltani S. Antifungal agent utilization evaluation in hospitalized neutropenic cancer patients at a large teaching hospital. Drug Healthc Patient Saf 2015;7:97-102.

21. Lyu X, Zhao C, Yan ZM, Hua H. Efficacy of nystatin for the treatment of oral candidiasis: A systematic review and meta-analysis. Drug Des Devel Ther 2016;10:1161-71.

22. Orozco AS, Higginbotham LM, Hitchcock CA, Parkinson T, Falconer D, Ibrahim AS, et al. Mechanism of fluconazole resistance in Candida krusei. Antimicrob Agents Chemother 1998;42:2645-9.

23. Krcmery V, Barnes AJ. Non-albicans Candida spp. causing fungaemia: Pathogenicity and antifungal resistance. J Hosp Infect 2002;50:243-60.

24. Rautemaa R, Rusanen P, Richardson M, Meurman JH. Optimal sampling site for mucosal candidosis in oral cancer patients is the labial sulcus. J Med Microbiol 2006;55:1447-51.

25. Afraseyabi S, Afkhamzadeh A, Sabori H, Verdi F, Khaksar N, Afraseyabi S, et al. Identification and antifungal susceptibility of Brazilian Candida isolates assessed by a disk diffusion method. Braz J Infect Dis 2002; 6:118-23.

26. Schelenz S, Abdallah S, Gray G, Stubbings H, Gow I, Baker P, et al. Oral Candidiasis amongst cancer patients at Qods hospital. Afr J Clin Exp Microbiol 2011;12;3:129-132

27. Sroussi HY, Epstein JB, Bensadoun RJ, Saunders DP, Lalla RV, Migliorati CA, et al. Common oral complications of head and neck cancer radiation therapy: Mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. Cancer Med 2017;6:2918-31.

28. Schelenz S, Abdollahi S, Gray G, Stubbings H, Gow I, Baker P, et al. Epidemiology of oral yeast colonization in patients with hematological malignancies, head neck and solid tumors. J Oral Pathol Med 2011;40:83-9.

29. Dahiya MC, Redding SW, Dahiya RS, Eng TY, Kirkpatrick WR, Coco BJ, et al. Oropharyngeal candidiasis caused by non-albicans yeast in patients receiving external beam radiotherapy for head-and-neck cancer. Int J Radiat Oncol Biol Phys 2003;57:79-83.

30. Kiampour S, Ardestani ME, Dehghan P. Identification of Candida albicans and Candida dubliniensis Species Isolated from bronchoalveolar lavage samples using genotypic and phenotypic methods. Adv Biomed Res 2018;7:66.

31. Wayne P. National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard. NCCLS Document M27-A3. Clinical and Laboratory Standards Institute; 2008.

32. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Fourth Informational Supplement M27-S4. PA, USA, Wayne: Clinical and Laboratory Standards Institute; 2012.

33. Wayne P. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard. CLSI Document M27-A2. Clinical and Laboratory Standards Institute; 2002.