Original Article

Isolation of *Candida* Species from Gastroesophageal Lesions among Pediatrics in Isfahan, Iran: Identification and Antifungal Susceptibility Testing of Clinical Isolates by E-test

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Abstract

Background: *Candida* species can become opportunistic pathogens causing local or systemic invasive infections. Gastroesophageal candidiasis may depend on the *Candida* colonization and local damage of the mucosal barrier. Risk factors are gastric acid suppression, diabetes mellitus, chronic debilitating states such as carcinomas, and the use of systemic antibiotics and corticosteroids. The aim of this study is collection and molecular identification of *Candida* species from gastroesophageal lesions among pediatrics in Isfahan, and determination of minimum inhibitory concentration (MIC) ranges for clinical isolates. **Materials and Methods:** A total of 200 patients underwent endoscopy (130 specimens from gastritis and 70 samples from esophagitis) were included in this study between April 2015 and November 2015. All specimens were subcultured on sabouraud dextrose agar, and genomic DNA of all strains was extracted using boiling method. Polymerase chain reaction and DNA sequencing of the ITS1-5.8SrDNA-ITS2 region were used for the identification of all *Candida* strains. MIC ranges were determined for itraconazole (ITC), amphotericin B (AmB), and fluconazole (FLU) by E-test. **Results:** Twenty of 200 suspected patients (10%) were positive by direct microscopy and culture. *Candida albicans* was the most common species (60%) followed by *Candida glabrata* (30%), *Candida parapsilosis* (5%), and *Candida kefyr* (5%). MIC ranges were determined for FLU (0.125–8 μg/mL), ITC (0.008–0.75 μg/mL), and AmB (0.008–0.75 μg/mL), respectively. **Conclusion:** Every colonization of *Candida* species should be considered as a potentially factor of mucocutaneous candidiasis and should be treated with antifungal drugs.

Keywords: *Candida species, E-test, Gastroesophageal lesions, Minimum inhibitory concentration, Sequencing*

Introduction

Candida species are opportunistic pathogens causing local or systemic invasive infections while the immune system is weakened or the mucocutaneous barrier is disrupted. After colonization, *Candida* spp. in low numbers may remain for several months or years in the absence of inflammation. *Candida* species are regularly isolated from the oral cavity and are found in 30–60% of healthy individuals. Colonization rates were usually increased with the hospitalization period and acuteness of illness. Predisposing factors are stem cell/organ transplantation, chemotherapy, corticosteroid therapy, acquired immune deficiency syndrome (AIDS), multiple intravenous catheters, and surgical wounds.[1,2] *Candida* infections are categorized into three groups depending on their localization: Cutaneous (the skin and its appendages), mucosal including oropharyngeal, esophageal, and vulvovaginal candidiasis, and systemic infection such as candidemia and different forms of invasive candidiasis. The esophagus is the second most frequent site of gastrointestinal candidiasis, after the oropharynx. Esophageal candidiasis (EC) usually occurs in AIDS patients with low CD4+ T-cells (<200 cells/μL).[2,3] Although *Candida albicans* still remains the main cause of candidiasis, however, the prevalence of non-*albicans Candida* species such as *Candida parapsilosis*, *Candida glabrata*, and *Candida krusei* is increasing.[3,4] *Candida* species that are obtained from the esophageal surface are ordinarily the same organisms recognized in oral secretions. With regard to the differences in susceptibilities to

How to cite this article: Salehi F, Esmaeili M, Mohammadi R. Isolation of *Candida* Species from Gastroesophageal Lesions among Pediatrics in Isfahan, Iran: Identification and Antifungal Susceptibility Testing of Clinical Isolates by E-test. Adv Biomed Res 2017;6:103.

Received: May, 2016. **Accepted:** June, 2016.

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Access this article online

Website: www.advbiores.net

DOI: 10.4103/2277-9175.213662

Quick Response Code:

antifungal agents among *Candida* spp., identification of clinical isolates to the species level is vital to quick and appropriate therapy. This study aimed to identify the clinical strains of *Candida* spp. isolated from gastro-EC among pediatrics in Isfahan, Iran, and determine the antifungal susceptibility testing of clinical isolates by E-test.

Materials and Methods

Specimens

A total of 200 patients underwent endoscopy (130 specimens from gastritis and 70 samples from esophagitis) were included in this study between April 2015 and November 2015. Ninety-eight suspected patients were male, and 102 were female. Biopsy specimens were transferred to the clinical laboratory for pathology test with periodic acid-Schiff (PAS) staining. Before microtomy and paraffin section preparation, a small part of samples were minced by means of a sterile scissors and mortar and divided into two parts for direct microscopy with potassium hydroxide 10% and cultured on sabouraud dextrose agar (Biolife, Milano, Italy).

Molecular identification

Genomic DNA of all strains was extracted using boiling method.[5] Polymerase chain reaction (PCR) amplification and DNA sequencing of the ITS1-5.8SrDNA-ITS2 region were used for the identification of all *Candida* strains. The universal fungal primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) were used to amplify the entire ITS rDNA region.[6,7] PCR mixture contained 5 μL of 10× reaction buffer, 0.4 mM of dNTPs, 1.5 mM of MgCl2, 2.5 U of Taq polymerase, 30 pmol of each ITS1 and ITS4 primers, and 2 μL of extracted DNA in a final volume of 50 μL. The PCR cycling conditions comprised: Initial denaturation at 94°C for 5 min, followed by thirty cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min.

Sequencing

The amplicons were purified using the ethanol purification method, and cycle sequencing reactions in forward direction were performed (Bioneer, Korea). The sequencing products were analyzed with Chromas 2.3 (http://chromas.software.informer.com/2.4/). Results were checked using NCBI BLAST searches against fungal sequences existing in DNA databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antifungal susceptibility testing

Agar diffusion E-test (Biomerieux, Sweden) was performed with RPMI 1640 (Sigma Chemical Co., St. Louis, MO, USA) (8.5 g/l), 2% glucose and 1.5% agar which buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid buffer (Sigma, St. Louis, MO, USA) in 90 mm diameter plates. Petri dishes were inoculated by immersion of a sterile swab into the inoculum suspension adapted to the turbidity of a 0.5 McFarland standard and streaking it across the surface of the plates in four directions. The plates were incubated at 37°C for 15 min before applying the E-test strips. After 48 h incubation period, minimum inhibition concentrations (MICs) endpoints were determined for itraconazole (ITC), amphotericin B (AmB), and fluconazole (FLU).

Results

Twenty of 200 suspected patients (10%) were positive by direct microscopy and culture. Nine patients (45%) had gastritis, seven patients (35%) had esophagitis, and four patients (20%) presented lesions on both organs. Eleven patients (55%) were male, and nine patients (45%) were female. Clinical symptoms included abdominal pain, nausea, vomiting, reflux, constipation, and dysphagia [Table 1]. *C. albicans* was the most common species (60%) followed by *C. glabrata* (30%), *C. parapsilosis* (5%), and *Candida kefyr* (5%). MIC ranges were determined for FLU (0.125–8 μg/mL), ITC (0.008–0.75 μg/mL), and AmB (0.008–0.75 μg/mL), respectively [Table 2].

Discussion

While the immune system, especially the cellular immunity, is weakened or the cutaneous or mucosal barrier is disturbed, *Candida* species can become opportunistic fungal pathogens causing local or systemic invasive infections. Gastro-EC distribution may depend on the *Candida* colonization and local disturbance of the mucosal barrier (esophagitis and gastritis). In such individuals, the presence of EC can usually be linked to other predisposing factors. Such conditions contain gastric acid suppression, diabetes mellitus, chronic debilitating states such as carcinomas, and the use of systemic antibiotics and corticosteroids.[8] *Candida* colonization in the digestive system is associated with a higher frequency of candidemia.[4,9,10] Patients with gastro-EC may present with one of the routine symptoms of the infectious such as dysphagia, esophagitis, odynophagia, gastritis, nausea, vomiting, and chest pain or may remain asymptomatic.[11,12] In this study, we also found two cases (10%) with dysphagia. Gastroesophageal plaques of patients in this study are meaningfully higher than those reported by Underwood *et al*. (0.71%),[13] Naito *et al*. (1.17%),[14] Choi *et al*. (0.32%),[11] and Mohammadi and Abdi (5.3%).[15] It is already well accepted that *Candida* colonization occurs in the esophagus of 20% of healthy adults.[16] In terms of pathogenesis, the development of gastro-EC is described as a two-step process containing of colonization of *Candida* spp. on the esophagus or stomach and following invasion of the yeast to the epithelial layer.[2,17,18] Once colonization has been occurred, and impaired cellular immunity allows invasion of the fungus to the epithelial layer. In this investigation, the invasion

Table 1: Features of patients in the present study

Sex	Age	Symptoms	Organ	*Candida* spp.	Use of antibiotic
Male	3	AP	Stomach	*C. kefyr*	−
Male	17	AP	Esophagus	*C. albicans*	−
Male	8	AP + nausea	Stomach	*C. albicans*	−
Male	8	AP	Stomach	*C. glabrata*	+
Female	4	AP	Esophagus	*C. albicans*	−
Female	12	AP + nausea	Stomach + esophagus	*C. albicans*	+
Female	11	AP + nausea	Stomach + esophagus	*C. glabrata*	−
Female	12	AP + nausea + reflux	Stomach	*C. albicans*	−
Female	13	AP + vomiting	Stomach	*C. glabrata*	−
Female	10	AP	Stomach	*C. albicans*	−
Male	13	AP + dysphagia	Esophagus	*C. albicans*	−
Male	9	AP	Stomach + esophagus	*C. albicans*	−
Male	5	AP + dysphagia	Stomach	*C. glabrata*	−
Male	9	AP	Stomach + esophagus	*C. albicans*	−
Male	5	AP	Stomach	*C. albicans*	−
Female	6	AP	Stomach	*C. parapsilosis*	−
Female	6	AP + constipation	Esophagus	*C. albicans*	−
Male	6	AP	Esophagus	*C. glabrata*	−
Male	7	AP	Esophagus	*C. albicans*	−
Female	14	AP + vomiting	Esophagus	*C. glabrata*	−

AP: Abdominal pain, *C. kefyr: Candida kefyr*, *C. albicans: Candida albicans*, *C. glabrata: Candida glabrata*, *C. parapsilosis: Candida parapsilosis*

Table 2: Minimum inhibitory concentration ranges for clinical isolates of *Candida* spp.

IT (μg/ml)	FL (μg/ml)	AMP-B (μg/ml)
0.25	0.5	0.19
0.5	0.25	0.094
0.5	4	0.25
0.094	0.125	0.008
0.5	0.5	0.19
0.25	0.25	0.094
0.5	16	0.5
0.094	0.25	0.023
0.75	8	0.125
0.5	0.5	0.19
0.25	1	0.125
0.5	0.5	0.5
0.5	0.125	0.19
0.094	1	0.75
0.008	0.5	0.094
0.094	1	1
0.25	0.125	0.19
0.125	2	0.025
0.094	4	0.5
0.38	0.25	0.023

AMP-B: Amphotericin-B, IT: Itraconazole, FL: Fluconazole

of *Candida* spp. to the epithelial layer was detected only in one patient (5%) by PAS staining. Although EC is well known to occur in immunosuppressed patients, it has only infrequently been reported in immunocompetent cases. In this study, we could not find any patient with cancer, HIV infection, and other immunocompromised condition, except for two cases (use of antibiotic). It might be associated with colonization of *Candida* species in pseudomembrane lesions as a microflora. The use of both systemic and inhaled corticosteroids and wide-spectrum antibiotics has also been associated with the development of EC by allowing colonization and overgrowth of the *Candida* species. The antifungal susceptibility testing of pathogenic fungi can manage the selection of adequate therapy and also provides an estimate of antifungal efficacy. AmB is the first systemic antifungal drug for treatment of invasive fungal infections,[19,20] but, due to nephrotoxicity effect, use of AmB has been limited.[21] Breakpoint for AmB has not been proposed because it can affect the immune system and motivates the body defenses against fungal infections. In this study, all isolates were susceptible to ITC and AmB; however, 10% of the clinical isolates were resistant to FLU. Similar to this investigation, Teseng *et al*.[22] showed all *Candida* strains were susceptible to AmB in their study. Long-term ITC and FLU prophylaxis were associated with a decrease in susceptibility to these antifungal agents. Susceptibility of *C. albicans* to FLU was 100% in this study (at MIC ≤4 μg/mL), in comparison with the susceptibility rates reported by Saporiti *et al*.[23] (87%), Bauters *et al*.[24] (79%), and Citak *et al*.[25] (87.5%). *C. glabrata* is clearly *Candida* species with the greatest potential to acquire resistance to FLU and other azoles.[26,27] In this investigation, 50% of *C. glabrata* isolates were resistant to FLU.

Conclusion

Although gastro-EC is connected to the suppression of cell-mediated immunity, however, every colonization of

Candida species should be considered as a potentially factor of mucocutaneous candidiasis and should be managed with an appropriate antifungal agent.

Financial support and sponsorship

The authors gratefully acknowledge the financial support for this work as a part of the thesis No. 394907 that was provided by Isfahan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

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