

## Anti-fungal resistance in candida isolated from oral and diaper rash candidiasis in neonates

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### Abstract:

The purpose of the present study is to evaluate the sensitivity of *Candida* species isolated from oral candidiasis and diaper dermatitis infections in children. The children referring to private and public clinics in Ilam, Iran were examined for oral candidiasis and diaper dermatitis. In this study, 248 oral candidiasis and diaper dermatitis samples were collected and cultured. *Candida* species were identified by using standard methods. Resistance and sensitivity to amphotericin B, nystatin, ketoconazole, fluconazole, itraconazole, clotrimazole, and posaconazole were determined using the CLSI M44-A standard disk diffusion method. From the 248 studied samples, 149 were positive for *Candida*, among which the *Candida albicans* was the most prevalent (64.4%). The resistance of different *Candida* species to nystatin, itraconazole, fluconazole, ketoconazole, clotrimazole, voriconazole, and posaconazole were 4, 43, 34.2, 34.9, 21.5, 6, and 6.7%, respectively. No resistance to amphotericin B was observed. Considering rather low resistance to nystatin, this drug is the best choice for oral candidiasis and diaper dermatitis.

**Keywords:** Drug resistance, Oral candidiasis, Diaper dermatitis.

### Background:

Diaper dermatitis is one of the most common forms of dermatitis in neonates [1], comprising 10-20% of dermatological problems in America with a prevalence of 7-35% in infants [2, 3]. The peak age for this complication is 6-12 months [1]. It can be primarily caused by diapers, increased skin pH, zinc deficiency, prolonged exposure to humidity, and feces and urine-induced irritation [1]. If dermatitis continues for more than three days, secondary infections such as *Candida* will occur [4]. As the name implies, diaper dermatitis occurs more frequently in diaper-covered areas [3]. Dermatitis caused by *Candida* species can be diagnosed clinically as well as by culture [5]. Topical anti-fungals such as nystatin, clotrimazole,

miconazole, and ketoconazole are used to treat diaper dermatitis [6]. Oral candidiasis is a local infection observed in infants and patients taking antibiotics, head and neck chemotherapy or radiotherapy, or immunocompromised individuals and those using inhalational corticosteroids [7, 8]. The most common cause of oral candidiasis is *Candida albicans* [8] and diagnosis is mostly done clinically [9]. However, preparing cultures from the lesions using gram staining or KOH can also be used as a diagnostic method [8]. Azoles such as fluconazole and itraconazole can be used for treatment. It has been shown that posaconazole is also effective in case of oral candidiasis [10], and nystatin is also used for treating mild forms this infection [10]. A previous study showed that

clotrimazole and fluconazole had the same effectiveness while nystatin had less [11]. The objective of this study was to assess susceptibility of *Candida* species to common antifungals used in the case of oral candidiasis and diaper dermatitis.

## Methodology:

### Patients

In this experimental study, samples from oral candidiasis and diaper dermatitis lesions were collected from children referring to private and public clinics in Ilam, Iran, using a census method during 2012. The patients' demographic characteristics were collected using a researcher-made questionnaire with confirmed reliability.

### Sample collection

Samples were obtained using sterile swabs during routine visits when oral candidiasis and diaper dermatitis lesions were clinically identified. We included patients who had not received antifungal treatment for the past three days in order to prevent false negative cases. The samples were put in distilled water and transferred to the laboratory. There the samples were incubated at 37°C for 48-72 hours on Sabouraud dextrose agar culture media, and then the cultured plates were evaluated. First, slides were prepared of the cultured colonies. In the case of finding yeast cells, the *Candida* species were identified by standard methods and the species were divided to groups; *C. albicans* and non-*C. albicans* [12, 13].

### Antifungal susceptibility testing

The antifungal susceptibility was determined based on the reference method M44-A suggested by the Clinical and Laboratory Standards Institute (CLSI) [14]. The *Candida albicans* strain ATCC 2091 as standard strain was used. The isolates were subcultured on Sabouraud Dextrose Agar (SDA) and incubated at 37°C overnight. Saline suspension of the organism was made and the turbidity was adjusted to 0.5 McFarland standards. The disks were placed on the media and the plates were incubated at 37°C. The zone of inhibition was recorded after 24 hours and 48 hours. We used nystatin 100 unit/disk, fluconazole 10 µg/disk, itraconazole 10µg/disk, ketoconazole 10µg/disk, amphotericinB 20µg/disk, clotrimazole 10µg/disk, posaconazole 5µg/disk, and voriconazole 1µg/disk (Sigma Aldrich, USA) [15].

### Statistical analysis

Data were analyzed using SPSS software, version 16. Chi-square and T test student were used as appropriated.  $P < 0.05$  was considered as significant.

## Results & Discussion:

The *Candida*-positive were 149 among all 248 samples including 60 oral candidiasis samples, 87 diaper dermatitis samples, and 2 samples having both infections. The most common infective agent was *Candida albicans* (64.4%). About 43.6% of the children were born through cesarean and 15.4% of the patients had experienced *Candida* infection more than once. The 24.8% of the infants were fed by formula, the 70.5% were breastfed, and the 4.7% were fed by combination of formula and breast milk. We did not find a significant relationship between type of feeding and *Candida* infection ( $P=0.42$ , **Table 1 (see supplementary material)**), which is consistent with most

other studies such as Li et al [14]. In contrast, another study showed that infants who are breastfed are at low-risk of suffering from moderate to severe dermatitis [15]. In this study the 62.4% of the children were 1-3 years old, which is a common age for diaper dermatitis infection. This finding is consistent with another previous study [16]. Fifty and thirteen percent of the infants had a history of taking antibiotic and antifungal, respectively. Moreover, 10.1% had previously used corticosteroids (30% topical and 70% systemic). However, use of antibiotics and corticosteroids did not have a significant relationship with *Candida* infection ( $P=0.7$ ). We did not find any significant relationship between history of taking antibiotics and corticosteroids and *Candida* infection. However, this could have affected the susceptibility of the study samples. In a study investigating the risk factors of candidiasis, the researchers found that antibiotics ( $P=0.015$ ) and corticosteroid ( $P=0.002$ ) usage had a significant relationship with candidiasis [17]. The *Candida albicans* species isolated from oral candidiasis lesions were 100% susceptible to nystatin, posaconazole, voriconazole, and amphotericin B **Table 2 (see supplementary material)**. We found that the resistance of *Candida albicans* to itraconazole, fluconazole, ketoconazole, voriconazole, clotrimazole, and posaconazole was 43.8%, 33.3%, 36.5%, 6.3%, 20.8%, and 7.3%, respectively. Also, *Candida albicans* susceptible to amphotericin B and nystatin. The resistance of non-*Candida albicans* species to itraconazole, fluconazole, ketoconazole, voriconazole, clotrimazole, posaconazole, and nystatin was 41.5%, 35.8%, 32.1%, 5.7%, 22.6%, 5.7%, and 11.3%, respectively. The non-*Candida albicans* species were not resistant to amphotericin B and only 1.9% were intermediate. The susceptibility of all different *Candida* species to nystatin, itraconazole, fluconazole, ketoconazole, amphotericin B, voriconazole, clotrimazole, and posaconazole were 95.3%, 38.3%, 63.1%, 38.9%, 99.3%, 89.9%, 75.8%, and 91.9%, respectively. In our study, the 4% resistance to nystatin was found, while no resistance to amphotericin B was observed, which is consistent with some other studies [18, 19]. Therefore, these two antifungals can be recommended to treat likewise *Candida* infections. In a recent study on 150 *Candida* species isolated from oral candidiasis lesions in patients with HIV/AIDS infection, the resistance of *Candida albicans* to fluconazole was 34.07% [20], which was similar to the resistance found in our study (33.3%). However, in another recent study on 595 *Candida* species, the resistance of 10.5% to fluconazole was reported [21]. This difference could be justified by the use of antifungals and the genetics of *Candida* species. Obaci et al., reported a 59.6% resistance to fluconazole for *C. albicans* [22]. Another study on 177 *Candida* species isolated from oral lesions in AIDS patients showed 7.7 and 4.7% resistance to ketoconazole and itraconazole, respectively [23], which is much less than what we found (34.9 and 43%, respectively). This difference could be justified by several reasons such as frequent usage of antifungals. Another study showed that all *Candida* isolated were resistant to itraconazole [24]. Similar to Pfaller et al [15], we found a lower resistance (6%) to voriconazole compared with Sharma et al, (10.99%) [18]. We found a 21.5% resistant ratio to clotrimazole, which was relatively similar to a previous study on 53 *Candida* vaginal samples [25]. All *Candida* species were resistant to clotrimazole in another study [24]. The resistance to posaconazole was reported to be 90% in a study on 3312 *Candida* species [26], which was similar to this study as well as another study by

Cárdenes *et al.*, [27]. This shows the universal resistance of *Candida* to this antifungal. This study showed that *Candida* species have most susceptible to amphotericin B and used too much of this medicine are makes *Candida* species resistant to this drug makes difficult the treatment of infectious diseases resistance.

## Conclusion:

The resistance of different *Candida* species to nystatin, itraconazole, fluconazole, ketoconazole, clotrimazole, voriconazole, and posaconazole were 4, 43, 34.2, 34.9, 21.5, 6, and 6.7%, respectively. No resistance to amphotericin B was observed and 0.7% was intermediate. Since nystatin is used topically and has less systemic side effects and drug resistance compared with other antifungals, it is a suitable medication for the treatment of candidiasis.

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## Competing interests:

Authors have declared that no competing interests exist.

## Ethical Considerations:

This research project was approved by the ethics committee of Ilam University of Medical sciences and name and Specifications of patient were kept confidential and Samples from patients was with Personal satisfaction.

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## Supplementary material:

**Table 1:** Frequency distribution of the patients' demographic variables

		Oral candidiasis	Diaper dermatitis %	Both	P Value
Age	1 <	3.3 (2)	11.5 (10)	-	0.07
	1-3	68.3 (41)	58.6 (51)	50 (1)	
	4-7	25 (15)	18.4 (16)		
	7 >	3.3 (2)	11.5 (10)	50 (1)	
Sex	Boy	66.7 (40)	69 (60)	50 (1)	0.82
	Girl	33.4 (20)	31 (27)	50 (1)	
Feeding type	Formula	18.3 (11)	29.9 (26)		0.42
	Breastfeeding	75 (45)	66.7 (58)	100 (2)	
	Both	6.7 (4)	3.4 (3)		
Antifungal use	Yes	11.7 (7)	13.8 (12)		0.8
	No	88.3 (53)	86.2 (75)	100 (2)	
History of hospitalization	Yes	10 (6)	10.3 (9)		0.89
	No	90 (54)	89.7 (78)	100 (2)	
Use of pacifier	Yes	21.7 (13)	13.8 (12)		0.37
	No	78.3 (47)	86.2 (75)	100 (2)	

**Table 2:** Frequency distribution of antifungal susceptibility and resistance of Candida species isolated from oral candidiasis and diaper dermatitis lesion

		Flu	Itr	Ket	Clot	Vori	Posa	Nys	Amph B	
Oral candidiasis	<i>C. albicans</i>	Susceptibility % (frequency)	71.4 (26)	28.6 (10)	22.9 (8)	91.4 (32)	100 (35)	100 (35)	100 (35)	100 (35)
		Dose-dependent % (frequency)	5.7 (2)	31.4 (11)	45.7 (16)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistance % (frequency)	22.9 (8)	40 (14)	31.4 (11)	8.6 (3)	0 (0)	0 (0)	0 (0)	0 (0)
	Non- <i>C. albicans</i>	Susceptibility % (frequency)	26.4 (14)	44 (11)	48 (12)	68 (17)	96 (24)	88 (22)	84 (21)	100 (25)
		Dose-dependent % (frequency)	4 (1)	24 (6)	12 (3)	4 (1)	4 (1)	8 (2)	4 (1)	0 (0)
		Resistance % (frequency)	40 (10)	32 (8)	40 (10)	28 (7)	0 (0)	4 (1)	12 (3)	0 (0)
Diaper dermatitis	<i>C. albicans</i>	Susceptibility % (frequency)	57.6 (34)	37.3 (22)	35.6 (21)	69.5 (41)	84.7 (50)	89.8 (53)	59 (100)	59 (100)
		Dose-dependent % (frequency)	1.7 (1)	16.9 (10)	23.7 (14)	3.4 (2)	5.1 (3)	0 (0)	0 (0)	0 (0)
		Resistance % (frequency)	40.7 (24)	45.8 (27)	40.7 (16)	27.1 (16)	10.2 (6)	10.2 (6)	0 (0)	0 (0)
	Non- <i>C. albicans</i>	Susceptibility % (frequency)	67.9 (19)	46.4 (13)	57.1 (16)	78.6 (22)	85.7 (24)	92.9 (26)	89.3 (25)	96.4 (27)
		Dose-dependent % (frequency)	0 (0)	3.6 (1)	17.9 (5)	3.6 (1)	3.6 (1)	0 (0)	0 (0)	3.6 (1)
		Resistance % (frequency)	32.1 (9)	50 (14)	25 (7)	17.9 (5)	10.7 (3)	7.1 (2)	10.7 (3)	0 (0)
<b>P Value</b>		0.28	0.49	0.19	0.12	0.003	0.01	0.55	0.34	