

Antifungal Susceptibility Pattern of the Candida Species in Nail Infections

Antifungal Susceptibility of the Candida in Nail Infections

Shahida Kashif¹, Behram Khan Khoso², Faiza Zeeshan³, Muhammad Khalid³, Wajid Hussain⁴ and Fakhur Uddin⁵

ABSTRACT

Objective: To determine the in-vitro susceptibility of Candida sp. against fluconazole, voriconazole and pasiconazole.

Study Design: A cross-sectional study

Place and Duration of Study: This study was conducted at the department of Microbiology, in collaboration with department of Dermatology, JPMC, Karachi from November 2018 to January 2020.

Materials and Methods: Total 358 nail samples has been collected. Sample was processed for direct microscopy with 20% potassium hydroxide and nail was inoculated on two set of Sabouraud's dextrose agar (SDA) supplemented with antibiotics and plain SDA plates & a selective dermatophytes test medium (Dermasil) plate. Candida isolates were identified by commercial identification kit (API ID 32C system) and germ tube test. Susceptibility to fluconazole, posaconazole and voriconazole was determined by disc diffusion test and minimal inhibitory concentration (MIC) of fluconazole was determined by Epsilometer (Etest) strip [bioMerieux, France]

Results: Out of 358 case onychomycosis of 253(70.76%) were positive for fungal culture and 253/109 (43.08%) were of candidal onychomycosis. *C. albicans* was the major isolated species 60/109(55.04%) followed by *C. tropicalis* was the predominant species account for 29/109(26.60%). Majority of candidal onychomycosis cases were in age groups of ≤ 25 years and 36-45 years 25 (26.9%) and 22 (23.7%) respectively. The cases were more common in female 67(66%). Antifungal susceptibilities reveal that 76 (69.73%) Candida species were susceptible, 33(30.27%) were resistant to the fluconazole. Fluconazole resistance was lower in *C. albicans* versus non-albicans species; *C. glabrata*, *C. parapsilosis* *C. tropicalis*, species presented 57.14%, 53.84%, 48.27%, respectively. All the species except *C. glabrata* were sensitive to voriconazole and pasiconazole.

Conclusion: Candida albicans was the main species isolated from onychomycosis. Significant number of Candida species was resistant to fluconazole, whereas most of species susceptible to voriconazole and pasiconazole.

Key Words: Candida, Dermatophytes, Non-albicans, Onychomycosis, Potassium hydroxide, Voriconazole

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INTRODUCTION

Onychomycosis is sort of broad with a universally expanded rate and recurrence state primarily in grown-up populace amid the past few decades.¹ It's a big public ill health due to its high frequency of occurrence, related dreariness and long-term therapeutic strategies.²

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It is mostly unremitting, upsetting to annul, highlights a inclination to return and increase the treatment cost by adding systemic therapy with antifungal agent.³ In spite of the fact that moderate in progression, if left unattended may cause total dystrophic onychomycosis.⁴ Dermatophytes were considered the foremost causative agents and amid which Trichophyton species were the preminent.⁵ Situation has moved from dermatophytes to yeasts and non-dermatophytes molds.⁶ Current reports have built a new narrative that Candida species are the rising pathogens in onychomycosis.⁴

The treatment for onychomycosis is individualized and based upon the degree of onychomycosis, types of fungi, co-morbidities, cost, and predilections.⁷ One of the negative prognostic nail findings have the Candida which is harder to treat than dermatophytes and other molds. Fluconazole is not approved for onychomycosis in United States (US) but sanctioned in the Europe and prescribed.⁷ Fluconazole is often the ideal medication for many Candidal infections especially caused by *C. albicans*, it has good compliance, inexpensive and low toxicity.⁸ Its efficacy and mycological cure rates

between 36% - 100% are established in onychomycosis. Fluconazole, itraconazole and ketoconazole are second generation azoles. In third generation azole group voriconazole and posaconazole is included.⁹ The posaconazole and voriconazole are traizoles having broader-spectrum and higher efficacy than the fluconazole. There are several important enzymes in the biosynthesis pathway of ergosterolone to ergosterol. It is responsible for the membrane integrity and permanence. The azoles have mode of action to constrain its synthesis by inhibiting 14- α -sterol demethylase and consequence are accumulation of 14 alpha-methylated precursor, inhibition of other pathways, cell membrane fragility and finally death.¹⁰ A resistance mechanism in *Candida* species against azoles group is due to point mutation in ERG11, ERG11p, ERG3, intrinsic resistant, efflux pump over expression, decrease expression of ERG3 and insertion or deletion of genes.¹¹

In vitro antifungal testing assays have time-honored role in therapeutic assessment, preparing guidelines and to know the epidemiology of antifungal resistance.^{8,12} Laboratory based antifungal susceptibility testing methodologies include disc diffusion method and commercially prepared dehydrated broth microdilution micro-well plates and E-test strips are available.¹³ Normally the antifungal susceptibility testing is rarely performed in Pakistan. Therefore a limited data of resistant profile of *Candida* isolates from onychomycosis is available. This study will add some data about the *Candida* species and their susceptibility pattern.

MATERIALS AND METHODS

The present research was piloted in the departments of microbiology and dermatology of Jinnah Postgraduate Medical Centre (JPMC), Karachi.

Sample Size: The sample size of this study was calculated by OpenEpi, Version 3 online. The parameters were set as confidence level 95% with bound of error 5% and prevalence of *Candida* was 36.94 % of a former study.¹⁴ Hence a total of 358 cases of onychomycosis were taken in this work.

Sample Selection: All the cases of clinically suspected onychomycoses were included regardless of sex and age. Patients on antifungal drugs were excluded.

Ethical Consideration: The ethical endorsement was acquired from Institutional Review Board (IRB) of JPMC, Karachi. The informed written consent was taken from enrolled participants and confidentiality of data was maintained.

Data Collection Procedure: Demographic data was collected on proforma design for this study and clinical assessment was done by the dermatologist. The socio-economic status of the patients was classified into low, middle and upper class on the basis of monthly income.¹⁵

Sample Collection and Processing: The nails and surrounding were sponged with distilled water and cleaned with 70% alcohol. Nail samples were collected on filter paper. The specimen was observed by direct microscopy with 10% KOH, for spores and hyphae, the remaining specimen was inoculated on SDA and Dermasil medium. The inoculated media vials were incubated at 37°C and 25°C for 15 days and regularly observed for fungal growth. Identification was based upon growth characteristics and microscopic characteristic includes pigmentation, colonial characteristic, hyphae, spore types and arrangements. *Candida* species were identified using germ tube test and API 20 C AUX *Candida* system (bioMerieux, France). Susceptibility of *Candida* species to fluconazole, posaconazole and voriconazole was determine by disc diffusion test and minimal inhibitory concentrations (MIC) of fluconazole was determined by Epsilon meter (Etest) strip [bioMerieux, France].¹³

RESULTS

Out of 358 onychomycosis cases 253(70.76%) were positive for fungus culture, these were dermatophytes, *Candida* and non-dermatophytes. Of these 253, in 253/109(43%) *Candida* species and remaining were dermatophytes (45%) and non-dermatophyte molds (12%). *C. albicans* was the major isolated species 60/109(55.04%) followed by *C. tropicalis* was the predominant species account for 29/109(26.60%), *C. parasilosis* 13(11.92%) and *C. glabrata* 7(6.42%) was the least frequent species (Fig.1).

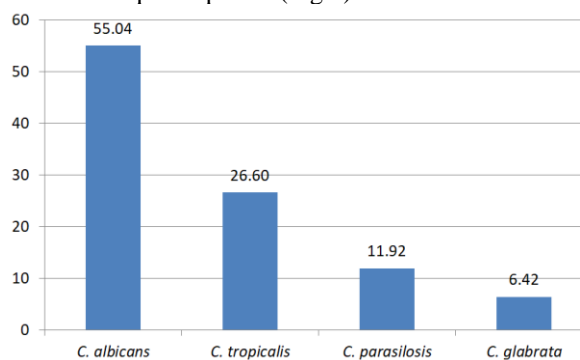


Figure No.1: Prevalence of *Candida* species in candidal onychomycosis cases (n=109)

The association of candidal onychomycosis with age, gender and other studied variables were observed. Majority of candidal onychomycosis cases were in age groups of ≤ 25 years and 36-45 years, 30(27.52%) and 26(23.85%) respectively. The cases were more common in female 76 (69.73%). The history of duration of infection at the time of specimen collection were 2-5 years in most of the cases, followed by the ≤ 1 year and 93(85.32%) cases were of low socioeconomic class and prevalence was higher 65(59.63%) in housekeeping patients. The association was statistically significant with age and duration (Table.1).

Table No.1: Association of Candida with age, gender and other variables (n=109)

Characteristics		Candidal onychomycoses (n=109)	Non-candidal onychomycoses (n=144)	Total (%)	p-value
		N (%)	N(%)		
Age Group	≤25 years	30 (27.52)	39 (27.08)	69(27.27)	0.04*
	26 - 35 years	21(19.26)	41(28.47)	62(25.50)	
	36 - 45 years	26(23.85)	46(31.94)	72(28.45)	
	46 -55 years	17(15.59)	14(9.72)	31(12.25)	
	>55 years	15(13.76)	4(2.77)	19(7.50)	
Total (%)		109(100)	144(100)	253(100)	
Gender	Female	76(69.73)	97(67.36)	173(68.37)	0.09
	Male	33(30.27)	47(32.64)	80(31.63)	
Total (%)		109(100)	144(100)	253(100)	
Duration of disease	≤1 year	40(36.70)	93(64.58)	133(52.56)	<0.01*
	2-5 years	49(44.96)	36(25.0)	85(33.59)	
	>5 years	20(18.34)	15(10.41)	35(13.85)	
Total (%)		109(100)	144(100)	253(100)	
Socioeconomic status	Low	93(85.32)	103(71.54)	196(77.47)	0.11
	Middle	12(11.0)	35(24.30)	47(18.57)	
	Upper	4(3.66)	6(4.16)	10(3.95)	
Total (%)		109(100)	144(100)	253(100)	
Occupation	House keeping	65(59.63)	73(50.69)	138(54.54)	0.20
	Labor	19(17.43)	31(21.52)	50(19.76)	
	Office worker	13(11.29)	15(10.41)	28(11.06)	
	Retired	8(7.33)	11(7.63)	19(7.50)	
	Students	4(3.66)	14(9.72)	18(7.11)	
Total (%)		109(100)	144(100)	253(100)	

*p<0.05 was considered significant using Pearson Chi Square test

Table No.2: Distribution of minimum inhibitory concentration (MIC) of fluconazole against Candida spp (n=109)

MIC µg/ml	C. albicans	C. tropicalis	C. parapsilosis	C. glabrata	Total (%)
0.16-0.125	13	-	-	-	13 (11.92)
0.19-2	39	15	6	-	60 (55.04)
3-12	8 ^a	14 ^a	7 ^a	3	32 (29.35)
16-64	-	-	-	4 ^a	4 (3.66)
Total	60(55.04)	29(26.60)	13(11.92)	7(6.42)	109 (100)

Note: ^a Resistant isolates

Overall fluconazole susceptibility pattern revealed that 76(69.72%) Candida species were susceptible and intermediate was 6(5.50%). Fluconazole was effective against C. albicans 52(86.66%) was susceptible whereas Candida non-albicans species were resistance (>50%) to fluconazole. The minimal inhibitory concentration (MIC) of fluconazole against Candida species was determined by Etest strips (bioMerieux, France). The results were similar to the disc diffusion method. The MIC of fluconazole was higher in Candida non-albicans and compared to the C. albicans (Table.2). Voriconazole and pasiconazole susceptibility pattern, results showed that C. albican, C. tropicalis, C. parapsilosis, and C. parapsilosis were 100% sensitive to voriconazole and pasiconazole while 50% and 75% C. glabrata were susceptible respectively (Fig. 2).

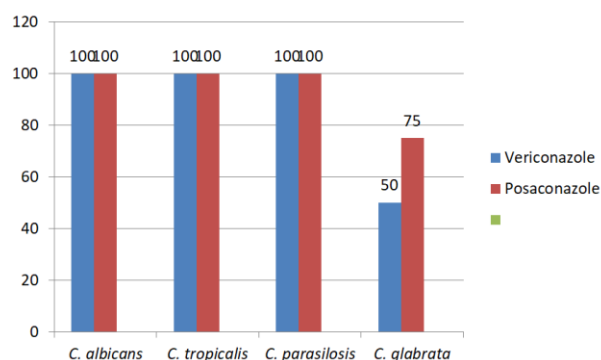


Figure No.2: Voriconazole and pasiconazole susceptibility pattern in Candida species

DISCUSSION

Onychomycosis happens around the world and a significant proportion (30%) of the superficial infectious diseases and about 50% of all nail variations from the norm.¹⁶ It hinders the eminence of life, ponderousness and other psychological conditions, in addition to irritation and setback.¹⁷ Among the foremost regular and well known pathogens are *Candida*, dermatophytes, and non dermatophyte molds.¹⁸ The essential pathogen of onychomycosis in past was the dermatophytes, whereas *Candida* were frequently counted as auxiliary cause. Presently the literature support the predominance of *Candida* as a leading pathogen in onychomycosis, which may be associated with extend introduction to damping nature of working conditions, injuries results from the physical and chemical factors.¹⁷ Thus, yeast ought to continuously be kept in intellect whereas examining, diagnosis and treatment formulation for onychomycosis and common hone of disposing of them as normal flora ought to be dodged.

Fluconazole is the best choice and a common in practice of dermatologists but unfortunately fluconazole resistant *Candida* species is reported.¹⁹ The frequency of resistance in *Candida* is varies from species to species 0.5–2% in *C. albicans* while frequencies are higher in other species, 4-9, 2-6, and 11-13% for *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* respectively.²⁰ *Candida auris* is newly identified *Candida* species, and more than 90% to this drug.²¹ In this study, *C. albicans* were 14.29% resistant to fluconazole. The similar resistance pattern has been reported by Otasevic et al. (2016) and Sav et al. (2018).^{4,17} In this study, *C. tropicalis* showed higher (48%) resistance to fluconazole. Alike results are stated by the former study.²² The resistance depends upon the different factors so it is not uniform to all over the world. These results revealed that the continuous monitoring and reporting of susceptibility at regional levels. The resistance to fluconazole was higher in *C. parapsilosis* (63.63%), and 50% *C. glabrata*, which is well-matched to the study done by Otasevic et al. (2016).^{4,23}

In this study, all isolates were susceptible to voriconazole, which is in strong agreement to Mello et al., (2021)²⁴ and Rather et al., (2021)²⁵ showed no resistant to voriconazole and pasiconazole that is similar to present work. The higher frequencies of resistance may vary from geographic regions and depends upon the different factors includes, misuse, overuse and availability over the counter may increase the resistance prevalence. Empowering information is that *C. albicans* is susceptible to fluconazole in vitro, whereas non-*albicans* species were resistant.

CONCLUSION

Candida albicans was the main species isolated from onychomycosis. Susceptibility pattern of *C. non albicans* species revealed that these species are mostly resistant to fluconazole. Pasiconazole and voriconazole are effective and a choice of treatment for *Candida* onychomycosis.

Author's Contribution:

Concept & Design of Study: Shahida Kashif
 Drafting: Behram Khan Khoso, Faiza Zeeshan
 Data Analysis: Muhammad Khalid, Wajid Hussain, Fakhur Uddin
 Revisiting Critically: Shahida Kashif, Behram Khan Khoso
 Final Approval of version: Shahida Kashif

Conflict of Interest: The study has no conflict of interest to declare by any author.

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