



Note

Species distribution and antifungal susceptibility among clinical isolates of *Candida parapsilosis* complex from India

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ABSTRACT

Background: *Candida parapsilosis* is recognized as a species complex: *Candida parapsilosis sensu stricto*, *Candida orthopsilosis* and *Candida metapsilosis* are three distinct but closely related species.

Aims: To determine the species and antifungal susceptibility of members of the *C. parapsilosis* complex, isolated from clinical samples.

Methods: Isolates identified as *C. parapsilosis* complex by VITEK[®] 2 system were included. Antifungal susceptibility test was done using the VITEK[®] 2 semi-automated system. The distribution of the species in the complex was determined by multiplex PCR.

Results: Among the seventy-seven *C. parapsilosis* complex isolates, *C. parapsilosis sensu stricto* (57.1%) was the commonest species, followed by *C. orthopsilosis* (40.2%) and *C. metapsilosis* (2.5%). All three species were susceptible to amphotericin B, caspofungin and micafungin. Among *C. parapsilosis sensu stricto* isolates, 16% were resistant to fluconazole while 2.2% showed dose dependent susceptibility. Also, 18.2% of *C. parapsilosis sensu stricto* isolates showed dose dependent susceptibility to voriconazole.

Conclusions: *C. parapsilosis sensu stricto* was the most commonly isolated member of the *C. parapsilosis* complex and it showed high resistance to fluconazole. A high prevalence of *C. orthopsilosis* (40.2%) was also noted.

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Distribución de especies y sensibilidad antimicótica de aislamientos clínicos del complejo *Candida parapsilosis* en India

RESUMEN

Antecedentes: *Candida parapsilosis* se reconoce como un complejo de especies compuesto por *Candida parapsilosis sensu stricto*, *Candida orthopsilosis* y *Candida metapsilosis*, tres especies distintas pero estrechamente relacionadas.

Objetivos: Establecer las especies y la sensibilidad antifúngica de aislamientos del complejo *C. parapsilosis* procedentes de muestras clínicas.

Métodos: Se incluyeron los aislamientos identificados como complejo *C. parapsilosis* por el sistema VITEK[®] 2. El estudio de la sensibilidad antimicótica se realizó mediante el sistema semiautomático VITEK[®] 2. La distribución de las especies del complejo se estableció mediante PCR múltiple.

Resultados: Entre los 77 aislamientos del complejo *C. parapsilosis*, *C. parapsilosis sensu stricto* (57,1%) fue la especie más común, seguida por *C. orthopsilosis* (40,2%) y *C. metapsilosis* (2,5%). Las tres especies fueron sensibles a anfotericina B, caspofungina y micafungina. Entre los aislamientos de *C. parapsilosis sensu stricto*, el 16% fue resistente al fluconazol, mientras que el 2,2% mostró sensibilidad dependiente de la dosis. Además, el 18,2% de los aislamientos de *C. parapsilosis sensu stricto* mostró una sensibilidad al voriconazol dependiente de la dosis.

Palabras clave:

Candida orthopsilosis

Candida metapsilosis

Fluconazol

Reacción en cadena de la polimerasa

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Conclusiones: *C. parapsilosis sensu stricto* fue la especie más aislada del complejo *C. parapsilosis* y mostró una elevada resistencia al fluconazol. *C. orthopsilosis* fue también aislada con una alta prevalencia (40,2%).

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Candida parapsilosis has emerged as the third most commonly isolated species from blood cultures of critically ill patients admitted to Indian intensive care units.⁴ It is known to persist in hospital environments, is spread by contaminated hands, and prior colonization is not a prerequisite for causing invasive infection.² In 2005 Tavanti et al. recognized *C. parapsilosis* as a complex of species comprising *C. parapsilosis sensu stricto*, *Candida orthopsilosis* and *Candida metapsilosis*.¹¹ Since the three species are phylogenetically and morphologically indistinguishable, only molecular techniques like multiplex PCR, randomly amplified polymorphic DNA analysis, and analysis of internal transcribed spacer sequences of DNA encoding ribosomes can reliably differentiate them.¹ Another closely related species, *Lodderomyces elongisporus*, is also often misidentified as *C. parapsilosis* by phenotypic methods.¹ Previous studies have shown *C. parapsilosis sensu stricto* to be the most commonly isolated species from clinical specimens among the members of the *C. parapsilosis* complex.¹ The members of the complex are also known to differ in their antifungal susceptibilities and virulence.¹² The aim of the study was to determine the prevalence of the three closely related species (*C. parapsilosis sensu stricto*, *C. orthopsilosis*, *C. metapsilosis*) comprising *C. parapsilosis* complex, as well as the phenotypically closely related species *L. elongisporus* using a single multiplex PCR (mPCR) assay.

The study was conducted from December 2015 to February 2017 in a tertiary care 1300-bed hospital in South India. Seventy-seven non-repetitive clinical isolates identified as *C. parapsilosis* complex by VITEK[®] 2 system (bioMérieux, Marcy l' Etoile, France) were collected from different clinical specimens like, blood, urine, sputum, pus, skin, wound tissue and nail. Antifungal susceptibility for fluconazole, amphotericin B, caspofungin, micafungin, flucytosine, and voriconazole was done using the VITEK[®] 2 semi-automated system (AST-YSO1 cards, bioMérieux). Susceptibility of *C. parapsilosis* complex was interpreted using CLSI M27-S4 guidelines.⁷ The minimum inhibitory concentrations (MIC) of all fluconazole-resistant isolates were confirmed using Etest.

Genomic DNA was extracted using an established CTAB method. Briefly, three to five isolated colonies were placed in 1.5-ml microcentrifuge tubes containing 700 µl of 2 × CTAB buffer (100 mM Tris-HCl [pH 8], 1.4 M NaCl, 25 mM EDTA, 2% CTAB). After vortexing the cell mixture, 1 volume of phenol-chloroform was added. The mixture was vortexed again and centrifuged at 12,000 × g for 5 min. DNA was precipitated from the upper aqueous phase by adding an equal volume of isopropanol and centrifuging at 12,000 × g for 10 min. After washing the DNA pellet once in 70% ethanol, it was resuspended in 100 µl of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA).¹³ Species characterization of *C. parapsilosis* complex was performed by multiplex PCR using a previously published protocol.¹ The PCR consisted of species-specific forward primers for *C. parapsilosis sensu stricto*, *C. orthopsilosis*, *C. metapsilosis* and *L. elongisporus* and a common reverse primer resulting in products of 171 bp, 109 bp, 217 bp and 258 bp respectively (Fig. 1).

Seventy-seven *C. parapsilosis* complex isolates were included in the study. Out of these, 26% (20/77) were recovered from wound tissue samples, 22% (17/77) each from blood and nail samples, and 9% (7/77) each from skin and urine samples. Species distribution in various samples is represented in Table 1. Male patients accounted

for 54.5% of the cases. There was a solitary case of corneal ulcer caused by *C. orthopsilosis*. The most common species isolated was *C. parapsilosis sensu stricto* (57.1%) followed by *C. orthopsilosis* (40.2%) and *C. metapsilosis* (2.5%). None of the isolates were identified as *L. elongisporus*. All three species of *C. parapsilosis* complex were susceptible to amphotericin B, caspofungin and micafungin (Table 2). Among *C. parapsilosis sensu stricto* isolates 16% were resistant to fluconazole while 2.2% showed dose dependent susceptibility (SDD). Notably, all isolates (18.2%) that were either resistant or SDD to fluconazole were also SDD to voriconazole. Though all isolates were susceptible to caspofungin the MICs of 68% of the *C. parapsilosis sensu stricto* isolates were on the higher side (1 µg/ml).

Since 2005, when *C. parapsilosis* species complex was divided into *C. parapsilosis sensu stricto*, *C. orthopsilosis*, and *C. metapsilosis*, several studies have reported their distribution, antifungal resistance and virulence.^{1,5,6,8,10–12} Recent studies have reported an increase in the prevalence of human infections caused by *C. parapsilosis* species complex. A large study among critically ill patients in Indian intensive care units showed this species complex was the second most common cause of candidemia.⁴ Studies have shown that there is a considerable variation in the distribution of *C. metapsilosis* and *C. orthopsilosis* at several geographic locations.^{1,5,6,8,10–12} A recently published study from India on eye infections due to *Candida* species reported *C. parapsilosis* species complex was the commonest cause (44%) followed by *C. albicans*.¹⁰ The distribution of *C. metapsilosis* and *C. orthopsilosis* in other clinical specimens from Indian hospitals is unknown due to non-availability of molecular techniques and MALDI-TOF for species differentiation.

Though *C. parapsilosis sensu stricto* was found to be the most common species in our study, the prevalence of 57% is the lowest recorded when compared to previous published studies (60–95%).^{1–3,5,6,8,10–12} The prevalence of *C. orthopsilosis* (40.2%) in our study is the highest reported in literature with all previous studies having reported figures of 2–35%.^{1–3,5,6,8,10–12} Our findings are also not in agreement with a recently published study from India which reported a prevalence of 95.5% and 4.5% for *C. parapsilosis sensu stricto* and *C. orthopsilosis*, respectively.¹⁰ Though more than half (58%) of our *C. orthopsilosis* isolates were from older patients (>50 years), statistical analysis using chi-square test showed that the association was not significant ($p = 0.051$). Two previous studies have reported *C. orthopsilosis* infections exclusively in older patients.^{3,12} *C. orthopsilosis* is known to be more heterogenous genotypically than *C. parapsilosis sensu stricto* which is predominantly clonal.¹¹ The prevalence of *C. metapsilosis* in our study (2.5%) is similar to what has already been reported in literature.^{1,5,8,12} Studies have shown that *C. metapsilosis* is the least virulent among the species of the *C. parapsilosis* complex. A recent study on expression of virulence factors found that 34.2% of *C. parapsilosis sensu stricto* were protease positive while none of the *C. orthopsilosis* and *C. metapsilosis* expressed protease activity.¹²

The members of the *C. parapsilosis* complex exhibit different MICs to the antifungal drugs, therefore warranting species-specific identification.^{1,5,8,12} None of the *C. metapsilosis* and *C. orthopsilosis* isolates were resistant to any of the antifungal agents tested, which is in contrast with previous reports.^{1,5,8,12} Fluconazole resistance in *C. metapsilosis* has been reported in many previous studies.^{12,14} A

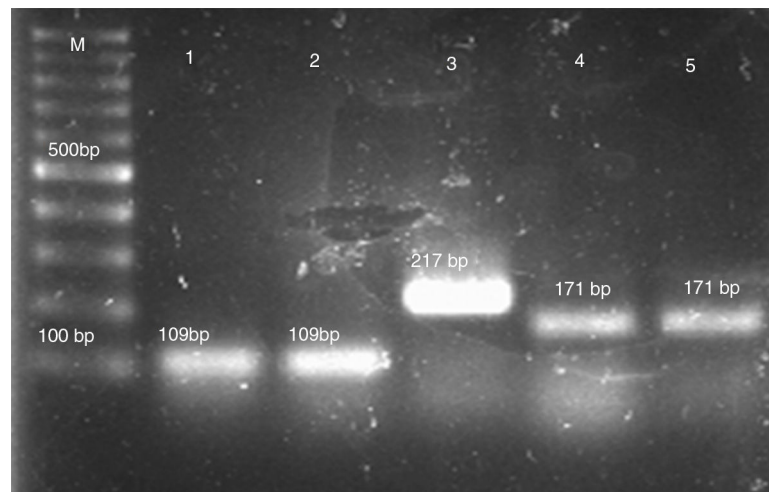


Fig. 1. Multiplex PCR amplification products of *C. orthopsilosis* (lanes 1, 2: 109 bp), *C. metapsilosis* (lane 3: 217 bp), and *C. parapsilosis* (lanes 4, 5: 171 bp). M: 100 bp ladder.

Table 1

Sample wise distribution of *C. parapsilosis* complex isolates.

Sample	<i>C. parapsilosis sensu stricto</i> (n)	<i>C. orthopsilosis</i> (n)	<i>C. metapsilosis</i> (n)	Total (n)
Tissue	8	10	2	20
Blood	13	4	0	17
Nail	11	6	0	17
Skin	3	4	0	7
Urine	4	3	0	7
Nasal discharge	3	1	0	4
Pus	1	1	0	2
Synovial fluid	0	1	0	1
Catheter tip	1	0	0	1
Corneal scraping	0	1	0	1
Total (%)	44 (57.1%)	31 (40.2%)	2 (2.5%)	77

Table 2

Antifungal susceptibility of *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis* isolates.

Species (n)	Antifungal agents	S (%)	SDD (%)	R (%)
<i>C. parapsilosis sensu stricto</i> (44)	Amphotericin B	44 (100)	0	0
	Fluconazole	36 (81.8)	2 (4.54)	6 (13.63)
	Voriconazole	36 (81.8)	8 (18.18)	0
	Caspofungin	44 (100)	0	0
	Micafungin	44 (100)	0	0
<i>C. orthopsilosis</i> (31)	Amphotericin B	31 (100)	0	0
	Fluconazole	31 (100)	0	0
	Voriconazole	31 (100)	0	0
	Caspofungin	31 (100)	0	0
	Micafungin	31 (100)	0	0
<i>C. metapsilosis</i> (2)	Amphotericin B	2 (100)	0	0
	Fluconazole	2 (100)	0	0
	Voriconazole	2 (100)	0	0
	Caspofungin	2 (100)	0	0
	Micafungin	2 (100)	0	0

S: susceptible; R: resistant; SDD: susceptible-dose dependent; breakpoints: voriconazole S/SDD/R, $\leq 0.12 \mu\text{g/ml}/0.25\text{--}0.5 \mu\text{g/ml}/\geq 1 \mu\text{g/ml}$; fluconazole S/SDD/R, $\leq 2 \mu\text{g/ml}/4 \mu\text{g/ml}/\geq 8 \mu\text{g/ml}$; caspofungin, micafungin S/I/R, $\leq 2 \mu\text{g/ml}/4 \mu\text{g/ml}/\geq 8 \mu\text{g/ml}$; amphotericin B S, $\leq 1 \mu\text{g/ml}$.

recent study by Chen et al. reported higher MICs to voriconazole in *C. metapsilosis*.⁶ However, caspofungin MICs of *C. parapsilosis sensu stricto* and *C. orthopsilosis*, though susceptible, were on the higher side, which is in agreement with previous studies.^{12,14} Resistance to fluconazole among *C. parapsilosis sensu stricto* isolates was 16%, which is contrary to the low levels of resistance reported in previous studies.^{1,5,8} All fluconazole-resistant or SDD isolates (18%) showed SDD to voriconazole also. The high azole resistance may be due to the widespread use of fluconazole in our hospital. The major limitation of our study was that broth microdilution was not

used to determine the MIC values. However, previous studies have shown excellent quantitative and qualitative agreement (within 2 dilutions) for fluconazole and voriconazole between the reference CLSI or EUCAST broth microdilution and VITEK[®] 2 MICs.⁹

A recent report has described the emergence of fluconazole-resistant *C. parapsilosis sensu stricto* during prolonged antifungal treatment due to increase in the expression of *MRR1* transcription factor resulting in overexpression of *MDR1*.¹⁴ Fluconazole resistance is known to emerge in settings of complicated infections, suboptimal dosing and extensive use of antifungal agents. Since

C. parapsilosis complex is known to show high MIC to echinocandins, the emergence of azole resistance has significant clinical implications.

In conclusion, ours is the second study to report the species distribution of *C. parapsilosis* species complex from India. Though *C. parapsilosis sensu stricto* was the most common species isolated, the prevalence of *C. orthopsilosis* at 40.2% is the highest reported in literature. Resistance to fluconazole and voriconazole in *C. parapsilosis sensu stricto* is also high. Our data have shown that species distribution and fluconazole resistance among members of *C. parapsilosis* complex in India is different from those of other countries. This knowledge could be of clinical relevance in guiding therapeutic decisions.

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Conflict of interest

We declare that there is no entity (among us) with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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