

Identification to Species Level of *Candida parapsilosis* Complex into Sites Oral Cavity in a Cohort of Argentinians Patients

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Abstract

Candida parapsilosis is a complex of three species (*Cp. sensu stricto*, *Candida orthopsilosis* and *Candida methapsilosis*) due to genetic heterogeneity. Currently, it is the second most isolated in yeast bloodstream infections in Latin America, Asia and Europe. In Argentina and globally is no data on the distribution and behavior of the species that make up this complex in oral niches. Knowledge becomes important in the event that the mouth can be a potential source of candidemia and / or invasive infections by this yeast in patients with other risk factors source.

Objective: To identify to species *C. parapsilosis* complex in clinical isolates obtained from various oral niches, of a cohort of Argentinians patients with different buccodentary clinical situations.

Methods: Retrospective, transversal and descriptive study using 31 clinical isolates of oral cavity, cryopreserved and obtained from immunocompetent patients with and without periodontal disease, and presence or absence of intraoral appliances; previously recognized by conventional methods such as *C. parapsilosis*, and recovered for molecular characterization endpoint PCR using specific primers.

Results: 100% (31/31) of the isolates were positive for *Candida parapsilosis sensu stricto*. 77.5% of these strains were recovered in oral inflammatory conditions, and 22.5% in terms of oral health. Being statistically significant difference ($p=0$).

Conclusions: *C. parapsilosis sensu stricto* is a common colonizing the oral mucosa, especially in pathological conditions.

Keywords: *Candida parapsilosis* complex; *Candida parapsilosis sensu stricto*; *Candida orthopsilosis*; *Candida methapsilosis*; Oral niches

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Introduction

Candida parapsilosis is a complex of three species (*Candida parapsilosis sensu stricto*, *Candida orthopsilosis*, and *Candida methapsilosis*) due to its heterogeneity genética [1]. In recent years *C. parapsilosis* has emerged as an emerging nosocomial pathogen, and currently is the second most isolated in yeast bloodstream infections in Latin America, Asia and Europe [2]. It is the kind *sensu stricto* the most prevalent, especially in immunocompetentes [3] subjects. It is known from literature that this species is the second more asylee after *Candida albicans* in buccal cavity [4]. In Argentina and globally are no data on the distribution and behavior of the species that make up this complex in oral niches, knowledge becomes important in the

event that the mouth can be a potential source of candidemia and / or invasive infections this yeast in patients with other risk factors.

Objective

Perform identification to species level of *Candida parapsilosis complex* on clinical a isolates into sites oral cavity, in cohort of Argentinians patients with different buccodentary clinical situations.

Methodology

Retrospective, transversal and descriptive study, that used 31 clinical isolates of oral cavity, cryopreserved and obtained

ambulatory and immunocompetent patients with and without periodontal disease, and with presence or absence of intraoral appliances; previously recognized by conventional methods such as *C. parapsilosis*, and recovered for molecular characterization with endpoint PCR using specific primers (CPAR, CPAF, COOR, COOF, CMEF, CMER) [5] (Table 1), derivatives of unique sequences contained in the internal transcriptional spacer 1 (ITS 1-5.8rRNA-ITS2) ribosomal DNA fúngico [5]. Data were processed in Microsoft Excel 2010. For quantitative and qualitative analysis, Stadicx 7.0 and SPSS programs were used; with a confidence interval of 95% and alpha error of 0.05, statistical significance considering a p-value minor to alpha error. The statistical association was assessed with chi square test and prevalence ratio.

Results

A total of 31 *C. parapsilosis* strains were recovered and molecularly evaluated in this study; obtained from different oral niches as buccal mucosa, palate, tongue and gingival sulcus. Of the 31 strains, seven were obtained from the collection of isolates of Mycology Center-UBA, and 24 came from Gandulfo Hospital (Table 2). All (100%) isolates were positive for *Candida parapsilosis sensu stricto* (Figure 1). 77.5% of these strains were recovered in oral pathological conditions, with clinical forms of gingivitis and periodontitis (Table 3). It was statistically significant difference (p=0); with a prevalence ratio of 3.35, which means that there are three times more likely to isolate *C. parapsilosis sensu stricto* in an oral cavity under inflammatory conditions compared to oral cavity health conditions (Table 4). On the other hand, 54.8% (17/31, p=0.6115) strains came from patients with prosthetic or orthodontic devices, however not statistically significant was obtained (Table 5).

Discussion

Of the complex, *C. parapsilosis sensu stricto* is the most isolated species in clinical isolates derived from immunocompetent patients both pathological conditions and such commensal [6,7]. This is knowledge that has been supported by many studies worldwide. However there are few studies reporting the prevalence and distribution of species of this complex health conditions and disease to level oral. So far it is known that *C. parapsilosis sensu stricto* is the most prevalent species in oral niche in conditions of immune-competence, regardless of geographic region. This has been reported studies from USA [4], Portugal [8], Turkey [9] and China [10]. While a single work in Brazil, investigated

the distribution of species of this complex in oral cavity of chronic immunocompromised patients for HIV, in which *C. methapsilosis* was the most isolated species followed by *C. parapsilosis sensu stricto*, although the difference it was not statistically significant, and also the sample used was very escasa [2].

In the present study, was isolated only *C. parapsilosis sensu stricto*, with 100% prevalence of total recruited samples of oral cavity from immunocompetent patients. This result is similar to that reported by other authors as Ghanoun et al. [4]; Enger et al. [11]; Silva et al. [8]; Ge et al. [10]; and Tosun et al. [9] (Table 6).

By relating the species found with oral clinical conditions at the time of sampling, according to data recorded in the medical history of each patient, we obtained that *C. parapsilosis sensu stricto* dominated inflammatory oral conditions compatible with forms clinics gingivitis and periodontitis, predominating in the latter group in particular (Tables 3 and 4). On the other hand, by correlating of the isolate recovered with the use of intraoral devices, although, *C. parapsilosis sensu stricto* predominated in the group of patients with some form of intraoral appliances, however, the difference was not statistically significant compared to non-carriers (Table 5). There are no published data to contrast our results.

Conclusions

1. *C. parapsilosis sensu stricto* is a regular colonizing the oral mucosa, especially in pathological conditions. In this context, the mouth becomes a potential source of candidemia or invasive infections by this yeast; besides being a possible source of transmission for this fungus, by direct contact from person to person.
2. It's probably that *C. methapsilosis* and *C. orthopsilosis* are two strange species in niche oral cavity under both health and disease.
3. Until now, this is the first study that studies the distribution of the species complex in oral niches in a collection of more than 20 clinical isolates, in addition, the study considers the situation dental clinic at the time of sampling, which is not seen in other reports concerning the subject.

Recommendations

It is suggested to apply the formatting of the study in a larger sample and a prospective model to validate these reported in this paper results.

Table 1 Sequence of primers used for the rapid identification at the species level *C. parapsilosis* complex.

Source: Asadzadeh et al. [5]

Primers	Gene	Direction	Species specificity	Sequence	Amplicon
CPAF	ITS 1	Forward	<i>C. parapsilosis</i>	TTTGCTTTGGTAGGCCTTCTA	379pb
CPAR	ITS 2	Reverse		GAGGTCTGAATTTGGAAGAAGT	
CORF	ITS 1	Forward	<i>C. orthopsilosis</i>	TTTGGTGGCCACGGCCT	367pb
CORR	ITS 2	Reverse		TGAGGTCTGAATTTGGAAGAATT	
CMEF	ITS 1	Forward	<i>C. methapsilosis</i>	TTTGGTGGCCACGGCT	374pb
CMER	ITS 2	Reverse		GAGGTCTGAATTTGGAAGAATGT	

Table 2 Molecular identification of 31 isolates previously defined by phenotypic methods as *C. parapsilosis*.

Strain	Source	Site	Species
10.2	Gandulfo	Buccal mucosa	Sensu stricto
13.2	Gandulfo	Buccal mucosa	Sensu stricto
15A	Gandulfo	Subgingival	Sensu stricto
51.2	Gandulfo	Buccal mucosa	Sensu stricto
14.2	Gandulfo	Buccal mucosa	Sensu stricto
15.1	Gandulfo	Buccal mucosa	Sensu stricto
16.1	Gandulfo	Buccal mucosa	Sensu stricto
23.2	Gandulfo	Buccal mucosa	Sensu stricto
7.2	Gandulfo	Buccal mucosa	Sensu stricto
11.1	Gandulfo	Buccal mucosa	Sensu stricto
36.1	Gandulfo	Buccal mucosa	Sensu stricto
53A	Gandulfo	Subgingival	Sensu stricto
40A	Gandulfo	Subgingival	Sensu stricto
12A	Gandulfo	Subgingival	Sensu stricto
50A	Gandulfo	Subgingival	Sensu stricto
50.1	Gandulfo	Buccal mucosa	Sensu stricto
73A	Gandulfo	Subgingival	Sensu stricto
16A	Gandulfo	Subgingival	Sensu stricto
23B	Gandulfo	Subgingival	Sensu stricto
32B	Gandulfo	Subgingival	Sensu stricto
75CA	Gandulfo	Cheek	Sensu stricto
78LE	Gandulfo	Tongue	Sensu stricto
6LE	Gandulfo	Tongue	Sensu stricto
6PA	Gandulfo	Palate	Sensu stricto
4220	Gandulfo	Buccal mucosa	Sensu stricto
4757	Micology center	Buccal mucosa	Sensu stricto
5299	Micology center	Buccal mucosa	Sensu stricto
5301	Micology center	Buccal mucosa	Sensu stricto
5462	Micology center	Buccal mucosa	Sensu stricto
6912	Micology center	Buccal mucosa	Sensu stricto
7066	Micology center	Buccal mucosa	Sensu stricto

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Table 3 Relationship between *parapsilosis* species complex and oral clinical condition: 77.4% of the isolates were isolated in inflammatory oral conditions.

Species	Gingivitis	Periodontitis	Oral health	Total
Sensu stricto	6(19.4%) IC95%:7.5-37.5	18(58.1%) IC95%:39.3-74.9	7(22.5%) IC95%:9.6-41.1	31(100%)
Orthopsilosis	0	0	0	0
Methapsilosis	0	0	0	0
Total	19.4%	58.1%	22.5%	100%

Table 4 Distribution of *Candida parapsilosis sensu stricto* according to presence or absence of oral inflammation.

CHI2 (Yates)=16,5161; GL=1; p=0; Prevalence ratio: 3.35

Inflamación	<i>Cp sensu stricto</i>	
oral	Nº	%
Presente	24	77
Ausente	7	23
Total	31	100

Table 5 Distribution of the species *C. parapsilosis sensu stricto* according to presence or absence of intraoral devices.

**CHI2 (Yates)=0,258 1; GL=1; p=0.6115 Prevalence ratio: 1.21*

Intraoral device	Species sensu stricto
	Nº (%)
Present	17 (54.8%)
Absent	14 (45.2%)
Total	31 (100%)



Figure 1 Amplification products agarose gel *C. parapsilosis sensu stricto* with primers CPAR-CPAF.

Note: Mk: Weight marker; +: Positive control; Streets 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17: Clinical samples; Streets 8, 18: Negative controls

Table 6 Distribución de especies del complejo *C. parapsilosis* en nichos de cavidad bucal, resumido de estudios publicados.

Author	Nº de aislamientos (%) por especies			Área de origen	Referencia
	<i>C. parapsilosis</i>	<i>C. orthopsilosis</i>	<i>C. metapsilosis</i>		
Ghannoum et al.	3	0	1	USA (2010)	[4]
Ge et al.	2	0	1	China (2012)	[10]
Moris et al.	7	0	8	Brasil (2014)	[2]
Enger et al.	9	5	0	Global (2001)	[11]
Silva et al.	65	0	4	Portugal (2009)	[8]
Tosun et al.	2	0	0	Turkia (2012)	[9]
Presente estudio	31	0	0	Argentina	-

References

- 1 Treviño R, Garza E, Gonzales J (2012) Molecular characterization and antifungal susceptibility of the *Candida parapsilosis* species complex of clinical isolates from Monterrey, Mexico. *Med Mycol* 50: 781-784.
- 2 Moris D, Melhem M, Martins M (2012) Prevalence and antifungal susceptibility of *Candida parapsilosis* complex isolates collected from oral cavities of HIV-infected individuals. *J Med Microbiol* 61: 1758-1765.
- 3 Constante C, Monteiro A, Alves S (2014) Different risk factors for candidemia occurs for *Candida* species belonging to the *C. parapsilosis* complex. *Med Mycol* 52: 403-406.
- 4 Ghannoum M, Jurevic R, Mukherjee P (2010) Characterization of the Oral Fungal Microbiome (Mycobiome) in Healthy Individuals. *PLoS Pathogens* 6: 1-8.
- 5 Asadzadeh M, Ahmad S, Al-Sweih N, Khan Z (2009) Rapid molecular differentiation and genotypic heterogeneity among *Candida parapsilosis* and *Candida orthopsilosis* strains isolated from clinical specimens in Kuwait. *J Med Microbiol* 58: 745-752.
- 6 Ataide FS, Costa CR, Souza LK, Fernandes OD, Jesuíno RS, et al. (2015) Molecular identification and antifungal susceptibility profiles of *Candida parapsilosis* complex species isolated from culture collection of clinical samples. *Rev Soc Bras Med Trop* 48: 454-459.
- 7 Banerjee B, Saldanha Dominic RM, Baliga S (2015) Clinico-microbiological study of candidemia in a tertiary care hospital of southern part of India. *Iran J Microbiol* 7: 55-61.
- 8 Silva A, Miranda I, Lisboa C, Pina-Vaz C, Rodrigues A, et al. (2009) Prevalence, Distribution, and Antifungal Susceptibility Profiles of *Candida parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* in a Tertiary Care Hospital. *J Clin Microbiol* 47: 2392-2397.
- 9 Tosun I, Akyuz Z, Guler N, Gulmez D, Bayramoglu G, et al. (2012) Distribution, virulence attributes and antifungal susceptibility patterns of *Candida parapsilosis* complex strains isolated from clinical samples. *Med Mycol* 51: 483-492.
- 10 Ge Y, Boekhout T, Zhan P, Lu G, Shen Y, et al. (2012) Characterization of the *Candida parapsilosis* complex in East China: species distribution differs among cities. *Medi Mycol* 50: 56-66.
- 11 Enger L, Joly S, Pujol C, Simonson P, Pfaller M, et al. (2002) Cloning and characterization of a complex DNA fingerprinting probe for *Candida parapsilosis*. *J Clin Microbiol* 39: 658-669.