



Original article

Prevalence, virulence factors and antifungal susceptibility of oral isolates of *Candida albicans* from patients with cystic fibrosis in Mexico

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ABSTRACT

Background: *Candida* species are frequently isolated from the oral cavity of patients with cystic fibrosis. However, the information on the role of *Candida* in cystic fibrosis is scarce.

Aims: This study aimed to evaluate the prevalence, virulence profile and antifungal susceptibility of oral isolates of *Candida albicans* recovered from patients with cystic fibrosis.

Methods: Oropharyngeal swab samples were collected from sixty-five cystic fibrosis patients and sixty-five healthy individuals. *Candida* isolates were identified by MALDI-TOF VITEK-MS. Proteinase, phospholipase and esterase activity, biofilm production and level expression of *ALS*, *SAP* and *PLB* genes in *C. albicans* were evaluated. Minimal inhibitory concentration values were determined by means of an antifungal susceptibility test.

Results: Oral *Candida* colonization in cystic fibrosis patients was 66.15%, while in healthy individuals was 36.92%. *C. albicans* was the most frequently isolated species. *C. albicans* strains from cystic fibrosis patients were high producers of protease and biofilm, and had higher expression levels of adhesin and protease-associated genes in comparison with healthy subjects. Among the *C. albicans* strains isolated from cystic fibrosis patients, 18.91% were resistant to itraconazole, while 16.21% exhibited resistance to ketoconazole and fluconazole, and only one strain was resistant to voriconazole.

Conclusions: This work represents a surveillance study on virulence patterns and antifungal susceptibility of *Candida* from the oropharyngeal tract in cystic fibrosis.

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Prevalencia, factores de virulencia y sensibilidad antifúngica de aislamientos orales de *Candida albicans* de pacientes con fibrosis quística en México

RESUMEN

Palabras clave:

Candida albicans

Fibrosis quística

Factores de virulencia

Biopelícula

Sensibilidad antifúngica

Antecedentes: Las especies de *Candida* se aíslan con frecuencia de la cavidad oral de pacientes con fibrosis quística; sin embargo, la fisiopatología de *Candida* en la fibrosis quística es desconocida.

Objetivos: En este estudio evaluamos la prevalencia, perfil de virulencia y sensibilidad antifúngica de aislamientos orales de *Candida albicans* procedentes de pacientes con fibrosis quística.

Métodos: Se tomaron hisopados orofaríngeos en sesenta y cinco pacientes con fibrosis quística, y sesenta y cinco individuos sanos. Los aislamientos de *Candida* se identificaron mediante MALDI-TOF VITEK-MS. Se determinó la actividad de proteinasas, fosfolipasas, esterases, la producción de biopelícula y la expresión de los genes *ALS*, *SAP* y *PLB* de los aislamientos de *C. albicans*. Se determinó la concentración mínima inhibitoria mediante una prueba de sensibilidad a los antifúngicos.

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Resultados: La colonización oral por *Candida* en pacientes con fibrosis quística fue del 66,15%, mientras que en individuos sanos fue del 36,92%, y *C. albicans* fue la especie más frecuentemente aislada. Los aislamientos de *C. albicans* procedente de pacientes con fibrosis quística eran fuertes productores de proteasas y biopelículas, y tenían mayores niveles de expresión de genes asociados a adhesinas y proteasas en comparación con los aislamientos de sujetos sanos. El 18,91% de los aislamientos de *C. albicans* aislados de pacientes con fibrosis quística eran resistentes al itraconazol, mientras que el 16,21% lo eran al ketoconazol y al fluconazol, y sólo un aislamiento fue resistente al voriconazol.

Conclusiones: Este trabajo representa un estudio de vigilancia sobre patrones de virulencia y sensibilidad antifúngica de aislamientos de *Candida* del tracto orofaríngeo en la fibrosis quística.

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Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*cftr*) gene. The impairment of the CFTR protein reduces the secretion of chloride ions through the epithelial cells, which leads to an accumulation of dehydrated mucus and defective mucociliary clearance in the airways of patients.²⁸ CF patients are highly susceptible to respiratory tract infections, mainly by bacteria and fungi.¹³ Among the clinically significant fungi, *Aspergillus* and *Scedosporium* are the most frequent molds, while *Candida* species are the most common yeasts recovered from CF patients.⁴⁰

Candida species are opportunistic fungi isolated from oral cavity in up to 75% of CF patients; the high colonization is probably linked to both the use of inhaled steroids and recurrent antibiotic treatment.³⁹ The clinical significance of *Candida* airway colonization in CF patients remains poorly understood. Some studies have shown that the persistence of *Candida albicans* or *Candida dubliniensis* in the respiratory tract of CF patients is associated with decreased pulmonary function.^{1,9} The pathogenicity of *C. albicans* is attributed to virulence factors, such as the activity of the enzymes proteinase, phospholipase and esterase, as well as the ability of producing biofilm; these virulence factors facilitate the adherence, penetration in the tissues and invasion.³ Currently, there are few studies evaluating the activity of *C. albicans* virulence factors in CF. Moreover, being the emergence of antifungal-resistant fungi an issue of concern, several CF centers have evidenced increased antifungal resistance in *Aspergillus fumigatus*.²² However, antimicrobial susceptibility trend in *C. albicans* isolates is limited. Therefore, studies on the virulence and the antifungal susceptibility of *Candida* strains from CF patients are needed.

This study has evaluated the prevalence of *Candida* in the oropharynx of CF patients and healthy individuals, the enzymatic virulence factors displayed by the *C. albicans* strains in culture media, and their susceptibility to several antifungal drugs.

Material and methods

Patients and *Candida* isolates

This study was conducted on sixty-five CF patients (33 males and 32 females, aged 1.5 months to 30 years) referred to the Regional Centre for Infectious Disease Control of the University Hospital, Universidad Autónoma de Nuevo León, Mexico. The patients included had a genetic CF diagnosis; CF patients that received an antifungal treatment over the previous 30 days were excluded. Sixty-five healthy individuals (31 males and 34 females) were matched to cystic fibrosis patients and included as controls ($p=0.98$). Suffering from diabetes mellitus or other diseases, the use of orthodontic appliances or having had any treatment with antimicrobials over the previous 30 days were non-inclusion criteria for healthy subjects. This study was authorized and supervised by the Ethics and Research Committee (MB22-00006).

Oropharyngeal swab samples from CF patients and healthy individuals were collected, and then plated out in duplicate on Sabouraud-dextrose agar (SDA) supplemented with amikacin (0.1 mg/mL) and *Candida* agar (CHROM agar). Plates were incubated at 37 °C for 48 h, and the yeast colonies grown were counted afterwards. Oral colonization of *Candida* was determined as: low (<10 colonies), moderate (10–50 colonies) and high (>50 colonies).¹⁵ The isolates were incubated on SDA at 37 °C for 48 h, identified by germ tube formation, and microculture in cornmeal-Tween 80 agar. The identification was confirmed by means of MALDI-TOF VITEK-MS (bioMérieux, France). Fungal strains were stored in Sabouraud-dextrose broth (SDB) with 20% glycerol at –80 °C. *C. albicans* isolates were then subjected to both the virulence and susceptibility testing.

Enzymatic activity assays

C. albicans strains were cultured on SDA for 24 h at 37 °C. Then, the activity of proteinase, phospholipase and esterase were performed as previously reported by Sriphannam et al.³⁵ using plates with specific media depending on the enzyme, such as YCB-BSA medium, egg-yolk agar, and Tween 80 opacity test medium. Five microliters of a 10⁷ CFU/mL adjusted suspension were inoculated onto each test medium. Plates were incubated for 5 and 7 days at 37 °C. Each enzymatic activity was evaluated using the Pz index, which is calculated by dividing the colony diameter by the colony diameter plus the precipitation zone. The degree of enzyme production was classified as follows: high (Pz < 0.40), medium (Pz = 0.41–0.60), low (Pz = 0.61–0.80), very low (Pz = 0.81–0.99) and none (Pz = 1.00).³⁵

Biofilm assay

Biofilm production was evaluated by crystal violet assay. Briefly, *C. albicans* isolates were cultured overnight on 4 mL of SDB with 8% glucose at 37 °C with shaking. Afterwards, 20 µL of each *Candida* suspension containing 10⁷ CFU/mL were inoculated into each well of a sterile flat-bottomed 96-well microtiter plate containing 180 µL SDB with 8% glucose; the plates were incubated for 24 h at 37 °C. Then, the medium was discarded and wells were washed twice. The biofilms formed were stained with 200 µL of 0.2% crystal violet for 15 min. The excess dye was discarded and wells were washed twice; the dye was solubilized with 200 µL of 96% ethanol. Absorbance was measured at 595 nm, and blanks were used as negative control. Biofilm formation was classified into four categories based on cut-off value (ODc), which was obtained from the mean values of the negative controls plus three standard deviations. The biofilm density categories are the following: OD < ODc (no biofilm formation), ODc < OD ≤ 2 × ODc (low-biofilm formation), 2 × ODc < OD ≤ 4 × ODc (moderate-biofilm formation), 4 × ODc < OD (high-biofilm formation).³⁵

Table 1Prevalence and oral colonization of *Candida* species in cystic fibrosis patients and healthy subjects.

	Cystic fibrosis patients n (%)	Healthy individuals n (%)	p
<i>Candida</i> cultures from oral cavity			
Positive cultures	43 (66.15%)	24 (36.92%)	0.024*
Degree of <i>Candida</i> colonization			
Low	5 (11.63%)	11 (45.83)	0.017*
Moderate	21 (48.84%)	12 (50.00%)	0.488
High	17 (39.53%)	1 (4.17%)	0.003*
<i>Candida</i> species			
<i>Candida albicans</i>	37 (86.04%)	20 (83.33%)	–
<i>Candida tropicalis</i>	3 (6.98%)	ND	–
<i>Candida parapsilosis</i>	2 (4.66%)	3 (12.5%)	–
<i>Candida krusei</i>	1 (2.32%)	ND	–
<i>Candida glabrata</i>	ND	1 (4.17%)	–

ND: not detected; –: not analyzed.

* Statistically significant: $p \leq 0.05$.

Virulence gene expression

Total mRNA from *C. albicans* strains was isolated with TRIzol reagent (Invitrogen, USA) according to manufacturer's instructions, and then were quantified by measuring the optical density at 260 nm. Total mRNA quality was estimated by electrophoresis on agarose gel stained with ethidium bromide. Reverse transcription was performed employing 1 µg of total mRNA with SensiFAST cDNA Synthesis (Bioline, USA) according to manufacturer's instructions. cDNA obtained was used for conventional PCR amplification using gene-specific primers for different virulence factors: secreted aspartyl proteinase (*SAP1–8*), phospholipase B (*PLB1–2*), agglutinin-like sequence (*ALS1–7*) and actin gene (*ACT1*). The primers used are listed in [Supplementary Table 1](#). RT-PCR was carried out using MyTaq DNA polymerase (Bioline) according to manufacturer's instructions, and relative expression of each virulence gene was semi-quantified in relation to the housekeeping gene actin.

Antifungal susceptibility testing

The antifungal susceptibility profiles of *C. albicans* isolates were determined by the broth microdilution reference method.⁵ The protocol from the Clinical and Laboratory Standards Institute (CLSI) was applied for determining the minimal inhibitory concentrations (MICs) for azoles (fluconazole, ketoconazole, itraconazole and voriconazole), echinocandins (anidulafungin and micafungin), and amphotericin B. MICs were recorded after 24 h of incubation with the antifungals. *Candida parapsilosis* ATCC 22019 was used as quality control.

Statistical analysis

The experiments were performed independently at least two times. To test the normality of the data, the Shapiro–Wilk test was used. Concerning the interpretation of the studied virulence factors, the Fisher's exact test (two-tailed) was used to determine statistical differences between CF patients and healthy subjects. Comparisons of the MIC values of strains from the CF and non-CF groups were done by the Mann–Whitney test (GraphPad Software, USA).

Results

The incidence of oral *Candida* colonization in CF patients was 66.15% (43 of 65 patients), while in healthy individuals was 36.92% (24 of 65 individuals); this difference was statistically significant

($p = 0.003$). The distribution of *Candida* species evidenced that *C. albicans* was the most frequently isolated species ([Table 1](#)).

Since we found a higher prevalence of *C. albicans* isolates in the oral cavity of CF patients than in healthy individuals, we evaluated the virulence profiles of *C. albicans* ([Table 2](#)). The results showed that all isolates were proteinase producers to any extent. Significant differences in protease activity were observed among both study groups: 37.84% of *C. albicans* isolates from CF patients were high protease producers, in contrast to 15% of isolates from healthy subjects ($p = 0.013$), whereas no significant differences in phospholipase and esterase activity were found between the two groups. Regarding biofilm formation ([Table 2](#)), 43.25% of *C. albicans* from CF patients were high biofilm producers, compared to 10% of the *C. albicans* isolates from healthy subjects ($p = 0.009$). Results of virulence genes study showed that *ALS3*, *ALS4*, *SAP1*, *SAP3*, *SAP7*, and *PLB1* were more frequently expressed in oral *C. albicans* from CF patients than in those *C. albicans* isolated from healthy subjects ($p < 0.05$) ([Table 3](#)). These results evidenced that *C. albicans* from the oropharyngeal tract of CF patients had increased protease activity, higher level of biofilm formation, and higher expression levels of adhesins and protease-associated genes.

Regarding the antifungal susceptibility, oral isolates of *C. albicans* from CF patients showed 18.91% resistance to itraconazole, while 16.21% of the isolates exhibited resistance to ketoconazole and fluconazole; only one strain was resistant to voriconazole. In contrast, all *C. albicans* isolates recovered from healthy individuals were susceptible to azoles ([Table 4](#)). All *C. albicans* isolates from both study groups were susceptible to micafungin, anidulafungin and amphotericin B ([Table 4](#)).

Discussion

The recent advances in the management and treatment of CF have positively changed the prognosis and life expectancy of CF patients. However, these advances have facilitated the fungal colonization of the respiratory tract.^{8,40} The genus *Candida* comprises opportunistic fungi that are frequently isolated from CF patients, and cases of oral, esophageal and respiratory candidiasis have been reported.^{9,38} Despite their increasing presence in the airway of CF patients, the role of *C. albicans* in the disease remains unclear, posing a major challenge to clinicians and researchers.⁴ Therefore, studies evaluating the oral fungal burden and virulence factors of *C. albicans* in CF are needed.

In our study, the prevalence of *Candida* species in the oral cavity of CF patients was higher if compared with healthy subjects, and *C. albicans* was the most frequently isolated species; similar data were reported in previous studies.^{7,12,19,26,40} We found a

Table 2Virulence factors of *Candida albicans* isolates from cystic fibrosis patients and healthy subjects.

Virulence factors	Cystic fibrosis patients		Healthy individuals		p
	n = 37	%	n = 20	%	
<i>Proteinase</i>					
High	14	37.84%	3	15%	0.013*
Medium	18	48.65%	9	45%	0.725
Low	5	13.51%	6	30%	0.042*
Very low	0	0%	2	10%	–
None	0	0%	0	0%	–
<i>Phospholipase</i>					
High	0	0%	0	0%	–
Medium	20	54.05%	9	45%	0.691
Low	9	24.33%	7	35%	0.085
Very low	4	10.81%	3	15%	0.243
None	4	10.81%	1	5%	0.092
<i>Esterase</i>					
High	6	16.23%	2	10%	0.164
Medium	25	67.57%	10	50%	0.372
Low	3	8.1%	4	20%	0.088
Very low	1	2.7%	1	5%	0.193
None	2	5.4%	3	15%	0.072
<i>Biofilm production</i>					
High	16	43.25%	2	10%	0.009*
Medium	15	40.55%	8	40%	0.865
Low	5	13.5%	9	45%	0.036*
None	1	2.7%	1	5%	0.193

Enzymatic activity was classified according to the Pz index: high (Pz < 0.4), medium (Pz = 0.41–0.6), low (Pz = 0.61–0.8), very low (Pz = 0.81–0.99) and none (Pz = 1.00).

The biofilm was quantified according to cut-off values (ODc): OD < ODc (no biofilm formation), ODc < OD ≤ 2 × ODc (low-biofilm formation), 2 × ODc < OD ≤ 4 × ODc (moderate-biofilm formation), 4 × ODc < OD (high-biofilm formation).

–: not analyzed.

* Statistically significant: $p \leq 0.05$.**Table 3**Differential expression of virulence factors of *Candida albicans* isolates from CF patients and healthy subjects.

Virulence gene expression	Cystic fibrosis patients n = 37 (%)	Healthy individuals n = 20 (%)	p
ALS1	16 (43.24)	8 (40)	0.702
ALS2	21 (56.75)	12 (60)	0.568
ALS3	37 (100)	13 (65)	0.026*
ALS4	36 (97.3)	10 (50)	0.019*
ALS5	37 (100)	20 (100)	0.995
ALS6	15 (40.54)	7 (35)	0.236
ALS7	15 (40.54)	9 (45)	0.618
SAP1	33 (89.19)	5 (25)	0.008*
SAP2	34 (91.89)	18 (90)	0.895
SAP3	30 (81.08)	4 (20)	0.001*
SAP4	16 (43.24)	11 (55)	0.636
SAP5	29 (78.37)	14 (70)	0.355
SAP6	15 (40.54)	8 (40)	0.955
SAP7	31 (83.78)	3 (15)	0.001*
SAP8	14 (37.83)	6 (30)	0.355
PLB1	35 (94.59)	4 (20)	0.007*
PLB2	17 (45.94)	8 (40)	0.568

* Statistically significant: $p \leq 0.05$.**Table 4**Antifungal susceptibility profiles of *Candida albicans* isolates from CF patients and healthy subjects. Concentration ranges and MIC values are in µg/mL.

Antifungal agent	Cystic fibrosis patients				Healthy individuals			
	Concentration range	MIC50	MIC90	No. resistant (%)	Concentration range	MIC50	MIC90	No. resistant (%)
Ketoconazole	0.06–64	2	16	6 (16.21)	0.03–0.25	0.06	0.25	–
Itraconazole	0.06–64	2	16	7 (18.91)	0.03–0.5	0.03	0.25	–
Fluconazole	0.06–64	1	32	6 (16.21)	0.03–1	0.125	0.5	–
Voriconazole	0.06–2	0.12	0.25	1 (2.7)	0.03–0.12	0.06	0.12	–
Micafungin	0.03–0.12	0.06	0.12	–	0.03–0.12	0.06	0.12	–
Anidulafungin	0.03–0.25	0.06	0.12	–	0.03–0.25	0.06	0.12	–
Amphotericin B	0.03–0.25	0.03	0.12	–	0.03–0.25	0.03	0.12	–

MIC: minimum inhibitory concentration; MIC50: lowest concentration that inhibits the growth of 50% of the isolates; MIC90: lowest concentration that inhibits the growth of 90% of the isolates.

low presence of non-*C. albicans* *Candida* species, such as *Candida tropicalis*, *C. parapsilosis* and *Pichia kudriavzevii* (formerly *Candida krusei*), which is in line with others reports.^{7,12,19,26} The clinical practice guidelines for the management of candidiasis, proposed by the Infectious Diseases Society of America, states that, “growth of *Candida* from respiratory secretions usually indicates colonization and rarely requires treatment with antifungal therapy”.²⁹ However, previous studies have shown that persistent and high levels of *C. albicans* or *C. dubliniensis* colonization in CF patients are associated with decreased lung function and severe cases of exacerbated pulmonary infection.^{1,9} We observed that the oral fungal carriage was higher in the CF group (39.53%) than in the healthy group (4.17%), similar to that reported by Lepesqueur et al.¹⁹ These studies show that CF patients have a higher fungal burden in the airways, which could contribute to the pathophysiology of the disease or the development of mycosis.

Some virulence factors of *C. albicans* have been used as biomarkers for issuing the prognosis and outcome in cases of candidiasis.³⁰ Our results showed that the *C. albicans* isolates from the oropharyngeal tract of CF patients exhibited higher protease activity and biofilm production than those isolates recovered from healthy subjects. Whereas we did not observe differences in phospholipase and esterase activity among the study groups, Karaman et al. reported that *C. albicans* isolates from CF patients showed protease, phospholipase, esterase, and biofilm activity; however, they did not compare their results with isolates from healthy individuals.¹⁵ On the other hand, previous studies have shown that *C. albicans* from patients with oral candidiasis had a higher proteinase production than those isolated from the control group.^{17,30} Biofilm formation by *C. albicans* allows a persistent colonization of the tissues and may promote the development of mycosis.³⁰ It has been observed that *C. albicans* isolates from recurrent vulvovaginal candidiasis have higher biofilm-forming activity than isolates from non recurrent-vaginal candidiasis,⁶ and clinical isolates of *C. albicans* from patients with dental caries and periodontitis have also higher biofilm-forming activity.^{33,37} These studies evidenced that *C. albicans* virulence plays a crucial role in infections, and that virulence profiles can be used as biomarkers for both monitoring *C. albicans* colonization and issuing a prognosis in case of infection.

In the virulence-related genes assays, such as *ALS*, *SAP* and *PLB*, we found that the isolates from CF patients showed higher expression levels of *ALS3* and *ALS4* when compared with healthy individuals. *ALS1–5* genes are upregulated during oral candidiasis; particularly, *ALS3* is responsible for the adherence, invasion and biofilm formation during mucocutaneous infection.²⁷ Furthermore, we found that 100% of the *C. albicans* isolates from both study groups exhibited *ALS5* gene expression. It has been reported that *ALS5* is involved in the adhesion of the yeast phase of *C. albicans* to human buccal epithelial cells.²⁷ The differential expression of adhesins found in the *C. albicans* isolates of both groups may contribute to the higher levels of colonization and persistence, as well as increased biofilm activity in CF patients. In our study, the oral *C. albicans* isolates from CF patients had higher expression levels of *SAP1*, *SAP3*, *SAP7* and *PLB1*. Previous studies have shown that patients with oral candidiasis carried strains that exhibited higher expression levels of *SAP1*, *SAP3*, and *SAP4–6* than the strains from healthy carriers.^{21,25} High levels of *SAP1–3* also contribute to tissue damage.³⁴ The expression of *SAP1* and *SAP3* has been associated with the phenotypic transition from commensal to virulent pathogens in oral candidiasis.²⁵ The airways of CF patients exhibit physiological, biochemical and molecular changes that condition the adaptation of microorganisms to this microenvironment, allowing them to colonize and cause chronic infections.² For example, the pH of the airway surface fluid of CF patients is slightly acidic,²⁰ and the acidic pH has a significant impact on the adhesion and biofilm formation of *Candida* in the oral cavity.¹¹ Furthermore,

C. albicans adapts to the acidic microenvironment through the differential expression of *PHR1*, *PHR2* and *PRA1* genes (surface proteins associated with stress and fungal morphology), that allow chronic colonization,³¹ so possibly the low pH of the oral mucosa in CF patients may be a key factor in the prevalent colonization and high fungal burden of *Candida* species. The composition of glucans and glycoproteins on the surface of the respiratory tract of CF patients is abundant and complex, and *C. albicans* must adapt to this niche.³² Previous studies have shown that glucan enrichment is a key factor in enhancing *C. albicans* adherence and biofilm formation, but may negatively affect the expression of virulence genes.^{14,18} On the other hand, the availability of essential nutrients in the CF airways, such as iron, is limited, thus affecting the microorganisms. Previous studies have shown that *Candida* species exhibit mutations in transcriptional regulators, such as *Rob1* and *Mrs4*, which increase their fitness and adaptive growth in the CF lung environment.^{10,23} *Rob1* and *Nrg1* are transcriptional regulators that are also associated with the expression of *C. albicans* virulence genes. Kim et al. found that mutations in the *Nrg1* regulator in *C. albicans* isolates from CF patients lead to increased filamentous growth capacity, possibly associated with the mechanism of adaptation to the CF lung environment.¹⁶ These results demonstrate that the airway microenvironment in cystic fibrosis is complex and may condition the expression of *C. albicans* virulence genes to enable adaptation and colonization. Thus, the role of *C. albicans* virulence factors in the adaptation to the host or in the pathophysiology processes during CF remains poorly understood and further studies are needed.

In this study, *C. albicans* strains from the oral cavity of CF patients exhibited resistance to azoles: 18.91% to itraconazole, 16.21% to ketoconazole and fluconazole, and one isolate was resistant to voriconazole. In our study, only two CF patients had received antifungal treatment (several months before) prior to *C. albicans* isolation; however, we were unable to demonstrate an association between azole prophylaxis and the development of azole resistance in *C. albicans*. Previously, a study conducted at the Stanford CF Center found resistance of *A. fumigatus* to voriconazole in the absence of voriconazole use in patients.³⁶ Previous studies have shown a low incidence of *C. albicans* strains resistant to fluconazole, itraconazole and voriconazole in CF patients,^{7,15,24} unlike other studies in which 100% susceptibility to azoles has been observed.^{19,26} According to Chotirmall and McElvaney, the resistance of *Candida* to the antifungal drugs may be influenced by the ability of the strains to grow within a biofilm structure.⁴ In our study, approximately 80% of the isolates exhibited moderate to strong biofilm formation. Finally, the most effective antifungals against *C. albicans* isolates were amphotericin B and the echinocandins (micafungin and anidulafungin). These findings are in accordance with those of previous studies.^{7,15,19,24,26}

Our study provides new data on the epidemiology of *Candida* species, virulence factors and antifungal susceptibility of *C. albicans* from the oropharyngeal tract of CF patients. This data may be useful to understand the clinical significance of *Candida* strains in these patients, and may help in implementing surveillance studies focused on early diagnosis, prevention and control of infections in CF patients.

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Conflicts of interest

The authors declare having no financial or commercial conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version available at <https://doi.org/10.1016/j.riam.2024.09.001>.

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