



Investigation of Oral and Fecal Colonization with *Candida* Species and Associated Factors in Human Immunodeficiency Virus-Infected Children in Türkiye

Esma Akkoyun Bilgi¹  Gonca Erköse Genç¹  Manolya Kara²  Eda Kepenekli Kadayıfçı³ 
Selda Hançerli Törün²  Canan Baydemir⁴  Ayper Somer²  Ali Ağaçfidan¹  Zayre Erturan¹ 

¹Department of Medical Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Türkiye

²Division of Pediatric Infectious Diseases, Department of Pediatrics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Türkiye

³Division of Pediatric Infectious Diseases, Department of Pediatrics, Marmara University School of Medicine, Istanbul, Türkiye

⁴Department of Biostatistics and Medical Informatics, Kocaeli University Faculty of Medicine, Kocaeli, Türkiye

Address for correspondence Esma Akkoyun Bilgi, MD, Department of Medical Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Türkiye (e-mail: esma.akkoyun@hotmail.com).

J Pediatr Infect Dis 2023;18:132–138.

Abstract

Objective The risk of endogenous infections in human immunodeficiency virus (HIV)-infected individuals increases with *Candida* species colonized in mouth and intestinal areas. The predisposing factors for colonization and the prevalence of different *Candida* spp. in HIV-infected Turkish children remain unknown. This study aimed to determine the colonization frequency and risk factors of colonization with *Candida* species in oral and fecal samples of HIV-infected pediatric patients in relation to a control group.

Methods Oral and feces samples of 22 HIV-infected and 52 healthy children were plated onto CHROMagar and CHROM-Pal-agar. Yeasts were identified by conventional methods, and strains with insufficient identification were identified by molecular techniques.

Results *Candida* spp. were detected in oral/fecal samples of 50%/68.2% HIV-infected and 36.5%/73.1% healthy children. The most common species was *Candida albicans* in oral and fecal samples of HIV-infected (31.8 and 31.8%) and healthy (26.9 and 48.1%) children. The most frequently non-albicans species in oral samples was *Candida dubliniensis* (18.2%) in HIV-infected children and *Candida parapsilosis* (3.8%) in healthy children. In feces samples, *C. parapsilosis*, *Candida glabrata*, and *Candida krusei* were most frequent (13.6%, each) in HIV-infected patients, and *Candida kefyr* (11.5%) was most frequent in controls. There was a significant association between oral *C. dubliniensis* colonization and HIV infection ($p = 0.006$). Yeast carriage was not associated with gender and viral load in HIV-infected patients.

Conclusion The isolation of *C. dubliniensis* from oral and fecal samples of pediatric HIV patients was done for the first time in Türkiye in the present study. Additional studies are needed to clarify the factors associated with oral and fecal *Candida* colonization in these children.

Keywords

- HIV
- children
- *Candida*
- oral colonization
- fecal colonization

received
October 19, 2022
accepted after revision
February 22, 2023
article published online
March 31, 2023

DOI <https://doi.org/10.1055/s-0043-1767737>.
ISSN 1305-7707.

© 2023. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution, and reproduction so long as the original work is properly cited. (<https://creativecommons.org/licenses/by/4.0/>)
Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Introduction

There were globally estimated to be 38.4 million people living with HIV, including 2.73 million children aged 0 to 19 years in 2021.¹ Although our country is among the countries where human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) is less frequent, the number of cases has increased in recent years. The total number of HIV-positive people in Türkiye was reported to be 21,520, and 199 of these were children aged 0 to 14 years.²

Candida species are commensal microorganisms of the oral and gastrointestinal mucosa; however, in the presence of predisposing factors, these may become pathogenic and cause infection. More than 72% of HIV-infected children develop oral candidiasis during the course of their illness, and this infection is considered an important marker of HIV disease and its progression.³ *Candida albicans* is the most frequent species isolated, but it has been reported that oral carriage of non-*albicans Candida* (NAC) is on increase, especially in HIV seropositive children.^{4,5} *Candida* spp. have also been implicated in the etiology of chronic diarrhea in HIV patients.⁶

Oral colonization by *Candida* spp. has been thoroughly investigated in HIV-positive adults, but the number of studies with HIV-positive children is still small.⁷ Also little is known about lower gastrointestinal colonization in this HIV-infected age group.⁸ In addition, there are no reported studies on these topics from HIV-infected children in Türkiye. Therefore, the present study was designed to investigate the prevalence of, and predisposing factors for, oral and fecal yeast carriage in Turkish pediatric HIV-infected patients and to compare the prevalence of different *Candida* spp. in HIV-infected and noninfected children.

Materials and Methods

Oral mouthwash or swab samples (from young children) and feces samples of children who are followed up in the outpatient clinics of Istanbul University, Istanbul Faculty of Medicine, Pediatric Infectious Diseases and Marmara University, Pendik Training and Research Hospital, Pediatric Infectious Diseases were evaluated between January 2018 and December 2019. The study protocol was approved by the Ethical Committee of Istanbul Faculty of Medicine (24.05.2017/535), and written informed consent was obtained from the parents of each patients prior to enrolment. Inclusion criteria were having no clinical signs of oropharyngeal candidiasis (OFC) and no ongoing treatment with antifungals or antimicrobials. Individuals with a history of prolonged antibiotic or steroid therapy or diabetes mellitus were excluded from the study. Oral wash samples were collected by asking the subjects to rinse their mouth with 10 mL of sterile 0.9% saline for 5 s and to return the specimen in a sterile container.⁹ Oral swabs were collected using sterile swab sticks, and fecal samples were collected in a sterile specimen pot with an integral spoon. Age, sex, weight, eating habits, presence of intraoral lesions and caries, CD4 count, and HIV-RNA viral load that was determined on the same day were recorded. Children with a diet mainly

rich in kefir and yogurt were included in the healthy eating group, while participants with high carbohydrate and simple sugar consumption made up the unhealthy eating group. Individuals with no particular preference were classified as people with normal nutrition.

All specimens were processed in the Mycology Laboratory of Istanbul Faculty of Medicine, Department of Medical Microbiology. Feces specimens were processed by emulsifying 1 g of feces in 10 mL of sterile water and a loopful (0.001 mL) of this was streaked onto the surface of the media.¹⁰ Each oral rinse was centrifuged at $2,000 \times g$ for 10 minutes, and 100 μ L of the deposit was inoculated onto CHROM-agar *Candida* (CAC) (Becton-Dickinson, Paris, France) and CHROM-Pal-agar (CAP) to differentiate *Candida dubliniensis*.¹¹ The plates were incubated at 37°C and 30°C for 48 hours. CAP medium was prepared by mixing equal volumes of prepared CAC and Pal's agar.¹² Yeast colonies were identified by microscopic morphology on cornmeal-tween 80 agar, growth at 45°C and by the API ID 32 C (Biomérieux, Marcy l'Etoile, France) system.¹³ Strains which gave an insufficient identification profile by the API system were identified by DNA sequencing. The sequencing results were used for homology comparison using the Basic Local Alignment Search Tool search (<http://www.ncbi.nlm.nih.gov/BLAST>).

Statistical evaluation was made with IBM SPSS 20.0 (IBM Corp., Armonk, NY, United States) package program. Numerical variables were given as mean \pm standard deviation and median (25th–75th percentile) and frequency (percentages). Differences between groups were compared using the Mann–Whitney U Test for numerical variables that did not have a normal distribution. Fisher's exact chi-square test, Yates' chi-square test, and Monte Carlo chi-square test were used for categorical variables to evaluate the differences between groups. $p < 0.05$ was considered sufficient for statistical significance in two-sided tests.

Results

The demographic characteristics of the study population are shown in ►Table 1. Overall, 22 HIV-infected children and 52 healthy controls were included in this research. No significant differences were found in the median age, sex distribution, and weight of the HIV-positive and control group ($p > 0.05$). All HIV-infected patients were receiving highly active antiretroviral therapy. The median plasma HIV-1 RNA burden and median CD4 T-lymphocyte count of HIV-infected children were 45 copies/mL (range: 45–1,605,235) and 486.50 cells/mm³ (range: 18–1,427), respectively.

Rates of yeast isolation from oral samples of patients and healthy controls were 50% (11/22) and 38.5% (20/52) ($p = 0.508$), and isolation rates from feces samples were 68.2% (15/22) and 80.4% (42/52) ($p = 0.382$), respectively. The distribution of yeast species isolated is shown in ►Table 2. *Candida* spp. were isolated from oral and feces samples of the HIV-infected group and controls in 11 (50%) and 19 (36.5%), and 15 (68.2%) and 38 (73.1%) children, respectively.

Table 1 Characteristics of the children participating in the study

	HIV-infected group <i>n</i> = 22 (%)	Control group <i>n</i> = 52 (%)	<i>p</i> -Value
Median age (months)	156 (range 2–204)	99 (range 24–204)	0.069
Gender			
Female	11 (50)	25 (48.1)	1.000
Male	11 (50)	27 (51.9)	1.000
Median weight	43 kg (range 5–77)	27.5 kg (range 11–68)	0.096
Tooth decay	0	4 (7.7)	–
Mucositis	1 (4.45)	0	–
Nutrition			
Healthy	0	13 (25)	–
Unhealthy	0	8 (15.4)	–
Normal	22 (100)	31 (59.6)	–

C. albicans was the most common species isolated from oral samples of HIV-infected and healthy children, being isolated from 7 (31.8%) and 14 (26.9%), respectively ($p = 0.885$). NAC were isolated from oral samples of seven (31.8%) HIV-infected children and seven (13.5%) healthy children ($p = 0.102$). The most frequent NAC in oral samples of HIV-positive children was *C. dubliniensis* (four isolates), whereas this species was not found in oral samples of healthy children ($p = 0.006$).

C. albicans was isolated from the feces of 7 (31.8%) and 25 (48.1%) HIV-infected and uninfected children, respectively ($p = 0.885$). NAC were isolated from 14 (63.6%) HIV-infected children and 22 (42.3%) controls ($p = 0.155$). The most common NAC species isolated from feces samples of HIV-infected children were *Candida parapsilosis*, *Candida glabrata* and *Candida krusei* that were isolated equally from three samples each (13.6%), while in healthy children *Candida kefyr* in six samples (11.5%). Although non-*Candida* yeasts were isolated from the feces of one (4.5%) of the HIV-infected patients and 13 (25%) healthy children, this difference was not statistically significant ($p = 0.052$). The most common non-*Candida* yeast grown from feces was *Rhodotorula* spp., isolated from 4.5 and 15.4% of patients and healthy children, respectively.

Mixed yeast growth was observed in the oral and fecal samples of 13.6% (3) and 22.7% (5) of HIV infected, 5.7% (3) and 28.9% (15) of healthy children (→ **Table 3**). No statistically significant difference was found between these rates ($p = 0.354$ and 0.776).

Candida isolation in oral and feces samples was observed as 0% (0/5) and 60% (3/5) in children with CD4 counts ≤ 200 cells/mm³, while it was found to be 65% (11/17) and 70.5% (12/17) in those with CD4 counts > 200 cells/mm³, respectively. This inverse relationship was attributed to the insufficient number of children with CD4 counts ≤ 200 cells/mm³, and statistical evaluation could not be made.

While no significant association was found between the sex and oral *Candida* colonization in HIV-infected children ($p = 0.395$), oral colonization of healthy girls was significantly more common than that of healthy boys ($p = 0.043$). Likewise, there was no significant difference between the sexes in rates

of oral NAC colonization ($p = 1.000$), but NAC colonization was significantly higher in healthy girls than in healthy boys ($p = 0.046$). No significant relationships were found between the sexes in relation to fecal *Candida* and NAC colonization, in both HIV-infected and healthy children ($p = 1.000$, $p = 1.000$, $p = 0.582$; 1.000 , respectively).

No significant differences were found between the oral samples of patients aged ≤ 120 months and ≥ 121 months in terms of yeast, *Candida* spp., *C. albicans*, NAC, and mixed and *C. dubliniensis* colonization. However, there were differences between HIV-infected children aged ≤ 120 and ≥ 121 months in terms of colonization rates of yeasts, *Candida*, NAC and *C. parapsilosis* complex ($p = 0.022$; 0.022 ; 0.024 ; 0.036 , respectively). These differences were unrelated to viral load.

Discussion

Oral lesions are common among HIV-infected children, and the most common of these is due to candidiasis.¹⁴ It has been reported that more than 72% of these children develop oral candidiasis during the course of the disease.^{3,15} Early detection of oral carriage of *Candida* spp. is important to initiate antifungal therapy before overt OFC and, additionally, to identify individuals who tend to develop a rapid progression in HIV infection.^{3,7,14} In addition, disseminated OFC cases have been reported in children.^{16,17}

Although there are many studies about oral *Candida* colonization in adult HIV-infected patients, studies in children are limited. In studies investigating oral *Candida* isolation from oral wash or swab samples in HIV-infected and HIV-negative children, colonization rates were reported to be 45.5 to 80% and 30 to 57.5%, respectively.^{3,15,18–20} In the present study, although oral *Candida* colonization was more common in HIV-infected (50%) than healthy children (36.5%), the difference was not significant ($p = 0.413$). This result is consistent with the findings of Jabra-Rizk et al¹⁹ and Pongsiriwet et al¹⁸ who reported colonization rates of 50% and 45.5% in HIV-positive and 30% and 40% in HIV-negative children. It has been noted that the variability in the resulting isolation rates may be due to factors such as the methods of

Table 2 Distribution of yeast species isolated from HIV-infected and healthy children according to the specimen

Culture distribution	HIV-infected group (n=22)		Control group (n=52)		p-Values	
	Oral specimen n (%)	Feces n (%)	Oral specimen n (%)	Feces n (%)	Oral specimen n (%)	Feces n (%)
Yeast species	11 (50)	15 (68.2)	20 (38.5)	42 (80.4)	0.508	0.382
<i>Candida</i> spp.	11 (50)	15 (68.2)	19 (36.5)	38 (73.1)	0.413	0.885
<i>C. albicans</i>	7 (31.8)	7 (31.8)	14 (26.9)	25 (48.1)	0.885	0.301
Non- <i>albicans Candida</i>	7 (31.8)	14 (63.6)	7 (13.5)	22 (42.3)	0.102	0.155
<i>C. kefyr</i>	–	–	1 (1.9)	6 (11.5)	1.000	0.170
<i>C. parapsilosis</i>	–	3 (13.6)	2 (3.8)	4 (7.7)	1.000	0.418
<i>C. lusitaniae</i>	–	–	1 (1.9)	3 (5.8)	1.000	–
<i>C. famata</i>	1 (4.5)	–	1 (1.9)	–	NE	–
<i>C. guilliermondii</i>	–	–	1 (1.9)	–	1.000	–
<i>C. lipolytica</i>	–	–	1 (1.9)	–	1.000	–
<i>C. chodatii</i>	–	–	1 (1.9)	–	1.000	–
<i>C. lambica</i>	–	–	–	1 (1.9)	–	1.000
<i>C. norvegensis</i>	–	–	–	2 (3.8)	–	1.000
<i>C. glabrata</i>	–	3 (13.6)	–	2 (3.8)	–	0.152
<i>C. krusei</i>	–	3 (13.6)	–	2 (3.8)	–	0.152
<i>C. valida</i>	–	–	–	1 (1.9)	–	1.000
<i>C. zeylanoides</i>	1 (4.5)	–	–	–	NE	–
<i>C. globosa</i>	–	–	–	1 (1.9)	–	1.000
<i>C. dubliniensis</i>	4 (18.2)	2 (9.1)	–	–	0.006 ^a	0.086
<i>C. colliculosa</i>	1 (4.5)	–	–	–	NE	–
<i>C. inconspicua</i>	–	1 (4.5)	–	–	–	NE
<i>C. rugosa</i>	–	1 (4.5)	–	–	–	NE
<i>C. tropicalis</i>	–	1 (4.5)	–	–	–	NE
Non- <i>Candida</i> yeasts	–	1 (4.5)	2 (3.8)	13 (25)	1.000	0.052
<i>Rhodotorula</i> sp.	–	1 (4.5)	1 (1.9)	8 (15.4)	1.000	0.265
<i>Saccharomyces cerevisiae</i>	–	–	–	1 (1.9)	–	1.000
<i>Trichosporon asahii</i>	–	–	–	1 (1.9)	–	1.000
<i>Schwanniomyces etchellsii</i>	–	–	–	2 (3.8)	–	1.000
<i>Hypopichia burtonii</i>	–	–	1 (1.9)	–	1.000	–
<i>Pichia fermentans</i>	–	–	–	1 (1.9)	–	1.000

Abbreviation: HIV, human immunodeficiency virus.

Note: NE: Could not be evaluated due to the low number

^aStatistically significant.

collecting and processing clinical samples used and pluralistic and ethnic differences.^{21,22} It is reported that the most sensitive method is the inoculation of concentrated oral wash water, which is used in the present study.²³

In most of the studies about the yeast colonization of the oral cavity of pediatric HIV-infected patients, *C. albicans* has been the most common species, with rates of 40 to 94.2%.^{3,5,15,18–20} Some researchers reported that the prevalence in HIV-infected children was significantly higher.^{3,15,20} Although the oral isolation of *C. albicans* was found to be higher in patients (31.8%) than in healthy children (26.9%) in the present study, this difference was not significant ($p=0.885$).

Most commonly isolated NAC species were reported as *Candida guilliermondii* (23.1%), *Candida tropicalis* (15.4%), and *Candida lusitaniae* (7.3%) by Cerqueira et al³; *C. krusei* (11.4%) and *C. parapsilosis* (7.14%) by Alves et al²⁰; *C. guilliermondii* (23%), *C. tropicalis* (17%), and *C. lusitaniae* (8%) by Pomarico et al¹⁵; *Candida glabrata* (33%), *C. kefyr* (13%), *C. lipolytica* (7%), and *C. tropicalis* (7%) by Castillo-Martínez et al.²⁴ In the present study, unlike other studies, these *Candida* species were not isolated from oral samples of HIV-infected patients. It was found that all the cultured strains were rare species, including *C. dubliniensis* (18.2%), *Candida famata*, *Candida zeylanoides*, and *Candida colliculosa*.

Table 3 Distribution of samples with mixed yeast growth according to the patients and control group

Mixed yeast cultures	HIV-infected group (n = 22)		Control group (n = 52)		p-Values	
	Oral specimen n (%)	Feces n (%)	Oral specimen n (%)	Feces n (%)	Oral specimen	Feces
<i>C. albicans</i> + NAC	2 (9.1)	2 (9.1)	2 (3.8)	6 (11.6)	0.630	1.000
<i>C. albicans</i> + <i>C. famata</i>	1 (4.5)	–	–	–	NE	–
<i>C. albicans</i> + <i>C. parapsilosis</i>	–	–	1 (1.9)	1 (1.9)	NE	NE
<i>C. albicans</i> + <i>C. chodatii</i>	–	–	1 (1.9)	–	NE	–
<i>C. albicans</i> + <i>C. dubliniensis</i>	1 (4.5)	–	–	–	NE	–
<i>C. albicans</i> + <i>C. glabrata</i>	–	1 (4.5)	–	1 (1.9)	–	NE
<i>C. albicans</i> + <i>C. kefyr</i>	–	–	–	2 (3.8)	–	NE
<i>C. albicans</i> + <i>C. krusei</i>	–	–	–	1 (1.9)	–	NE
<i>C. albicans</i> + <i>C. valida</i>	–	–	–	1 (1.9)	–	NE
<i>C. albicans</i> + <i>C. inconspicua</i>	–	1 (4.5)	–	–	–	NE
<i>C. albicans</i> + non-Candida yeast	–	–	1 (1.9)	3 (5.8)	NE	0.550
<i>C. albicans</i> + <i>Rhodotorula</i> spp.	–	–	–	2 (3.8)	–	NE
<i>C. albicans</i> + <i>T. asahii</i>	–	–	–	1 (1.9)	–	NE
<i>C. albicans</i> + <i>Hypopichia burtonii</i>	–	–	1 (1.9)	–	NE	–
NAC + non-Candida yeast	–	–	–	3 (5.8)	–	0.550
<i>C. parapsilosis</i> + <i>Rhodotorula</i> spp.	–	–	–	1 (1.9)	–	NE
<i>C. kefyr</i> + <i>Rhodotorula</i> spp.	–	–	–	1 (1.9)	–	NE
<i>C. globosa</i> + <i>Schwanniomyces etsyelsii</i>	–	–	–	1 (1.9)	–	NE
<i>C. albicans</i> + NAC + non-Candida yeast	–	1 (4.5)	–	2 (3.8)	–	1.000
<i>C. albicans</i> + <i>C. lambica</i> + <i>C. parapsilosis</i> + <i>Rhodotorula</i> spp.	–	–	–	1 (1.9)	–	NE
<i>C. albicans</i> + <i>C. kefyr</i> + <i>Saccharomyces cerevisiae</i>	–	–	–	1 (1.9)	–	NE
<i>C. albicans</i> + <i>C. glabrata</i> + <i>C. krusei</i> + <i>Rhodotorula</i> spp.	–	1 (4.5)	–	–	–	NE
NAC + NAC	1 (4.5)	2 (9.1)	–	1 (1.9)	0.297	0.209
<i>C. colliculosa</i> + <i>C. dubliniensis</i>	1 (4.5)	–	–	–	NE	–
<i>C. lusitanae</i> + <i>C. lambica</i>	–	–	–	1 (1.9)	–	NE
<i>C. rugosa</i> + <i>C. parapsilosis</i>	–	1 (4.5)	–	–	–	NE
<i>C. krusei</i> + <i>C. tropicalis</i>	–	1 (4.5)	–	–	–	NE
Total	3 (13.6)	5 (22.7)	3 (5.7)	15 (28.9)	0.354	0.776

Abbreviations: HIV, human immunodeficiency virus; NAC, non-albicans *Candida*.

Note: NE: Could not be evaluated due to the low number.

(4.5% each). Although in the present study the number of different NAC species isolated from the oral cavity of healthy children (7 species) was higher than the patients (4 species), similar to the results of the studies mentioned above, the rate of NAC species was found to be higher in HIV-infected (31.8%) than in healthy children (13.5%); however, this difference was not significant ($p = 0.102$).

Carqueira et al³ reported that *C. parapsilosis* was more prevalent in uninfected siblings of HIV-infected children.

Compatible with that study, Pomarico et al¹⁵ reported that the rate of *C. parapsilosis* was significantly higher in HIV-negative (25%) than in HIV-positive (4.6%) children. In the present study although this species was isolated from two of healthy (3.8%) but none from the HIV-positive children, there was no meaningful difference ($p = 1.000$).

C. dubliniensis is most commonly isolated from the oral cavity of HIV-positive patients with reported rates of 1.2 to 48%,²⁵ mainly in adults. The few studies in pediatric

HIV-positive patients have reported isolation rates of 0 to 17.24%.^{5,7,14,15,19,20,26–28} This yeast shows increased adherence to human buccal epithelial cells.²⁸ Also, this species can rapidly develop resistance when exposed to fluconazole, and fluconazole-resistant strains have been identified in HIV patients receiving antifungal agents.²⁹ It is often difficult to distinguish *C. dubliniensis* from *C. albicans*, which is the most frequently isolated species in routine laboratory since many phenotypic and morphological features are similar.²⁵ CAC is helpful to distinguish these two species in primary culture due to different pigmentation.^{25,30} In the present study, *C. dubliniensis* was isolated from the oral cavity of four (18.2%) HIV-positive and none from the control patients. It was found that the difference between these two groups was significant in terms of *C. dubliniensis* isolation ($p=0.006$). This is the first report of the isolation of *C. dubliniensis* from pediatric HIV-positive patients in our country. Sahand et al¹² reported CHROM-Pal medium was more sensitive in the distinction of *C. albicans* and *C. dubliniensis* than CAC with 100% accuracy. They determined 18.5% prevalence of this species in adult HIV-positive individuals. In the present study, CHROM-pal agar was used in addition to CHROM-agar and an isolation rate (18.2%) similar to that of Sahand et al was obtained.¹² However, using only CHROM-agar, Blignaut⁵ reported a similar isolation rate (17.24%). These findings indicate that, besides the medium used, geographic and population-dependent factors are also important.

Some researchers have investigated whether oral *Candida* colonization was associated with viral load or CD4 cell count, again generally in adult patients. It is thought that increased HIV-1 level may cause increased *Candida* colonization by suppressing the immune response, and that increased local HIV replication has a direct effect on *Candida* colonization.³¹ Some researchers reported that oral *Candida* colonization significantly correlated with the increase in viral load and the decrease in the number of CD4 cells.^{24,28} De Brito Costa et al³² found that oral *C. albicans* colonization correlated with a decrease in CD4 cell count in their study of HIV-positive children. In the present study, *Candida* colonization was not detected in oral samples of five HIV-positive children with CD4 count of ≤ 200 cells/mm³ but was found in 65 of those with CD4 count >200 cells/mm³. This apparent inverse relationship is likely due to the low number of patients with CD4 counts ≤ 200 cells/mm³, making statistical evaluation impossible. If oral *Candida* colonization was compared in children with viral loads of ≤ 45 and >45 copies (61.5 and 33.3%, respectively), no relationship was found between viral load and oral *Candida* colonization ($p=0.387$) in the present study.

Candida spp. are considered as gastrointestinal opportunistic pathogens and some authors blame them for the onset of diarrhea.^{33,34} Diarrhea is among the important clinical manifestations of AIDS and may cause malnutrition, growth retardation, and death especially in young children.³⁵ Most of the research on this subject has been done in adults and patients with diarrhea. Fecal *C. albicans* isolation in HIV-positive and diarrheal patients from different countries has been reported to vary between 7.6 and

39.1%.^{35,36} In the present study, the isolation rate of *C. albicans* from feces of HIV-infected children was 31.8%. However, unlike the aforementioned studies, none of the patients in the present study had diarrhea. We also noted a significant difference between patients aged ≤ 120 and ≥ 121 months in terms of *C. parapsilosis* colonization in feces, with a higher rate in the younger age group ($p=0.036$).

We found no previous reports of detection of *C. dubliniensis* in the feces of pediatric HIV patients. In the present study, although *C. dubliniensis* was not found in the feces of healthy children, it was isolated from two (9.1%) HIV-positive children ($p=0.086$). Additionally, this species was isolated from both the oral wash and the feces samples of one of these patients. There are also limited data on this subject with respect to adults. Awoyeni et al⁸ reported in their study of 154 HIV-infected patients aged 18 to 72 years that the isolation rate of *C. dubliniensis* from feces was 13.1%.

In conclusion, the presence of *C. dubliniensis* in oral and fecal samples in pediatric HIV patients was shown for the first time in Türkiye in the present study. Since this species is the most frequently isolated NAC species in Turkish patients and was found to be significantly more common in the mouths of HIV-positive children than healthy individuals, clinicians should be mindful of the risk of selecting for fluconazole-resistant *Candida* spp. while choosing antifungals as treatment or prophylaxis. We also recommend the use of chromogenic agar to detect *C. dubliniensis* in cultures for suspected fungal infection in these patients. Since *C. parapsilosis* is found to be significantly higher in feces samples of patients aged 10 years or younger, treatment should be arranged considering this species capable of forming biofilms in catheters especially in case of fungemia. Additional studies should be conducted in relation to oral and fecal *Candida* colonization in children, and additional predisposing factors should be investigated.

Funding

This work was supported by the Research Fund of Istanbul University, Project No: 26586.

Conflict of Interest

None declared.

References

- 1 UNICEF data. (2021): HIV statistics-global and regional trends. Accessed September 7, 2022 at: <https://data.unicef.org/topic/hiv/aids/global-regional-trends>
- 2 Türkiye HIV/AIDS Control Program (2019–2024). Turkish Ministry of Health Publication; 2019;1131, Ankara
- 3 Cerqueira DF, Portela MB, Pomarico L, de Araújo Soares RM, de Souza IPR, Castro GF. Oral *Candida* colonization and its relation with predisposing factors in HIV-infected children and their uninfected siblings in Brazil: the era of highly active antiretroviral therapy. *J Oral Pathol Med* 2010;39(02):188–194
- 4 Costa CR, Cohen AJ, Fernandes OF, et al. Asymptomatic oral carriage of *Candida* species in HIV-infected patients in the highly active antiretroviral therapy era. *Rev Inst Med Trop São Paulo* 2006;48(05):257–261

- 5 Blignaut E. Oral candidiasis and oral yeast carriage among institutionalised South African paediatric HIV/AIDS patients. *Mycopathologia* 2007;163(02):67–73
- 6 Scerpella EG, Gould SS, Mathewson JJ, DuPont HL, Dupont LH. Methods for detection of an intestinal secretory immunoglobulin A response to *Candida* spp. and their preliminary application in human immunodeficiency virus-infected patients with chronic diarrhea. *Clin Diagn Lab Immunol* 1995;2(02):246–248
- 7 Domaneschi C, Massarente DB, de Freitas RS, et al. Oral colonization by *Candida* species in AIDS pediatric patients. *Oral Dis* 2011;17(04):393–398
- 8 Awoyeni A, Olaniran O, Odetoyin B, et al. Isolation and evaluation of *Candida* species and their association with CD4⁺ T cells counts in HIV patients with diarrhoea. *Afr Health Sci* 2017;17(02):322–329
- 9 Tooyama H, Matsumoto T, Hayashi K, et al. *Candida* concentrations determined following concentrated oral rinse culture reflect clinical oral signs. *BMC Oral Health* 2015;15:150
- 10 Esebelahie NO, Enweani IB, Omoregie R. *Candida* colonisation in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria. *Libyan J Med* 2013;8(01):20322
- 11 White PL, Williams DW, Kuriyama T, Samad SA, Lewis MA, Barnes RA. Detection of *Candida* in concentrated oral rinse cultures by real-time PCR. *J Clin Microbiol* 2004;42(05):2101–2107
- 12 Sahand IH, Maza JL, Eraso E, et al. Evaluation of CHROM-Pal medium for the isolation and direct identification of *Candida dubliniensis* in primary cultures from the oral cavity. *J Med Microbiol* 2009;58(Pt 11):1437–1442
- 13 Walsh TH, Hayden RT, Larone DH. *Larone's Medically Important Fungi: A Guide to Identification*. Washington DC: ASM Press; 2018.
- 14 Pongsiriwet S, Iamaroon A, Kanjanavanit S, Pattanaporn K, Krisanaprakornkit S. Oral lesions and dental caries status in perinatally HIV-infected children in Northern Thailand. *Int J Paediatr Dent* 2003;13(03):180–185
- 15 Pomarico L, Cerqueira DF, de Araujo Soares RM, et al. Associations among the use of highly active antiretroviral therapy, oral candidiasis, oral *Candida* species and salivary immunoglobulin A in HIV-infected children. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;108(02):203–210
- 16 Leibovitz E, Rigaud M, Chandwani S, et al. Disseminated fungal infections in children infected with human immunodeficiency virus. *Pediatr Infect Dis J* 1991;10(12):888–894
- 17 Chiou CC, Groll AH, Gonzalez CE, et al. Esophageal candidiasis in pediatric acquired immunodeficiency syndrome: clinical manifestations and risk factors. *Pediatr Infect Dis J* 2000;19(08):729–734
- 18 Pongsiriwet S, Iamaroon A, Sriburee P, Pattanaporn K, Krisanaprakornkit S. Oral colonization of *Candida* species in perinatally HIV-infected children in northern Thailand. *J Oral Sci* 2004;46(02):101–105
- 19 Jabra-Rizk MA, Falkler WA Jr, Enwonwu CO, Onwujekwe DI Jr, Merz WG, Meiller TF. Prevalence of yeast among children in Nigeria and the United States. *Oral Microbiol Immunol* 2001;16(06):383–385
- 20 Alves TP, Simões AC, Soares RM, Moreno DS, Portela MB, Castro GF. Salivary lactoferrin in HIV-infected children: correlation with *Candida albicans* carriage, oral manifestations, HIV infection and its antifungal activity. *Arch Oral Biol* 2014;59(08):775–782
- 21 Erköse G, Erturan Z. Oral *Candida* colonization of human immunodeficiency virus infected subjects in Türkiye and its relation with viral load and CD4⁺ T-lymphocyte count. *Mycoses* 2007;50(06):485–490
- 22 Mushi MF, Bader O, Taverne-Ghadwal L, Bii C, Groß U, Mshana SE. Oral candidiasis among African human immunodeficiency virus-infected individuals: 10 years of systematic review and meta-analysis from sub-Saharan Africa. *J Oral Microbiol* 2017;9(01):1317579
- 23 Samaranayake LP, MacFarlane TW, Lamey PJ, Ferguson MM. A comparison of oral rinse and imprint sampling techniques for the detection of yeast, coliform and *Staphylococcus aureus* carriage in the oral cavity. *J Oral Pathol* 1986;15(07):386–388
- 24 Castillo-Martínez NA, Mouriño-Pérez RR, Cornejo-Bravo JM, Gaitán-Cepeda LA. [Factors related to oral candidiasis in HIV children and adolescents, species characterization and antifungal susceptibility]. *Rev Chilena Infectol* 2018;35(04):377–385
- 25 Loreto ES, Scheid LA, Nogueira CW, Zeni G, Santurio JM, Alves SH. *Candida dubliniensis*: epidemiology and phenotypic methods for identification. *Mycopathologia* 2010;169(06):431–443
- 26 Sano A, Vilela MM, Takahashi I, et al. Isolation of *Candida dubliniensis* from the oral cavity of an HIV-positive child in Brazil. *Nippon Ishinkin Gakkai Zasshi* 2000;41(03):177–181
- 27 Brown DM, Jabra-Rizk MA, Falkler WA Jr, Baqui AA, Meiller TF. Identification of *Candida dubliniensis* in a study of HIV-seropositive pediatric dental patients. *Pediatr Dent* 2000;22(03):234–238
- 28 Portela MB, Souza IP, Costa EM, Hagler AN, Soares RM, Santos AL. Differential recovery of *Candida* species from subgingival sites in human immunodeficiency virus-positive and healthy children from Rio de Janeiro, Brazil. *J Clin Microbiol* 2004;42(12):5925–5927
- 29 Perea S, López-Ribot JL, Wickes BL, et al. Molecular mechanisms of fluconazole resistance in *Candida dubliniensis* isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis. *Antimicrob Agents Chemother* 2002;46(06):1695–1703
- 30 Jabra-Rizk MA, Brenner TM, Romagnoli M, et al. Evaluation of a reformulated CHROMagar *Candida*. *J Clin Microbiol* 2001;39(05):2015–2016
- 31 Klein RS, Arnsten JH, Sobel JD. Oropharyngeal *Candida* colonization and human immunodeficiency virus type 1 infection. *J Infect Dis* 2000;181(02):812–813
- 32 de Brito Costa EM, dos Santos AL, Cardoso AS, et al. Heterogeneity of metallo and serine extracellular proteinases in oral clinical isolates of *Candida albicans* in HIV-positive and healthy children from Rio de Janeiro, Brazil. *FEMS Immunol Med Microbiol* 2003;38(02):173–180
- 33 Koffi-Akoua G, Ferly-Therizol M, Kouassi-Beugre MT, et al. [Cryptosporidium and candida in pediatric diarrhea in Abidjan]. *Bull Soc Pathol Exot* 1989;82(04):451–457
- 34 Chaudhury A, Nath G, Shukla B, Panda S, Singh TB. Diarrhoea associated with *Candida* spp.: incidence and seasonal variation. *J Diarrhoeal Dis Res* 1996;14(02):110–112
- 35 Rossit AR, de Almeida MT, Nogueira CA, et al. Bacterial, yeast, parasitic, and viral enteropathogens in HIV-infected children from São Paulo State, Southeastern Brazil. *Diagn Microbiol Infect Dis* 2007;57(01):59–66
- 36 Samé-Ekobo A, Lohoué J, Mbassi A. [A clinical and biological study of parasitic and fungal diarrhea in immunosuppressed patients in an urban and suburban area of Yaoundé]. *Sante* 1997;7(06):349–354