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Article

Prevalence and Antifungal Resistance of *Candida* Species in HIV-Positive Patients with Diarrhea: A Descriptive Cross-Sectional Study at a Single-Center in Ecuador

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Abstract

Background: *Candida* species are opportunistic fungal pathogens that frequently colonize the gastrointestinal tract of immunocompromised individuals, including people living with HIV (PLHIV). Gastrointestinal *Candida* colonization has been associated with persistent diarrhea and poor health outcomes. Despite the high burden of HIV/AIDS in Ecuador, there is limited research on *Candida* prevalence and antifungal resistance patterns in this population. This study aimed to determine the prevalence of *Candida* species in HIV-positive individuals with diarrhea and assess associations with clinical and immunological parameters. **Methods:** A descriptive, cross-sectional study was conducted at the José Daniel Rodríguez Infectious Hospital in Guayaquil, Ecuador, between April 2021 and May 2022. The study included 85 HIV-positive adults with diarrhea, categorized into those with and without *Candida* colonization. Fecal samples were analyzed using culture, microscopy (Gram staining, SEM, immunofluorescence), Sabouraud Glucose Agar and CHROMagar™ *Candida* for species identification. Antifungal susceptibility testing was performed using the VITEK® 2 Compact system following EUCAST guidelines. Due to the small sample size, only descriptive statistical analyses were performed. **Results:** The *Candida* species were detected in 64 of 85 patients (75.3%), with *Candida albicans* being the most prevalent species. *Candida* colonization was associated with severely low CD4 counts (<200 cells/μL, 71.0%), high viral loads (≥10,000 copies/mL, 46.6%), and poor ART adherence (55.3%). Antifungal susceptibility testing showed 94.5% fluconazole resistance (SEN1), while all isolates remained 100% sensitive to caspofungin (SEN4, SEN5, SEN6). **Conclusions:** This study highlights a high prevalence of *Candida* colonization in HIV-positive individuals with diarrhea, particularly those with advanced immunosuppression and poor ART adherence. The high levels of fluconazole resistance raise concerns about the efficacy of current antifungal treatments, suggesting a need for routine antifungal susceptibility testing. Given the study's descriptive nature and single-site design, further research with larger, multicentric cohorts is needed to improve clinical management and antifungal stewardship.

Keywords: HIV; *Candida* colonization; diarrhea; antifungal resistance; fluconazole resistance; ART adherence; immunosuppression; Ecuador

1. Introduction

The human immunodeficiency virus (HIV) remains a major global health challenge, with over 39 million people living with the virus and 1.3 million new infections annually [1–3]. A defining characteristic of HIV progression is the depletion of CD4+ T lymphocytes, leading to immunosuppression and an increased risk of opportunistic infections. Among these, fungal infections, particularly those caused by *Candida* species, are some of the most frequent and clinically significant complications in people living with HIV (PLHIV) [4–8]. *Candida* species are commensal fungi that under normal conditions inhabit the gastrointestinal tract without causing harm. However, in immunosuppressed individuals, they can overgrow and cause localized or systemic infections, further compromising patient health. Chronic diarrhea is a common symptom in PLHIV, and the presence of *Candida* in the gut has been associated with intestinal dysbiosis, malabsorption, systemic inflammation, and reduced antiretroviral therapy (ART) efficacy [9–12]. Despite its clinical relevance, gastrointestinal *Candida* colonization remains an underexplored area in HIV research, particularly in Latin America.

While *Candida albicans* is the most frequently isolated species in HIV/AIDS patients, recent studies have highlighted an increasing prevalence of non-*albicans* *Candida* species, such as *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*, many of which exhibit antifungal resistance, particularly to fluconazole [7,13,14]. The rise of drug-resistant *Candida* species poses a major challenge in clinical management, as treatment options become limited. Furthermore, *Saccharomyces cerevisiae*, a yeast widely used in probiotics and food production, has also been identified as an opportunistic pathogen in immunocompromised patients, indicating a potential link between intestinal yeast colonization and systemic fungal infections [15]. These findings underscore the need for robust epidemiological surveillance and improved diagnostic approaches to detect and characterize *Candida* species in PLHIV, particularly in resource-limited settings where treatment options are constrained.

The epidemiology of *Candida* infections varies across geographic regions, reflecting differences in environmental factors, healthcare access, and antifungal use. Studies have reported *Candida albicans* prevalence rates of 82.2% in China, 74.5% in Brazil, and 36% in Spain, while non-*albicans* *Candida* species predominate in regions such as Peru, where *C. tropicalis* accounts for 67.4% of cases [16–18]. In Ecuador, *C. albicans* represents 52.2% of isolates, followed by *C. tropicalis* (10.8%) and *C. glabrata* (4.2%), revealing a distinct species distribution compared to other Latin American countries [19,20]. Despite these findings, there is limited research on the role of *Candida* in HIV-related gastrointestinal infections in Ecuador. Given the high burden of HIV in certain urban and rural areas of the country, and the frequent lack of access to second-line antifungal treatments, understanding the prevalence and resistance patterns of *Candida* species in this population is essential for improving patient outcomes.

Most studies on *Candida* infections in PLHIV rely on conventional culture-based methods, which may fail to detect species diversity and antifungal resistance patterns. The integration of advanced molecular sequencing techniques, such as Illumina-NGS and ITS metagenomic analysis, provides a more comprehensive approach for identifying *Candida* species and detecting emerging resistance mechanisms [12,21]. This study aims to determine the frequency and distribution of *Candida* species in fecal samples from HIV/AIDS patients with chronic diarrhea, analyze the clinical, sociodemographic, and laboratory characteristics associated with *Candida* presence, and evaluate antifungal susceptibility profiles using a combination of culture-based methods, molecular sequencing, and microscopy. This is a single-site study conducted at the José Daniel Rodríguez Infectious Hospital in Guayaquil, Ecuador, which allows for an in-depth analysis of the population within this clinical setting. While the findings will provide important epidemiological insights, they may not be generalizable to all regions of Ecuador or to broader Latin American populations.

By identifying species-specific trends, risk factors, and antifungal resistance patterns, this study will contribute valuable data to guide diagnostic strategies, clinical management, and public health policies for opportunistic fungal infections in PLHIV. Given Ecuador’s unique epidemiological landscape, the limited access to advanced diagnostics, and the growing concerns over antifungal

resistance, this research represents a crucial step toward strengthening the country's response to HIV-related fungal infections.

2. Methods

2.1. Study Design and Setting

This study is a descriptive, cross-sectional analysis conducted at the José Daniel Rodríguez Infectious Hospital in Guayaquil, Ecuador, a specialized referral center for infectious diseases, including HIV/AIDS management. Data collection took place between April 2021 and May 2022. The study examines demographic, clinical, laboratory, lifestyle, and antifungal resistance characteristics associated with *Candida* presence in fecal samples from people living with HIV (PLHIV) who presented with chronic diarrhea.

2.2. Study Population

The study population consisted of HIV-positive adults aged 18 years or older with a confirmed HIV diagnosis and symptoms of diarrhea at the time of recruitment. Patients who had received antibiotics or antifungals within the last 30 days, had other confirmed causes of diarrhea unrelated to *Candida*, or were unable to provide informed consent were excluded. A total of 92 patients were initially enrolled, with 85 meeting the final inclusion criteria.

2.3. Data Collection and Laboratory Analysis

Clinical, sociodemographic, and lifestyle data were collected through structured interviews and medical record reviews. Additionally, we used a data collection form specifically developed for this study to systematically record general clinical, sociodemographic, and lifestyle characteristics, as well as detailed laboratory results from each participant (see Supplementary File). Fecal samples were obtained in sterile containers and stored at 4°C before being transported to the National Institute for Public Health and Research (INSPI) for processing.

2.4. Microbiological analysis of *Candida* spores

Several laboratory techniques were employed to detect and characterize *Candida* species. Gram staining was performed by heat-fixing fecal smears, staining them with gentian violet, applying Lugol's iodine, decolorizing with alcohol, and counterstaining with safranin. Microscopic examination at 1000x magnification allowed for the identification of *Candida* spores. Fig. 1

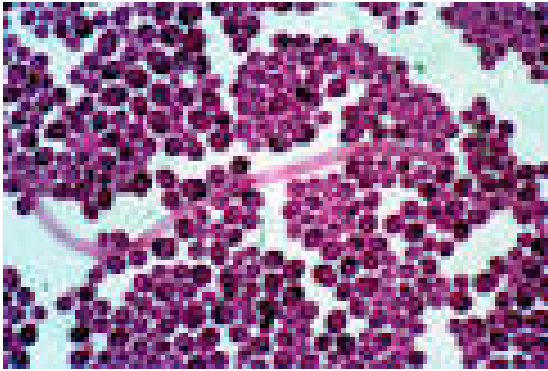


Figure 1. Purple *Candida* spores with Gram stain observed by optical microscopy at 1000x magnification.

Immunofluorescence microscopy with Uvitex 2A and Rylux BA Calcofluor white a 488 nm filter and 1000x magnification was used to identify *Candida* spores. Fig. 2

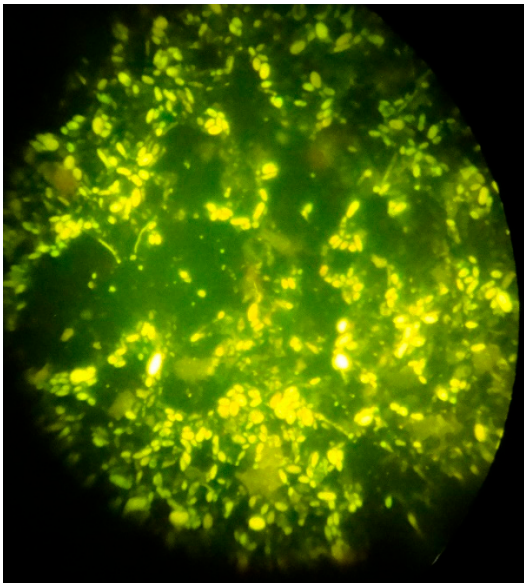


Figure 2. Photo of *Candida albicans* structures under fluorescence microscopy stained with Calcofluor white, bright green oval structures.

Another selective culture medium used is Sabouraud Dextrose Agar, for the isolation of mycelial fungi and yeasts from clinical samples. In this case, a stool sample was cultured. Its slightly acidic pH and the addition of chloramphenicol inhibit the growth of many bacterial species. Creamy white, soft, and smooth *Candida* colonies are observed in the culture medium. Fig. 3

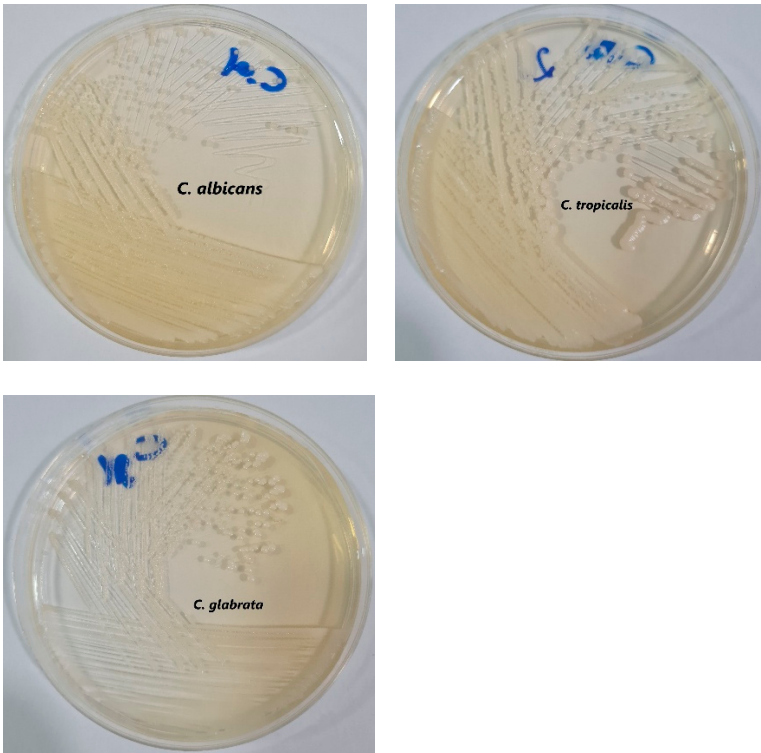


Figure 3. *Candida albicans*, *tropicalis* and *glabrata* on Sabouraud-Dextrose agar at 48h at 37°C.

CHROMagar™ *Candida* Plus was used because it is a selective chromogenic culture medium for the direct qualitative detection, visual differentiation and presumptive identification of *Candida* species by color change with a high degree of specificity and sensitivity. The color they take indicates

the species to which they correspond. Thus, if the colony turns bluish purple, it is *Candida tropicalis*, if it is apple green it is *Candida albicans*, and if it is pale pink, it is *Candida glabrata*. Fig 4

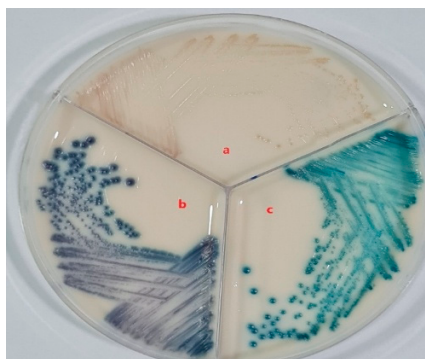


Figure 4. Differentiation of the main clinically significant *Candida* species; a) *glabrata*, b) *tropicalis* and c) *albicans* culture in CHROMagar™.

In addition, scanning electron microscopy was used, which allows the study of cell surfaces. The image is obtained by observing the sample with an ultra-fine electron beam. The generated signals are collected, amplified and captured in a cathode ray tube, and real three-dimensional images of the external structures of the *Candida* species can be observed. Fig 5

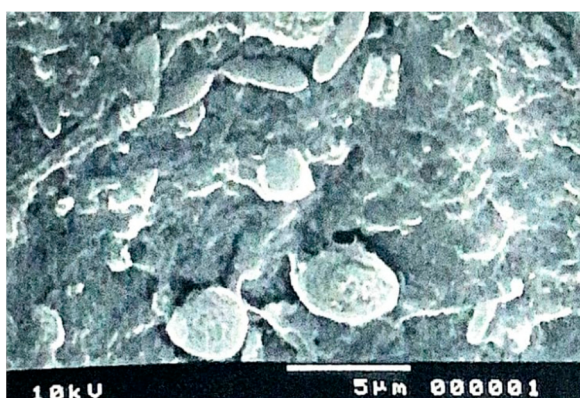


Figure 5. SEM Scanning electron microscope photo of candidiasis cell structures.

Molecular identification of *Candida* species was performed using Illumina-NGS sequencing with ITS metagenomic analysis. This technology is used to determine the order of nucleotides in entire genomes or in specific regions of DNA or RNA. NGS has revolutionized the biological sciences, allowing laboratories to perform a wide variety of applications and study biological systems at an unprecedented level. Based on the results of taxonomic annotation, the major taxa of each sample or group are displayed at each taxonomic rank (Phylum, Class, Order, Family, Genus, Species) using a relative abundance distribution histogram. Fig. 6

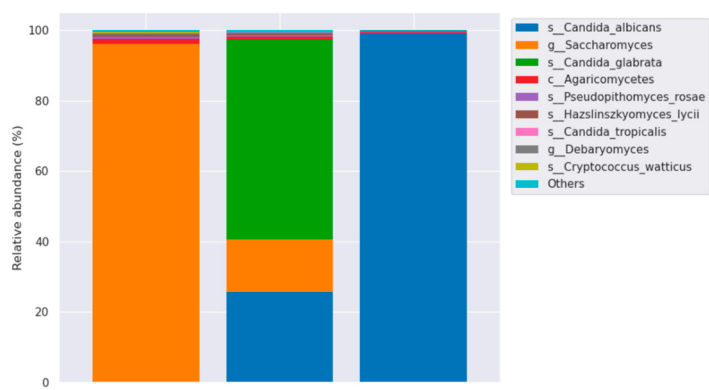


Figure 6. Molecular identification was performed using Illumina-NGS sequencing with ITS metagenomic analysis. Relative abundance distribution histogram.

2.5. Antifungal Susceptibility Testing

To determine antifungal resistance patterns, susceptibility testing was performed using the VITEK® 2 Compact BioMérieux automated system, following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Minimum inhibitory concentrations (MICs) were determined for fluconazole, itraconazole, voriconazole, amphotericin B, and caspofungin.

Isolates were classified as susceptible, intermediate, or resistant based on EUCAST breakpoints. Resistance was further assessed based on species-specific thresholds, with particular attention to *C. glabrata*, *C. tropicalis*, and *C. krusei*, which have shown higher fluconazole resistance rates in previous studies. The prevalence of resistant strains was compared between different *Candida* species and among patients with varying levels of immunosuppression.

2.6. Variables

The primary outcome variable was *Candida* presence, categorized as "No Presence" or "Active Presence", based on laboratory results. Subgroup analyses were conducted for the detection of *Candida albicans*, *Candida tropicalis*, and *Candida glabrata*.

Predictor variables included demographic characteristics (age, sex, city of residence, marital status, and education level), clinical characteristics (diarrhea type, adherence to ART, CD4 count, and viral load), and lifestyle factors (substance use).

Antifungal resistance variables included susceptibility status for fluconazole, itraconazole, voriconazole, amphotericin B, and caspofungin, classified as susceptible, or resistant based on EUCAST breakpoints.

2.7. Statistical Analysis

Descriptive statistics were used to summarize demographic, clinical, laboratory, and antifungal resistance data. Categorical variables were reported as frequencies and percentages, while continuous variables were described using means and standard deviations.

Comparisons were made between patients with *Candida* presence ("No Presence" vs. "Active Presence") and between different *Candida* species. The proportion of resistant isolates was evaluated across different species, and the association between resistance patterns and CD4 count was explored descriptively.

This study did not perform inferential statistical testing, as its primary aim was to describe prevalence, species distribution, antifungal resistance, and clinical associations within the study population.

2.8. Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Ethics Committee of Hospital Luís Vernaza in Guayaquil, Ecuador (HLV-CEISH-2021-011). Written informed consent was obtained from all participants before inclusion in the study. Strict confidentiality measures were implemented to ensure participant privacy.

3. Results

A total of 85 patients living with HIV and presenting with diarrhea were included in the study. The presence of *Candida* species was detected in 64 patients (75.3%), while 21 patients (24.7%) showed no evidence of *Candida* colonization. The demographic, clinical, and laboratory characteristics of the study population, stratified by *Candida* presence, are summarized in Table 1.

Table 1. Descriptive Characteristics of the Study Population, Stratified by *Candida* Presence.

Category	No Presence (n=21)	Active Presence (n=64)	Total (n=85)
Demographics			
Sex			
Female	4 (17.4%)	15 (24.2%)	19 (22.4%)
Male	19 (82.6%)	47 (75.8%)	66 (77.6%)
Age			
18-39	12 (50.0%)	27 (43.5%)	39 (45.9%)
40-59	11 (45.8%)	30 (48.4%)	41 (48.2%)
60+	1 (4.2%)	5 (8.1%)	6 (7.1%)
City of Residence			
Babahoyo	1 (4.3%)	0 (0.0%)	1 (1.2%)
Bahia de Caraquez	0 (0.0%)	1 (1.6%)	1 (1.2%)
Daule	0 (0.0%)	1 (1.6%)	1 (1.2%)
Duran	2 (8.7%)	5 (8.1%)	7 (8.2%)
Esmeraldas	0 (0.0%)	5 (8.1%)	5 (5.9%)
Guayaquil	14 (60.9%)	38 (61.3%)	52 (61.2%)
Lago Agrio	1 (4.3%)	0 (0.0%)	1 (1.2%)
Machala	1 (4.3%)	1 (1.6%)	2 (2.4%)
Milagro	1 (4.3%)	3 (4.8%)	4 (4.7%)
Nobol	0 (0.0%)	1 (1.6%)	1 (1.2%)
Playas	0 (0.0%)	1 (1.6%)	1 (1.2%)
Puerto Cayo	1 (4.3%)	0 (0.0%)	1 (1.2%)
Quevedo	1 (4.3%)	0 (0.0%)	1 (1.2%)
Riobamba	0 (0.0%)	3 (4.8%)	3 (3.5%)
Salitre	0 (0.0%)	1 (1.6%)	1 (1.2%)
Ventanas	0 (0.0%)	2 (3.2%)	2 (2.4%)
Yaguachi	1 (4.3%)	0 (0.0%)	1 (1.2%)
Marital Status (Married)			
No	9 (37.5%)	15 (24.2%)	24 (28.2%)
Yes	15 (62.5%)	47 (75.8%)	62 (72.9%)
Education Level			
Primary school	17 (73.9%)	37 (59.7%)	54 (63.5%)
High school	5 (21.7%)	23 (37.1%)	28 (32.9%)
Higher Education	1 (4.3%)	2 (3.2%)	3 (3.5%)
Clinical Characteristics			
Type of Diarrhea			
Acute	1 (4.3%)	2 (3.2%)	3 (3.5%)
Chronic	22 (95.7%)	60 (96.8%)	82 (96.5%)
Adherence			
Abandoned	10 (43.5%)	37 (59.7%)	47 (55.3%)
Continuous	5 (21.7%)	12 (19.4%)	17 (20.0%)
Started	8 (34.8%)	13 (21.0%)	21 (24.7%)
CD4			
Severely Low (<200)	15 (62.5%)	44 (71.0%)	59 (69.4%)

Moderate (200–349)	4 (16.7%)	8 (12.9%)	12 (14.1%)
Normal/High (≥350)	5 (20.8%)	10 (16.1%)	15 (17.6%)
Viral Load			
Undetectable/Low (<1,000 copies/mL)	15 (62.5%)	31 (53.4%)	46 (54.1%)
High/Very High (≥10,000 copies/mL)	9 (37.5%)	27 (46.6%)	36 (42.4%)
Laboratory Results			
Yeasts			
Negative	23 (100.0%)	2 (3.2%)	25 (29.4%)
+	0 (0.0%)	35 (56.5%)	35 (41.2%)
++	0 (0.0%)	20 (32.3%)	20 (23.5%)
+++	0 (0.0%)	5 (8.1%)	5 (5.9%)
Saccharomyces			
Negative	23 (100.0%)	46 (74.2%)	69 (81.2%)
Positive	0 (0.0%)	16 (25.8%)	16 (18.8%)
Cryptococcus			
Negative	23 (100.0%)	61 (98.4%)	84 (98.8%)
Positive	0 (0.0%)	1 (1.6%)	1 (1.2%)
Paras1			
Negative	21 (91.3%)	57 (91.9%)	78 (91.8%)
Positive	2 (8.7%)	5 (8.1%)	7 (8.2%)
Coli			
Negative	23 (100.0%)	57 (91.9%)	80 (94.1%)
Positive	0 (0.0%)	5 (8.1%)	5 (5.9%)
Giardia			
Negative	21 (91.3%)	61 (98.4%)	82 (96.5%)
Positive	2 (8.7%)	1 (1.6%)	3 (3.5%)
Diagnostic Techniques			
Immunofluorescence			
Negative	20 (87.0%)	49 (79.0%)	69 (81.2%)
Low positivity	3 (13.0%)	11 (17.7%)	14 (16.5%)
Moderate positivity	0 (0.0%)	2 (3.2%)	2 (2.4%)
TEM Microscopy			
Negative	20 (87.0%)	49 (79.0%)	69 (81.2%)
Low positivity	3 (13.0%)	11 (17.7%)	14 (16.5%)
Moderate positivity	0 (0.0%)	1 (1.6%)	1 (1.2%)
High positivity	0 (0.0%)	1 (1.6%)	1 (1.2%)
Gram chromotropic			
Negative	20 (87.0%)	49 (79.0%)	69 (81.2%)
Positive	3 (13.0%)	13 (21.0%)	16 (18.8%)
Chromagar			
Negative	23 (100.0%)	49 (79.0%)	72 (84.7%)
Positive	0 (0.0%)	13 (21.0%)	13 (15.3%)
Sabouraud			
Negative	23 (100.0%)	46 (74.2%)	69 (81.2%)
Positive	0 (0.0%)	16 (25.8%)	16 (18.8%)
Sequencing			
Negative		23 (100.0%)	23 (27.1%)
Positive	0 (0.0%)	31 (50.0%)	31 (36.5%)
Antifungal Sensitivity			
SEN1 (Fluconazole)			
Sensitive	–	4 (6.3%)	4 (4.7%)
Resistant	–	60 (93.7%)	60 (70.6%)
SEN2 (Itraconazole)			
Sensitive	–	57 (89.1%)	57 (67.1%)
Resistant	–	7 (10.9%)	7 (8.2%)
SEN3 (Voriconazole)			
Sensitive	–	8 (12.5%)	8 (9.4%)
Resistant	–	56 (87.5%)	56 (65.9%)
SEN4 (Amphotericin B)			

Sensitive	–	64 (100.0%)	64 (75.3%)
Resistant	–	0 (0.0%)	0 (0.0%)
SEN5 (Caspofungin)			
Sensitive	–	64 (100.0%)	64 (75.3%)
Resistant	–	0 (0.0%)	0 (0.0%)
<u>Lifestyle</u>			
Any Substance Use			0 (0.0%)
No	13 (54.2%)		13 (15.3%)
Yes	11 (45.8%)		11 (12.9%)

Demographic and Socioeconomic Characteristics

The study population was predominantly male (77.6%), with no significant difference between those with and without *Candida* presence (75.8% vs. 82.6%, respectively). The mean age was 40.8 years (SD: 10.6), with a slightly higher mean in those with *Candida* (41.2 years) compared to those without (39.6 years).

Most participants were from Guayaquil (61.2%), followed by smaller proportions from cities such as Durán (8.2%), Esmeraldas (5.9%), and Milagro (4.7%). The majority reported low economic status (100%), and 72.9% had never been married. Educational attainment was low, with 63.5% having only primary education, and only 3.5% having higher education.

Clinical Characteristics

Most participants had chronic diarrhea (96.5%), with no significant difference between groups. Antiretroviral therapy (ART) adherence was poor, with 55.3% of patients having abandoned treatment and only 20% maintaining continuous adherence.

In terms of immune status, 68.6% of patients had severely low CD4 counts (<200 cells/ μ L), with a higher proportion in the *Candida*-positive group (71.0%) compared to the *Candida*-negative group (62.5%). A high viral load ($\geq$ 10,000 copies/mL) was observed in 43.9% of patients, with a slightly higher prevalence in those with *Candida* (46.6%) compared to those without (37.5%).

Laboratory Findings

Microscopy and culture-based detection methods identified a range of *Candida* species. The presence of yeasts was detected in 70.6% of samples, with 41.2% showing low yeast presence (+), 23.5% moderate (++), and 5.9% high (+++).

Other fungal organisms, such as *Saccharomyces* spp., were found in 18.8% of patients, while *Cryptococcus* spp. was identified in only 1.2% of cases. Parasitic infections were rare, with *Coli* (5.9%) and *Giardia* spp. (3.5%) being the most frequently identified parasites.

Antifungal Susceptibility

Antifungal susceptibility testing revealed high levels of resistance to fluconazole, with 93.7% of active isolates classified as resistant (SEN1). Itraconazole (SEN2) showed lower resistance, with only 10.9% of isolates classified as resistant and 89.1% as sensitive. Voriconazole (SEN3) had a resistance rate of 87.5%. In contrast, all isolates were fully sensitive (100.0%) to amphotericin B (SEN4) and caspofungin (SEN5), with no resistant isolates detected for these antifungals.

Lifestyle Factors

Substance use was common, with 48.8% of participants reporting any substance use, though there was no significant difference between *Candida*-positive and *Candida*-negative groups.

4. Discussion

The present study investigated the prevalence of *Candida* species in HIV-positive patients presenting with diarrhea, revealing that *Candida* colonization was present in 75.3% of the participants. This finding underscores the high burden of fungal infections in immunocompromised individuals, particularly in resource-limited settings such as Ecuador. The study also identified significant associations between *Candida* presence and key clinical parameters, including low CD4 counts, high viral loads, and poor adherence to ART. These results align with previous studies, which have

demonstrated that *Candida* species frequently colonize the gastrointestinal tract of HIV/AIDS patients and contribute to persistent diarrhea and other gastrointestinal complications [8,11].

The high prevalence of *Candida* in this cohort aligns with prior studies that have documented increased fungal colonization in HIV-infected populations, particularly in individuals with advanced immunosuppression (Maheshwari et al., 2016; Zuza-Alves et al., 2017). Previous research has consistently shown that CD4 counts below 200 cells/ μ L are a critical risk factor for opportunistic infections, including fungal pathogens. The present study supports this observation, with a significantly higher proportion of patients with *Candida* falling into the severely low CD4 category (<200 cells/ μ L, 71.0%).

Moreover, the observed fluconazole resistance in *Candida* species is concerning and mirrors trends reported in other studies (Wang et al., 2021; Usher, 2024). 94.5% resistance in SEN1 and 87.3% resistance in SEN3 suggest that fluconazole monotherapy may no longer be a viable option for treating fungal infections in this population. These findings are consistent with previous reports indicating an increasing prevalence of fluconazole-resistant *Candida glabrata* and *Candida tropicalis*, particularly in Latin America [16,18]. The universal sensitivity to caspofungin (SEN4, SEN5, SEN6) suggests that echinocandins remain an effective therapeutic option, supporting existing guidelines for the management of invasive candidiasis [12].

The association between high viral loads and *Candida* colonization (46.6% vs. 37.5%) is also consistent with literature emphasizing that poor HIV viral suppression increases susceptibility to fungal infections (Awoyeni et al., 2017). The link between high viral loads and *Candida* colonization suggests that immune restoration through ART adherence is critical in reducing fungal burden. However, 55.3% of participants had abandoned ART, highlighting a significant public health concern. Studies have previously shown that ART nonadherence leads to increased risk of opportunistic infections and higher mortality rates in HIV/AIDS patients [5,10].

Study Limitations

Despite its contributions, this study has certain limitations. First, it is a single-site study, conducted at the José Daniel Rodríguez Infectious Hospital in Guayaquil, Ecuador. As such, the findings may not be fully generalizable to the broader HIV-positive population in Ecuador or other Latin American regions. A larger, multicentric study would provide a more comprehensive epidemiological profile. Additionally, the sample size of 85 participants, while informative, limits the statistical power to detect smaller associations.

This study is purely descriptive due to the small sample size, which limits the ability to perform inferential statistical analyses. With only 85 participants, the study does not have sufficient statistical power to establish causal relationships or to conduct multivariate analyses that adjust for potential confounders. As a result, while strong associations between *Candida* colonization and key clinical parameters were observed, these findings should be interpreted with caution. Future studies with larger sample sizes and advanced statistical modeling are necessary to confirm these associations and explore potential confounding factors.

Another limitation is that fungal species identification was based on culture and microscopy, which, while robust, may have missed certain non-culturable or emerging fungal pathogens that could have been detected using molecular techniques such as ITS metagenomics sequencing [17]. Given the emergence of cryptic and resistant fungal species, future studies should incorporate next-generation sequencing to improve diagnostic accuracy.

Lastly, while antifungal susceptibility testing was conducted, the mechanisms of resistance were not investigated. Molecular analysis of resistance genes, such as ERG11 mutations associated with fluconazole resistance in *Candida albicans*, would provide valuable insights into the evolving epidemiology of antifungal resistance in Ecuador [11,13].

Implications and Future Directions

These findings have several important clinical and public health implications. The high prevalence of *Candida* and its association with poor ART adherence and advanced immunosuppression highlight the need for enhanced patient education and adherence support

programs to reduce the burden of opportunistic infections. Health authorities should implement targeted interventions, such as routine fungal screening in HIV/AIDS patients with diarrhea, to facilitate early detection and treatment [15]. Additionally, the high fluconazole resistance suggests an urgent need to revise treatment guidelines and incorporate routine antifungal susceptibility testing in clinical practice. In Ecuador and other resource-limited settings, access to alternative antifungals such as echinocandins is limited, necessitating improved antifungal stewardship programs to ensure appropriate prescribing practices [12]. Given the very small sample size, this study is descriptive in nature and should be interpreted with caution. Future research should focus on expanding this study to multiple clinical sites to increase generalizability. Additionally, molecular analyses of antifungal resistance genes could provide deeper insights into resistance mechanisms, informing more targeted therapeutic strategies [16]. Given the emerging role of non-*Candida* species in immunocompromised populations, future studies should also consider next-generation sequencing approaches to identify a broader spectrum of fungal pathogens.

5. Conclusion

This study provides critical insights into the epidemiology of *Candida* colonization in HIV-positive patients with diarrhea, demonstrating a high prevalence, strong associations with immune suppression, and concerning antifungal resistance patterns. While the findings reinforce known risk factors, they also highlight urgent gaps in clinical management, particularly regarding fluconazole resistance and ART adherence. Addressing these gaps through better diagnostic practices, antifungal stewardship, and patient-centered interventions is essential to improving outcomes in this vulnerable population.

Ethics approval and consent to participate: This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Hospital Luis Vernaza in Guayaquil, Ecuador (HLV-CEISH-2021-011). Written informed consent was obtained from all participants before inclusion in the study.

Clinical Trial: Not applicable. This is not a clinical trial.

Consent for publication: Not Applicable.

Availability of data and material: The dataset generated and analyzed during the current study, which includes de-identified clinical and microbiological information from individuals living with HIV, has been deposited in both the NCBI Sequence Read Archive (SRA) and the Open Science Framework (OSF). To ensure participant confidentiality, all data have been fully anonymized. The dataset is accessible through the NCBI SRA under BioProject accession number PRJNA1251125 (<https://www.ncbi.nlm.nih.gov/bioproject/1251125>) and is mirrored on OSF at <https://osf.io/dq2a4/>.

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