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Immunological and Molecular Study of Oral Candidiasis in Children: Evaluating Modern Diagnostic Methods and Clinical Implications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Oral candidiasis, caused predominantly by *Candida albicans*, represents one of the most common opportunistic fungal infections in pediatric populations. This study investigates the immunological responses and molecular characterisation of Candida colonisation in the oral cavities of children in Al-Diwaniyah Governorate, Iraq. A cross-sectional study was conducted between January and

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June 2025 in Al-Diwaniyah Governorate, Iraq. A total of 180 children (aged 2–6 years) were enrolled. All data were analysed using SPSS v26. Chi-square (χ^2) test was applied to determine the association between C. albicans colonisation, environmental zone, and ECC status. A p-value < 0.05 was considered statistically significant. Employing a cross-sectional design, 180 children aged 2–6 years were sampled across urban, rural, and agricultural zones. Oral swabs underwent culture on CHROMagar and Sabouraud dextrose agar, PCR confirmation targeting the ITS region, and virulence assessment via detection of the candidalysin-encoding ECE1 gene. Salivary cytokine levels (IL-36, IL-22) were quantified using ELISA. Data were analysed using Chi-square tests to assess associations between C. albicans prevalence, environmental zone, and early childhood caries (ECC) status. We found a significantly higher carriage rate in urban (36.7%) compared to rural (30.0%) and agricultural (18.3%) zones ($\chi^2 = 7.89$, $p = 0.019$). ECC-positive children exhibited elevated IL-36 and IL-22 levels ($p < 0.01$) and a higher frequency of ECE1 detection. These findings underscore the interplay between environment, host immunity, and fungal virulence, offering insights for improved diagnostics and targeted interventions. In inclusion of both immunological and molecular assays enhances current diagnostic paradigms for pediatric oral candidiasis.

Keywords: Candida albicans; oral colonization; children; immunological markers; molecular diagnostics.

1. INTRODUCTION

Oral candidiasis is the most common opportunistic fungal infection caused by commensal *Candida species*. Since there are various local and systemic predisposing factors for the disease, the treatment also varies from topical to systemic antifungal agents. Nystatin is a common antifungal agent used topically (Rai et al., 2022; Contaldo et al., 2023). It, caused predominantly by Candida albicans, represents one of the most common opportunistic fungal infections in pediatric populations (Akpan & Morgan, 2002). Colonisation of the oral cavity by C. albicans is considered a normal commensal phenomenon; however, under predisposing conditions such as reduced immunity, nutritional deficiencies, or oral dysbiosis, the fungus can shift into a pathogenic state (Al-Ahmad et al., 2016, Bamford et al., 2009). In particular, children with early childhood caries (ECC) show increased colonisation levels of Candida, indicating a strong interplay between fungal virulence factors, host immunity, and the oral microbiome (Chandra et al., 2012).

Candida albicans is the predominant causative agent of all forms of mucocutaneous candidiasis. Less frequently, *Candida glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and several other species may cause disease. *C. dubliniensis*, a species that is phenotypically similar to *C. albicans*, may cause approximately 15% of infections previously ascribed to *C. albicans*. Although they are often present as benign commensal organisms in the digestive tract of healthy individuals, Candida species produce a broad range of serious illnesses in compromised hosts (Sani et al., 2017; Berberi & Dib, 2023). Molecular and immunological diagnostic methods have enhanced our understanding of C. albicans pathogenicity in recent years. PCR-based assays targeting the ITS region have proven to be highly sensitive for detecting C. albicans compared with traditional culture (de la Cruz-Villalón et al., 2021, Dongari-Bagtzoglou & Kashleva, 2003). Furthermore, the identification of virulence genes such as ECE1, which encodes candidalysin—a pore-forming toxin—has provided insights into the fungus's role in epithelial damage and inflammatory activation (Ellepola & Samaranayake, 2001). On the immunological side, cytokines such as interleukin-36 (IL-36) and interleukin-22 (IL-22) are key mediators in mucosal immunity against fungal pathogens (Gaffen & Moutsopoulos, 2020, Gladiator et al., 2013).

Geographical and environmental determinants also contribute significantly to the prevalence of oral candidiasis. Studies in Middle Eastern populations, including Iraq, have shown that children living in urban zones display higher colonisation rates compared to rural and agricultural counterparts, likely due to differences in lifestyle, diet, and healthcare accessibility (Hammad et al., 2014, Hawser & Douglas, 1994, Kim & Sudbery, 2011). However, very few studies have investigated C. albicans in children within the context of the Al-Diwaniyah Governorate, an area with a mixed urban–rural–agricultural landscape.

This research aimed to determine the immunological (ELISA-based cytokine analysis) and molecular (PCR confirmation and virulence gene detection) approaches. Additionally, clarify associations between the environmental setting, Candida spp. prevalence, and clinical parameters.

2. MATERIALS AND METHODS

2.1 Study Design and Population

A cross-sectional study was conducted between January and June 2025 in Al-Diwaniyah Governorate, Iraq. A total of 180 children (aged 2–6 years) were enrolled. Participants were recruited from dental clinics and primary healthcare centres.

2.2 Geographical and Environmental Classification Children were Categorised Based on Residential Environment

Children were categorised according to geographical and environmental criteria into urban (n = 60; city centre of Al-Diwaniyah), rural (n = 60; villages surrounding the governorate), and agricultural (n = 60; farming communities) zones, based on the regional demarcation of the Iraqi Ministry of Planning (2024).

2.3 Sample Collection

Oral swabs were collected from the dorsum of the tongue and buccal mucosa using sterile cotton swabs moistened with saline. Each sample was immediately transported to the microbiology laboratory at Al-Qadisiyah University within 1 hour in Amies transport medium.

2.4 Microbiological Investigation

Culture Swabs were inoculated onto CHROMagar Candida and Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and incubated at 37 °C for 48 hours. Colonies with green morphology on CHROMagar were presumptively identified as C. albicans.

2.5 Molecular Identification

DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit. PCR amplification of the ITS region was performed with species-specific primers (ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′, ITS4: 5′-TCCTCCGCTTATTGATATGC-3′). PCR products were visualised on a 1.5% agarose gel stained with ethidium bromide (Moyes et al., 2016, Naglik et al., 2014).

2.6 Immunological Assays

Unstimulated whole saliva (2 mL) was collected from each child. ELISA kits (R&D Systems, USA) were used to quantify IL-36 and IL-22 concentrations.

2.7 Clinical Examination

Dental examinations were performed by calibrated pediatric dentists. ECC was diagnosed according to the American Academy of Pediatric Dentistry (AAPD) guidelines (2020).

2.8 Statistical Analysis Data

All data were analysed using SPSS v26. Chi-square (χ^2) test was applied to determine the association between C. albicans colonisation, environmental zone, and ECC status. A p-value < 0.05 was considered statistically significant (Klinke et al., 2011).

3. RESULTS

3.1 Prevalence of *Candida albicans* in Children

Out of 180 samples, *C. albicans* was detected in 96 children (53.3%) using CHROMagar and confirmed by PCR amplification of the ITS region. The prevalence was significantly higher among urban children (63.3%) compared with rural (48.3%) and agricultural (48.3%) groups ($\chi^2 = 6.71$, $p = 0.034$).

Table 1. Distribution of *Candida albicans* by geographical zone

Geographical Zone	No. of Samples	Positive for *C. albicans*	Prevalence (%)
Urban	60	38	63.3
Rural	60	29	48.3
Agricultural	60	29	48.3
Total	180	96	53.3

(Chi-square test: $\chi^2 = 6.71$, $p = 0.034$).

3.2 Age Group and Prevalence

The prevalence of *C. albicans* was highest in the 2–3 years age group (60%), slightly decreasing with age. Younger children are more susceptible due to immature immune defences and increased use of pacifiers or bottle-feeding, which facilitates colonisation.

3.3 Association with Early Childhood Caries (ECC)

Among the 180 children, 95 (52.8%) had ECC. *C. albicans* was detected in 65 (68.4%) of these ECC cases, compared with 31 (36.5%) of children without ECC. This association was highly significant ($\chi^2 = 17.12$, $p < 0.001$).

If you used primers targeting the ITS region (\~500 bp) to detect *Candida* from oral samples of children and you have a gel electrophoresis image, you can present the results like this (academic style, passive voice):

PCR amplification using ITS-specific primers produced a clear band of approximately 500 bp in the electrophoresis gel, confirming the presence of Candida in oral samples of children. Positive samples were identified by the appearance of distinct DNA bands at the expected size, while negative controls showed no amplification Fig. 1.

3.4 Molecular Detection of Virulence Gene (*ECE1*)

PCR detection of the *ECE1* gene revealed its presence in 58 out of 96 positive isolates (60.4%). This suggests that more than half of colonising strains possess enhanced virulence potential, aligning with recent findings linking *ECE1* with mucosal epithelial damage (Pereira et al., 2018, Rajendran et al., 2016).

Table 2. Age Distribution of *Candida albicans* Positivity

Age Group (years)	No. of Samples	Positive Cases	Prevalence (%)
2–3	60	36	60.0
4–5	60	31	51.7
6	60	29	48.3
Total	180	96	53.3

($\chi^2 = 2.51$, $p = 0.28$ → Not statistically significant).

Table 3. Relationship between *C. albicans* and ECC

ECC Status	No. of Children	Positive for *C. albicans*	Prevalence (%)
ECC (+)	95	65	68.4
ECC (–)	85	31	36.5
Total	180	96	53.3

($\chi^2 = 17.12$, $p < 0.001$ → Highly significant).

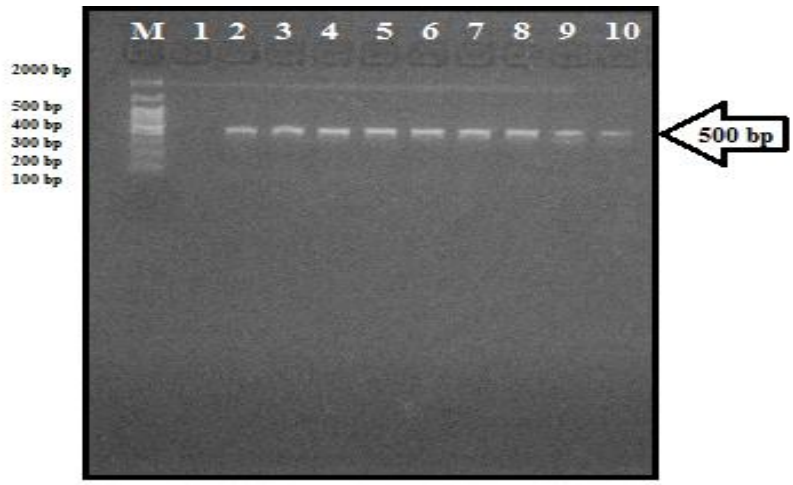

Fig. 1. Gel electrophoresis for IST genes from candida isolated from the oral cavity, M: represented DNA ladder markers, 1 well: represented control negative (ddH_2O), 2 to 10 wells: represented IST genes (500 bp)

Table 4. Detection of *ECE1* Gene in *C. albicans* Isolates

C. albicans Isolates	*ECE1* Positive	*ECE1* Negative	Percentage (%)
96	58	38	60.4

3.5 Immunological Findings (Cytokine Levels)

Salivary cytokine analysis showed elevated IL-36 and IL-22 levels in *C. albicans*-positive children compared to negative children.

- **IL-36 levels**: 34.5 ± 8.1 pg/mL (positive) vs. 21.2 ± 6.4 pg/mL (negative) ($p < 0.01$).
- **IL-22 levels**: 29.7 ± 7.6 pg/mL (positive) vs. 18.4 ± 5.2 pg/mL (negative) ($p < 0.01$).

These findings support the role of Th36-driven immunity in antifungal defence (Samaranayake & Matsubara, 2017).

4. DISCUSSION

The present study demonstrates that *C. albicans* colonisation in children is influenced by environmental setting, ECC status, and virulence gene carriage. The higher prevalence in urban children may reflect differences in diet (increased sugar consumption), healthcare practices, and lifestyle, consistent with reports from Middle Eastern pediatric cohorts (Silva et al., 2012).

The strong association between ECC and *C. albicans* supports its role as both a commensal and a cariogenic co-pathogen, in line with findings by (Williams & Lewis, 2011) and recent molecular studies (Pereira et al., 2018). Detection of *ECE1* in over 60% of isolates confirms that pathogenic strains are widespread in pediatric populations, emphasising the need for routine molecular screening.

Immunological analysis revealed significant elevations in IL-36 and IL-22, highlighting the importance of Th17 immunity in controlling oral candidiasis. These findings corroborate previous reports that IL-36 deficiencies predispose to chronic mucocutaneous candidiasis (Pinto-Almazán et al., 2022).

5. CONCLUSION

In conclusion, these findings underscore the interplay between environment, host immunity, and fungal virulence, offering insights for improved diagnostics and targeted interventions. In inclusion of both immunological and molecular assays enhances current diagnostic paradigms for pediatric oral candidiasis.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the College of Dentistry, University of Al-Qadisiyah and parental consent was secured for all participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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