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Morphological and genetic identification of yeasts from skin and oral infection in children in the Basrah province

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ABSTRACT

Introduction and aim. Human fungus infections are widespread and can lead to a variety of diseases in children. The purpose of this study was to isolate and identify yeasts from various places in children, including skin (diaper area) and oral cavity, utilizing morphological and molecular approaches for precise categorization.

Material and methods. One hundred swabs were collected from children clinically diagnosed with fungal skin infections. The collection period was October 2022 to August 2023. The isolated yeast species were examined, purified, and morphologically. The sequences have been deposited in GenBank of Japan as new strains under accession numbers LC790886 to LC79098. including *Candida albicans, Pichia kudriavzevii, Magnusiomces capitatus, Nakaseomyces glabratus, Kluyveromyces marxianus, Candida tropicalis, Meyerozyma guiliermonolii, Clavispora lusitaniae, Candida parapsilosis, Trichosporon ashii*.

Results. The presence of 10 yeast species, with *C. albicans* 56% representing the highest percentage of these, while the percentage of other yeasts was 44%. The Candida species was found to have the highest percentage of occurrence, 58% followed by the *C. tropicalis* species, 19%, which had a lower percentage of occurrence.

Conclusion. The phenotypical and genetic characteristics of yeast have been identified by the use of clinically isolated samples of children.

Keywords. *Candida*, newborns, oral candidiasis, skin infection

Introduction

Cutaneous candidiasis is a widespread fungal infection that affects people of all ages, while oral candidiasis is a common infection that affects primarily people with weakened immune systems, such as newborns, infants, those taking antibiotics or corticosteroids, *Candida* can be the main cause of skin disease or develop as a result of other skin conditions such as atopic dermatitis, psoriasis, or diaper rash.[1,2] It can affect any area of the body, and frequent symptoms include interdigital candidiasis, cheilitis, diaper dermatitis, and intertrigo.[3,4]

The genus *Candida* contains many species, but *Candida albicans* is the most common cause of candidiasis in humans, accounting for more than 80% of cases. Other less common species include *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata.*[5] The genus *Candida* has over 200 species, although only a tiny percentage of these are human opportunistic pathogens that infect people with compromised immune systems. Topical antifungal medications are an effective way to treat superficial Candida infections, which usually affect the skin or mucous membranes. Invasive fungal infections are frequently life-threatening, perhaps due to ineffective diagnostic techniques and inadequate first antifungal treatments.[6,7] More than 50% of surface *Candida* infections are caused by *C. albicans*, the most opportunistic fungal pathogen in humans.[8,9] It resides in the gastrointestinal and genitourinary tracts of approximately 70% of individuals as a benign commensal without inducing any symptoms of illness.[10] Children with oropharyngeal candidiasis can have acute or persistent symptoms, usually characterized by painful, erythematous, pseudomembranous plaques that can spread to the larynx and pharynx and make swallowing and eating difficult.[11]

Diaper dermatitis (DD) caused by candidiasis is a common problem, especially in newborns and infants. Diarrhea, which contributes to it, occurs in approximately one-third of affected children.[1] The affected area typically stays within the diaper zone, though severe cases can spread beyond it can extend beyond these limits.[5] Intertrigo, a fold skin condition, can develop due to either direct fungal infection or extension of the diaper rash. It can involve both major and minor skin creases.[12] Similar to DD, intertrigo manifests itself as reddened, scaly patches with potential blistering and swelling. Tiny bumps or pustules may also appear around the main lesion. In severe cases, ulceration and erosion can occur. Itching and/or pain are common.[5,12,13]

Aim

The aim of the study was to isolate and identify yeasts from various places in children, including skin, diaper area, and oral cavity, utilizing morphological and molecular approaches for precise categorization.

Material and methods

Collection of samples

During October 2022 to August 2023, One hundred swabs were collected from children who were clinically diagnosed with different fungal skin infections attending Al-Fayhaa General Hospital and Basrah Women's and Children's Hospital, in AL- Basrah, Iraq. These specimens were brought to the Mycology Laboratory of the University of Basrah for further examination. Samples were cultured on SDA media (Himedia, India) that included 250 mg/L of chloramphenicol added to SDA_C in order to prevent the contamination of samples with bacteria. After creating the samples, they were placed in an incubator at a temperature of 37 ° C for a period of time that ranged from two to thirty days. The cultures were then processed for inspection to determine the phenotypic and genetic characteristics of the organisms. The fungal isolates were examined macroscopically and microscopically using lactophenol cotton blue as a mounting material; this was done by preparing slides of each growing colony were prepared to observe under a light microscope with an objective lens magnification power of 40×. Observation of colony color and cell morphology on CHROM agar *Candida* (HiMedia, India) was used for yeast identification following the mycological literature.[14]

Examination and identification specimens

Using Chrome agar *Candida* medium for the identification of yeast isolates.

Preparation Chrome agar Candida medium as follows

Candida medium of Chrome agar (Himedia, India) 42.72 gm D.W 1000 ml (Table 1) .

Table 1. The color variation of *Candida spp*. in chrome agar candida medium [15]

No.	*Candida* species	Color
1	*C. albicans*	light green
2	*C. tropicalis*	blue to metallic blue
3	*C. glabrata*	cream to white smooth
4	*C. krusei*	purple fuzzy
5	*C. parapsilosis*	white to cream

Genetic diagnosis

A Presto Mini gDNA yeast kit that Geneaid/Korea gave was utilized to extract DNA from isolated cultures. For the amplification of the ITS region, universal primers ITS1 (5-TCCGTAGGTGAACCTGCGG-3), ITS4 (5`TCCTCCGCTTATTGATAT GC-3`) are utilized. The thermal Cycler, manufactured by Bioneer Corporation in Korea, is utilized for this process. A total volume of 25 µL is composed of 5 µL of DNA

Form, one microliter of F. Primer, one microliter of R. Primer, 1 microliters of Master Mix, 12.5 µL of nuclease-free water, and 5.5 µL of nuclease-free water .

PCR was carried out as follows: followed by 25 cycles at 94 ° C for thirty seconds, 56ºC for 45 seconds and 72ºC for one minute; the last extension was performed at 72 ° C for seven minutes.[16] The PCR result was observed by agarose gel electrophoresis, 2% agarose, 25 ml of TBE buffer, and 0.2 µL of green gel stain. Bioneer also used a 100 bp DNA ladder in Korea. Purific use and sequencing of the region ITS1-5.8S-ITS2 was performed by sending twenty microliters of the DNA of the PCR product for the ITS1-5.8S-ITS2 region to the Macrogen firm in South Korea.[17] Each yeast isolate that was discovered using the National Center for Biotechnology Information (NCBI) blast.

Ethical approval

All subjects gave their informed consent to be included before participating in the study. The study was carried out according to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Collage of Science in University of Basrah (1165 in Nov. 2022).

Results

On the basis of the morphological and genetic analysis of yeast isolates, it was discovered that isolated species are related to eleven different species of yeast, as indicated in Table 2.

Table 2. Species of yeasts with percentage of occurrence

No.	*Candida* species	Diaper rash (%)	Oral cavity (%)	Number of case	Occurrence %
1	*C. albicans*	30 (51.7%)	28 (48.2%)	58	58%
2	*C. tropicalis*	14 (73.6%)	5 (26.3%)	19	19%
3	*C. parapsilosis*	0	1 (100%)	1	1%
4	*C. lusitanaie*	2 (50%)	2 (50%)	4	4%
5	*Merozyma guiliermonolii*	2 (100%)	0	2	2%
6	*Pichia kudriavezevii*	3 (33.3%)	6 (66.6%)	9	9%
7	*Trichosporon ashii*	0	1 (100%)	1	1%
8	*Magunusiomyces capitatus*	0	3 (100%)	3	3%
9	*Nakaseomces glabratus*	1 (100%)	0	1	1%
10	*Kluyveromyces marxianus*	0	2 (100%)	2	2%
	Total	52	48	100	100%

C. albicans

Colony characteristics: colonies on SDAc after 2 days at 37 ° C. Colonies appear white to cream-colored, glistening or somewhat waxy, soft, and usually smooth. Some strains may become wrinkled and have a mycelial border. Color of the colony on chrome agar *Candida*, this species produces distinctive light green colonies. Microscopy: Budding cells (sub)spherical, (3−8×2−7) µm. pseudomyclium present (Fig. 1). [18]

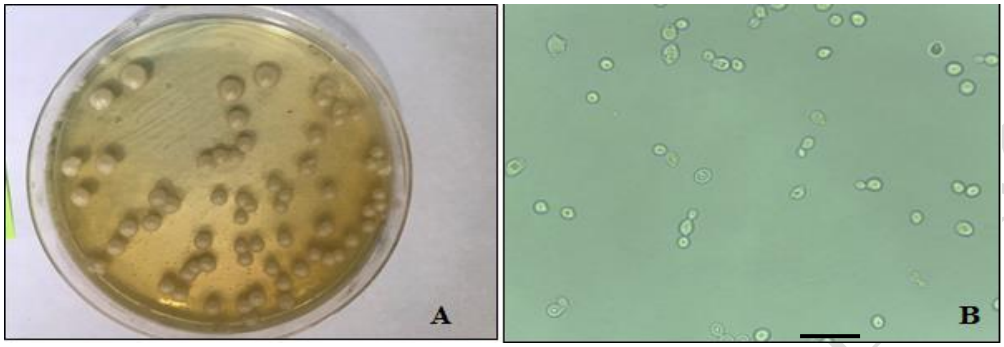

Fig. 1. A: Colonies of yeast *C. albicans*, B: yeast cells (B=13.5 µm)

C. tropicalis

Colony characteristics: colonies on SDA after 2 days at 37 ° C. Colonies appear cream-colored, off-white to grey dull, soft, smooth, and creamy or wrinkled and tough. Color of the colony on chrome agar *Candida*, steel blue to dark gray colonies. Microscopy: ellipsoidal budding cells, characterized by the presence of long, branching components that either bear conidia individually or in small chains or clusters (Fig. 2).[19]

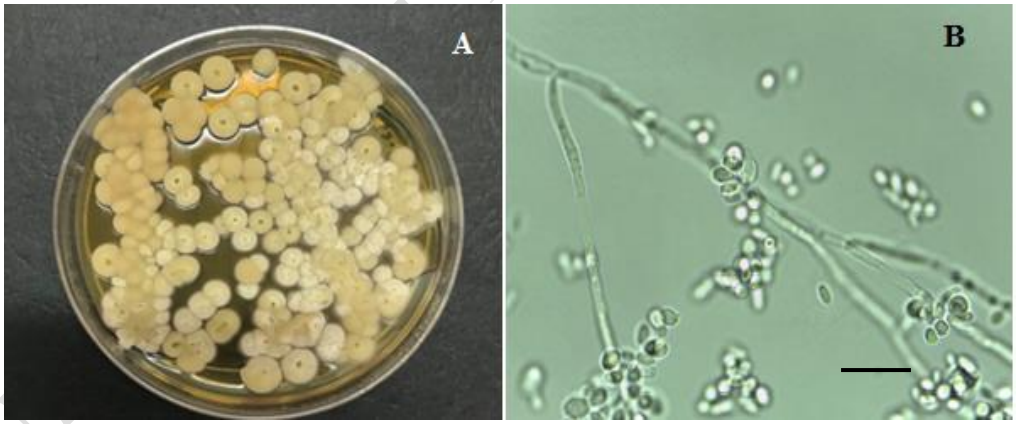

Fig. 2. A: Colonies of yeast *C. tropicalis*, B: yeast cells (B=13.5 µm)

C. lusitaniae

Colony characteristics: colonies on SDA after 2 days at 37 ° C. These colonies are white to cream-colored, shimmering, and soft and smooth. On chrome agar *Candida*, colonies of this species have a coloration ranging from pink to lavender, and some of them develop a waxy texture on this medium. Microscopy: ellipsoidal budding cells, and often pseudomycelium often present. containing 4 smooth- walled, clavate heterothallic (Fig. 3).[20]

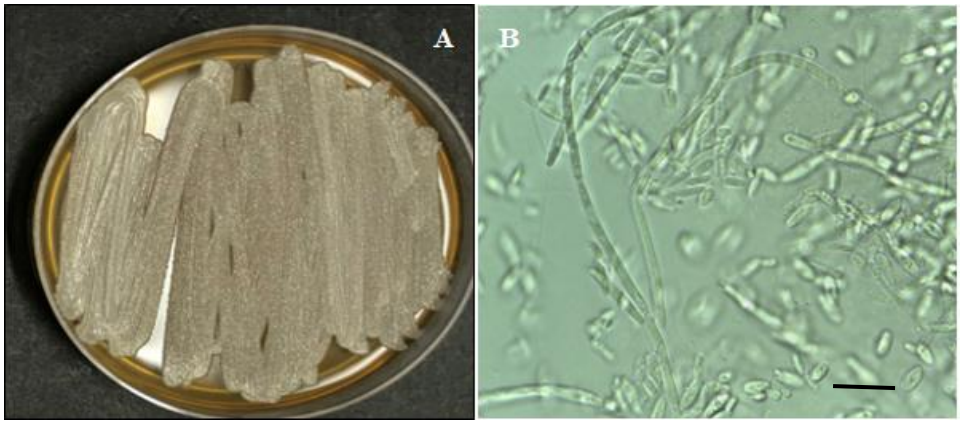

Fig. 3. A: Colonies of yeast *C. lusitaniae* , B: yeast cells (B=13.5 µm)

P. kudriavzevii

Colony characteristics: colonies on SDA after 2 days at 37 ° C. colonies appear white to cream coloured, colonies appear wrinkled and flat smooth. Microscopy reveals that blastoconidia are usually elongated, measuring up to 2 5µm in length. Pseudohyphae are visible with multilateral blossoming arranged in a varied pattern. These cells often exhibit a morphology resembling a matchstick. Pink colonies with rough texture are observed on Candida chromium agar (Fig. 4).[21,22]

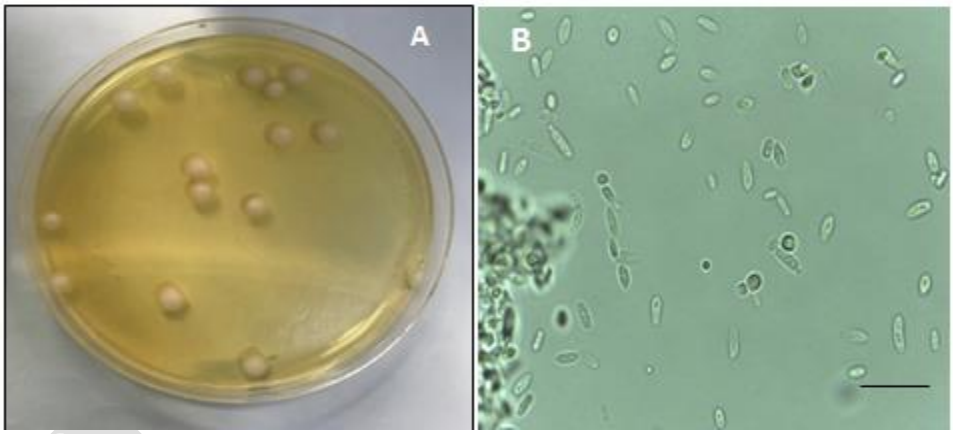

Fig. 4. A: Colonies of yeast *P. kudriavzevii*, B: yeast cells (B=13.5 µm)

T. asahii

Colony characteristics: colonies on SDA after 2 days at 37 ° C. Colonies moderately expending, dry, pustular with white to cream color, farinose or cerebriform surface with radial fissures, deep fissured marginal zone growth at 37 ° C. Microscopy shows that there are no lateral and budding cells, while arthroconidia are barrel-shaped. No appressoria is present (Fig. 5).[23]

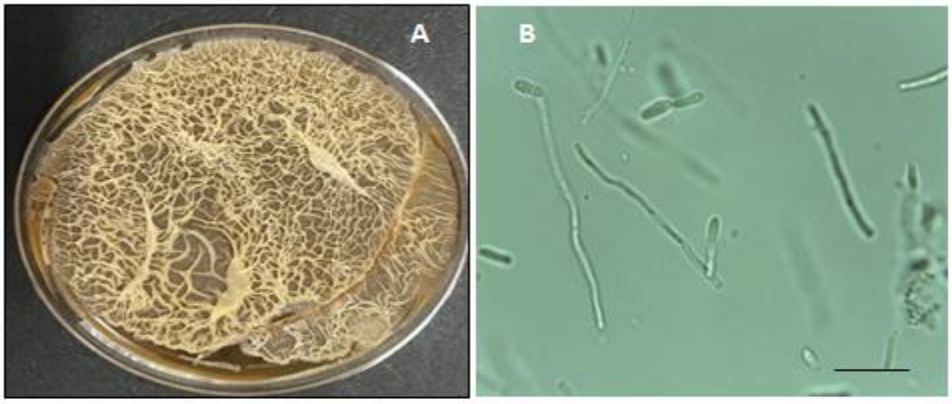

Fig. 5. A: Colonies of yeast *T. asahii*, B: yeast cells (B=13.5 µm)

M. capitatus

Colony characteristics: colonies on SDA after 2 days at 37 ° C. Colonies with moderate growth, whitish 24 Microscopy reveals conidiophores that are extensively branched at acute angles, measuring 180-500 µm long conidia cylindrical- clavate, hyaline, 1-celled, with a rounded apex and flat base, measuring (7–10×2.5–3.5) µm. In addition, rectangular arthroconidia are frequently observed. Endoconodia may be present at times (Fig.6).

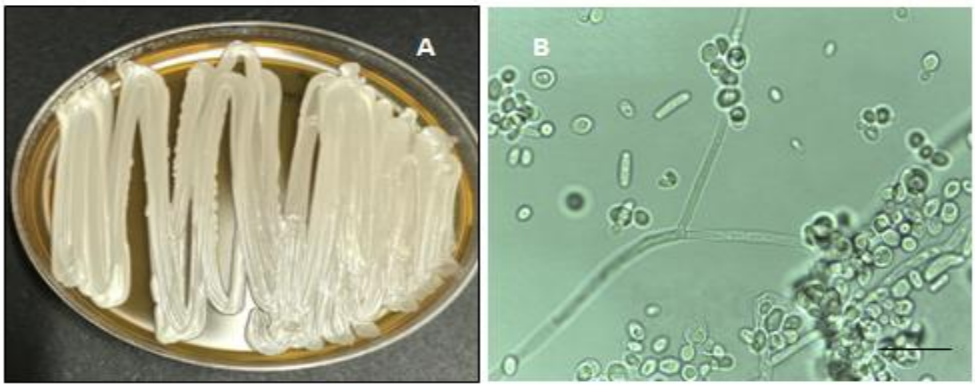

Fig. 6. A: Colonies of the yeast *M. capitatus*, B: yeast cells (B=13.5 µm)

C. parapsilosis

Colony characteristics: Colonies on SDA after 2 days at 37 ° C. The colonies are cream-colored to yellowish, shiny, and soft, typically smooth, but may be partially or fully wrinkled.[25] Microscopy: Consisting of branched chains of elongated cells with chains and clusters of spherical to ovoidal conidia forming at intervals along the hyphae (Fig. 7).

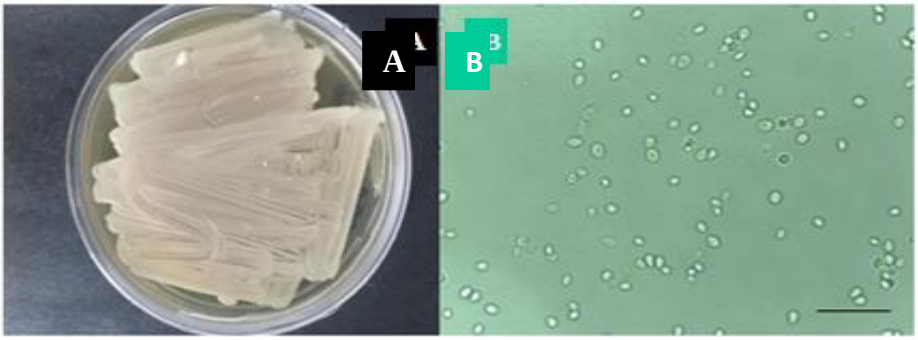

Fig. 7. A: Colonies of yeast *C. parapsilosis*, B: yeast cells (B=13.5 µm)

M. guiliermonolii

Colony characteristics: colonies on SDA after 2 days at 37 ° C. The colonies are white to cream-colored and butrous Microscopy shows budding cells that are subspherical to broadly ellipsoidal, measuring (3×6 and 2×4) µm. Pseudomycelium may be present, but hyphae are not generated. Colonies of *C. guilliermondii* appear pink to lavender on chrome agar *Candida* (Fig. 8).[26]

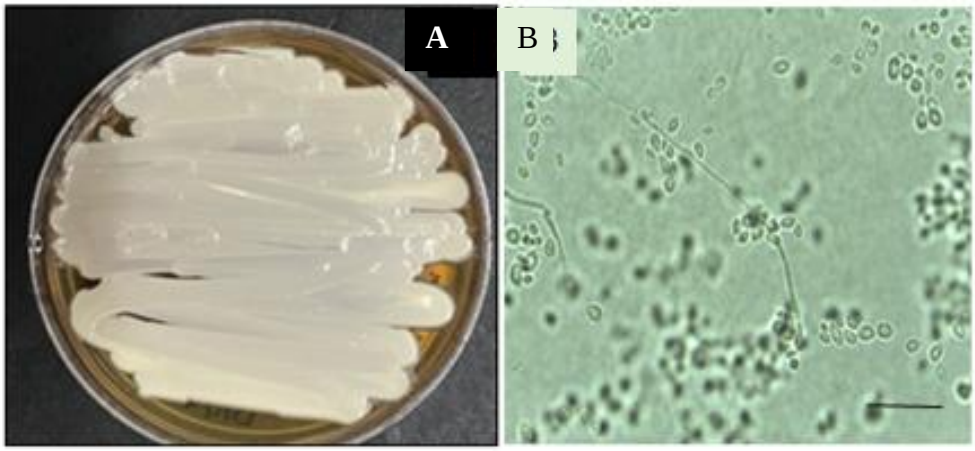

Fig. 8. A: Colonies of the yeast *M. guiliermonolii*, B: yeast cells (B=13.5 µm)

N. glabratus

Colony characteristics: colonies on SDA after 2 days at 37 ° C. Colonies cream-colored, soft, glossy and smooth. Microscopy: Some strains can produce a small number of branched chains of oval-shaped cells (Fig. 9).[27]

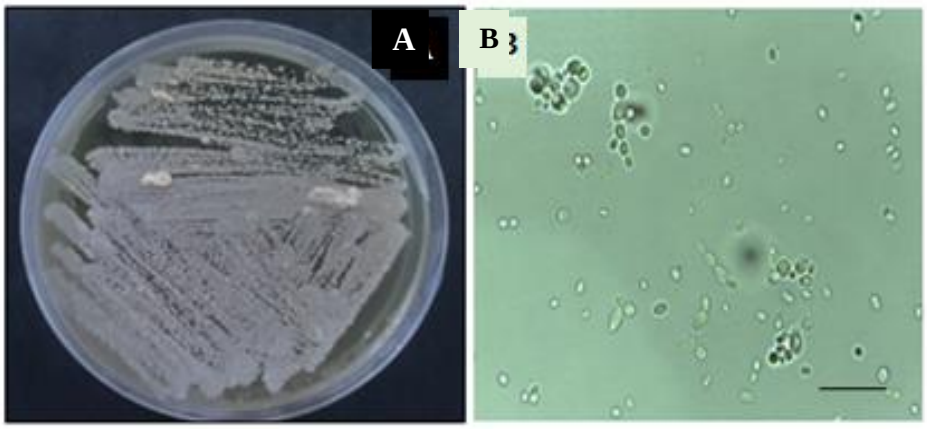

Fig. 9. A: Colonies of the yeast *N. glabratus*, B: yeast cells (B=13.5 µm)

K. marxianus

Colony characteristics: colonies on SDA after 2 days at 37 ° C. Colonies appear white to cream-colored butyrous. Microscopy: ellipsoidal cells of the budding cells ellipsoidal, (6−10×3−6) µm, hyphae absent, pseudomyclium absent or present pseudomyclium, containing 1-4 smooth-walled, ellipsoidal to reniform ascospores, homothalic (Fig. 10).[28]

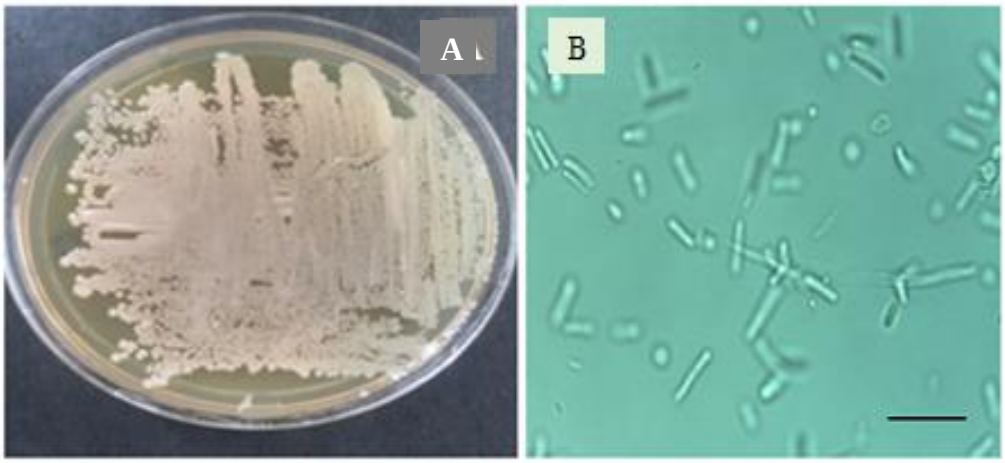

Fig. 10. A: Colonies of the yeast *K. marxianus*, B: yeast cells (B=13.5 µm)

Molecular identification

ITS1-5.8S-ITS2 rDNA is the gold standard for identifying yeast isolates. It is a fast reliability method compared to biochemical methods, as well as providing the formation of evolutionary relationships for the identification of yeast isolates.[29]

ITS1-5.8s-ITS2 rDNA

ITS1-5.8S-ITS2 rDNA of yeast isolates was displayed on agarose gel electrophoresis under UV trans-illuminator at the position 500 base pairs by comparing it with a standard DNA ladder (Fig. 11).

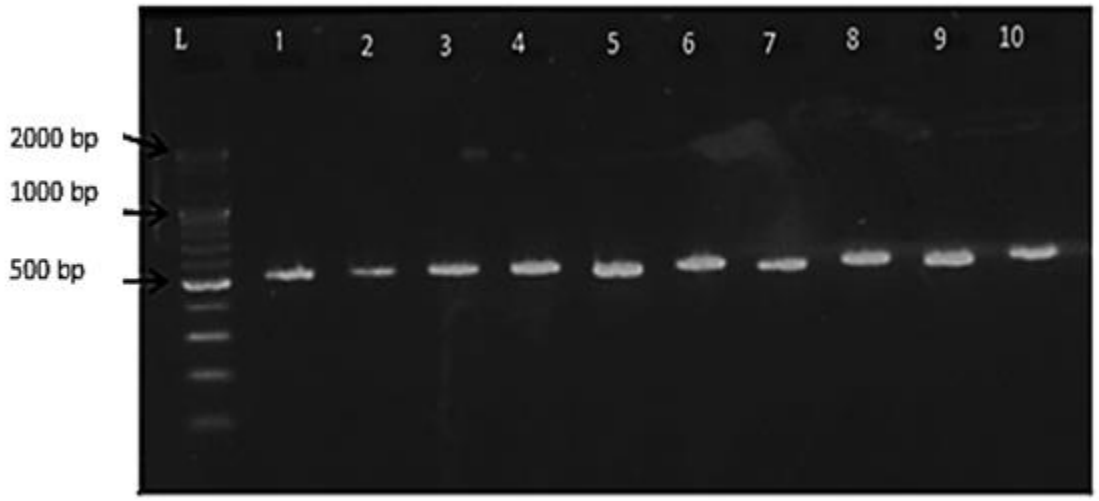

Fig. 11. Agarose gel electrophoresis 2% of PCR products for regions containing internal transcribed spacers (ITS1) and ITS2 (including 5.8S rDNA): 100 base pairs of the DNA ladder, lane L *C. albicans* (500 bp) *C. tropicalis* (500 bp), and *C. parapsilosis* (500 bp) are presented in lane 1, lane 2, and lane 3, respectively. Lane 4 has 500 bp of *C. lusitania*, lane 5 contains 500 bp of *M. guiliermonolii*, lane 6 includes 500 bp of *P. kudriavezevii*, and lane 7 contains 500 bp of *T. ashii* of the same species. Regarding yeast isolates, lane 8 contains *M. capitatus*, lane 9 contains *N. glabratus*, and lane 10 has *K marxianus*

Discussion

C. albicans

Table 2 revealed that *C. albicans* exhibited the highest incidence at 58%, indicating its predominance among the identified fungal species. This finding aligns with established knowledge, as *C. albicans* is a common member of the human oral microbiome, colonizing the oral cavity in virtually all individuals.[30] In fact, it is frequently the most prevalent *Candida* species in healthy mouths. In particular, *C. albicans* colonization often begins within the first week of life, affecting both the skin and oral mucosa.[31,32]

The study observed the incidence (48.2%) of oral candidiasis in infants associated with the use of pacifiers. This practice can increase the risk of oral thrush and potentially hinder the efficacy of treatment unless the pacifier is meticulously cleaned after each use.[33]

Furthermore, (51.7%) of diaper dermatitis caused by *C. albicans* were documented. This is attributed to the presence of *C. albicans* in the fecal flora of infants. Diarrhea can increase the risk of infection, particularly in the diaper area. Prolonged diaper contact can further aggravate the severity of the infection.[13]

C. tropicalis has emerged as the second most prevalent cause of invasive candidiasis in children, following *C. albicans*. This is consistent with the established role of *C. tropicalis* as a commensal organism that resides on the skin and in the oral cavity.[34]

C. tropicalis

Table 2 indicated that *C. tropicalis* represented approximately 19 cases among all fungal infections observed in this study. In particular, this species exhibited a high incidence (73.6%) in cases of diaper rash. Children, particularly newborns, are highly susceptible to *C. tropicalis* infections due to their immature immune systems. The neonatal intensive care unit (NICU) environment presents unique challenges, as the presence of medical devices can serve as potential entry points for this opportunistic fungus, facilitating the development of invasive candidiasis.

C. tropicalis was identified as the causative agent in five out of 19 cases of oral thrush (26.3%). Oral thrush, characterized by the presence of white or yellow patches on the tongue, inner cheeks, and oral mucosa, can cause discomfort and potentially lead to bleeding.[35]

C. lusitaniae

In this study, *C. lusitaniae* was found to be present at a rate of 4% of the total recorded species, which is a rather small percentage compared to *C. albicans* and *C. tropicalis*, where these fungi are generally harmless in healthy people, but they can cause cutaneous candidiasis in children when the skin is damaged, the conditions are warm and humid, or the immune system is weakened. *C. lusitaniae* is frequently found in healthcare settings, especially among children with hematologic malignancies and others receiving intensive care.[20,21]

P. kudriavzevii

P. kudriavzevii is the sexual form of *C. krusei*. A teleomorph is the stage of sexual reproduction of an organism, where *P. kudriavzevii* reproduces sexually by combining haploid cells. The nonsexual phase of a fungus is referred to as an "anamorph".[36] *P. kudriazvevii* may be commensal but can also exhibit pathogenicity. Some may classify *P. kudriavzevii* as an "opportunistic pathogen." *P. kudriavzevii* specifically infects individuals with weakened immune systems.[37] During this study, a species characterized as a rare species was detected, as approximately 9 isolates were diagnosed in the oral cavity and diaper area in infants, the percentage was 66.6% in the oral cavity and 33.3% in the diaper area. The infection is attributed to recurrent diarrhea in children. In some cases, the infection results from contact with animals or through the environment when they bite sources of this. The species or infection occurs as a result of some systemic diseases that cause a weakened immune system in children, such as cancerous diseases, because this species is opportunistic.[38-40]

T. asahii

This species appeared with an incidence of 1%, since it was diagnosed in the oral cavity, and this species colonizes human skin, gastrointestinal tract, and mucosal surfaces as part of the human microbiota.[41,42] Acquiring infection in children is the result of weak immunity, especially in young newborns and children with hereditary blood diseases.[43]

M. capitatus

This study showed that the incidence of this species is low compared to the total number of other isolates, as it was diagnosed in three cases, all in the oral cavity. This species lives symbiotically in the intestines, skin, and also in the respiratory system. The infection occurs in children due to weak immunity, and the disease is caused by the presence of cancerous diseases or antibiotics.[44]

C. parapsilosis

This species was diagnosed in the oral cavity of samples from hospitalized children, where its presence rate was 1% of the complex of isolated species. This species is usually found on the skin naturally in healthy people, but in the current study, the cause of infection in children was perhaps acquired from the hospital environment. From the central care unit, the infection occurred as a result of weak immunity in children, especially those who have systemic diseases such as cancer, as this species is opportunistic.[45,46]

M. guiliermonolii

This species was identified among other fungal species in this study, as about two isolates from diaper rash were diagnosed. This species is found in the mucous membrane and skin and is associated with people who suffer from serious diseases or who have undergone surgeries in the digestive system, heart, and blood vessels. It is also infected with children who suffer from the disease. Immunodeficiency and recent studies have shown a high resistance of this species to antibiotics in recent years.[47]

N. glabratus

This species appeared at a rate of 1% in the current study in the diaper area, as this species is considered a common species in the hospital environment, especially in children with HIV and cancer, those who have diabetes, and those who take chemical doses.[48]

K. marxianus

It is one of the diseases associated with immunodeficiency and blood diseases. Two isolates from the oral cavity in children during this study. This is due to its association with dairy products, as they can metabolize lactose.[49-51]

Conclusion

In this study, ten different pathogenic yeast species were isolated that cause infection in children in the Basra province. The phenotypical and genetic characteristics of the condition have been identified through the use of clinically isolated samples from children. Using PCR technology, pathogenic isolates were identified from the face, mouth, and diaper area; *C. albicans* recorded the highest incidence rate of 58%, followed by *C. tropicalis* at 19%, while *C. parapsilosis*, *T. ashii*, and *N. glabratus* recorded 1%. It has virulence characteristics that allow it to cause infection and it can develop at a temperature of 37 ° C. In addition to infections that are acquired in hospitals.

Declarations

Funding

The study was funded by the authors.

Author contributions

Conceptualization, F.T.M.A-M. and A.A.A.A-H.; Methodology, F.T.M.A-M.; Software, F.T.M.A-M.; Validation, A.A.A.A-H., N.W.J.A.A-M. and N.W.J.A.A-M.; Formal Analysis, F.T.M.A-M.; Investigation, A.A.A.A-H.; Resources, F.T.M.A-M.; Data Curation, F.T.M.A-M.; Writing – Original Draft Preparation, A.A.A.A-H.; Writing – Review & Editing, A.A.A.A-H.; Visualization, N.W.J.A.A-M.; Supervision, F.T.M.A-M.; Project Administration, F.T.M.A-M.; Funding Acquisition, N.W.J.A.A-M.

Conflicts of interest

There is no conflict of interest.

Data availability

The data used to support the results of this study can be found in (1165), but their availability is restricted because they were used under license for this work and are therefore not publicly available. However, the Basrah Health Department has granted the authors permission to make the data available on reasonable request.

Ethics approval

Before participating in the trial, each participant gave his informed consent. In November 2022, the Ethics Committee of 1165 accepted the protocol and the study was carried out in accordance with the Declaration of Helsinki.

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