



## Molecular detection of Virulence Factor Genes in *Candida Albicans* Isolated from the Oral Cavity of Local Chicken and Antifungal Susceptibility in Mosul Province

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**Keywords:** *Candida albicans*, molecular techniques, Local Chicken, Oral Cavity, Antifungal Susceptibility.

### Article History

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### Abstract:

*Candida* spp. is a naturally occurring bacteria in the GI tracts of numerous species, including birds. Candidiasis is an opportunistic illness that emerges when the normal microflora is disturbed. Clinical findings include thickening mucosa and white, elevated pseudomembranous areas. Gross lesions can serve as a presumptive diagnosis. This study aimed to detect and identify *Candida albicans* as of oral cavity of chicken. The Seventy samples were gathered using swabbing from the oral cavity of the Chicken and transported to the Mycology laboratory, using a cool container for handling. Samples were inoculated onto saturated dextrose agar for presumptive identification of *Candida albicans*. Based on molecular techniques, regarding the consequences of PCR amplification of the 18S rRNA gene in the identification of *Candida albicans*, this gene has existed in 50 samples, Metallo-aminopeptidase gene was present in 50/50 (100%) and the alkaline phosphatase sequence gene was once existing in 50/50 (100%). whilst alpha glucosidase used to be existing in 30/50 (60%) and sterol esterase genes 25/50 (50%). Antifungal sensitivity testing results showed that ketoconazole and itraconazole provided the most exceptional Sensitivity (100%) against *Candida albicans* isolates, followed by fluconazole and Amphotericin B. The outcomes of the present research showed that local poultry oral cavities likely contain pathogenic yeasts.

**Keywords:** *Candida albicans*, molecular techniques, Local Chicken, Oral Cavity, Antifungal Susceptibility.

# الكشف الجزيئي لجينات عامل الضرواة في المبيضات البيضاء المعزولة من تجويف الفم لدى الدجاج المحلي والحساسية للمضادات الفطرية في محافظة الموصل

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## الخلاصة:

أجناس المبيضات هي جزء من البكتيريا الطبيعية في الجهاز الهضمي للعديد من الأنواع، بما في ذلك الطيور. داء المبيضات هو عدوى انتهازية فرصية تحدث عند أي إعاقة للبكتيريا الطبيعية. تشمل العلامات السريرية سماكة الغشاء المخاطي مع ابيضاض وارتفاع بالغشاء الكاذب. قد يعتمد التشخيص الافتراضي على الآفات العينية. الغرض من هذه الدراسة هو عزل وتوصيف المبيضات البيضاء من تجويف الفم في الدجاج. جمعت السبعين عينة عن طريق أخذ مسحة من تجويف فم الدجاج ونقلها إلى مختبر الفطريات. باستخدام الصندوق البارد للمعالجة. وتم زراعة العينات على أكار سكر العنب من أجل التعرف الافتراضي على المبيضات البيضاء. بناءً على التقنيات الجزيئية، فيما يتعلق بأهمية تفاعل تضخيم البوليميرات المتسلسل لجين الرنا الريبوسومي S18 لتحديد المبيضات البيضاء، كان هذا الجين موجوداً في 50 عينة، وكان جين *Metallo-aminopeptidase* موجوداً في 50/50 (100%) وكان جين *alkaline phosphatase sequence* موجوداً في 50/50 (100%). بينما كان *alpha glucosidase* موجوداً في 50/30 (60%) وجين *sterol esterase* 25/50 (50%). أظهرت نتائج اختبار الحساسية للمضادات الفطرية أن الكيتوكونازول والإيتراكونازول قدموا الحساسية الأكثر استثنائية (100%). ضد عزلات المبيضات البيضاء، يليها الفلوكونازول والأمفوتريسين ب. نتائج هذا العمل توضح أن الدجاج المنزلي محتمل أن يحتوي على خمائر مسببة للأمراض في تجويف الفم.

**الكلمات المفتاحية:** المبيضات البيضاء، التقنيات الجزيئية، الدجاج المحلي، تجويف الفم، الحساسية للمضادات الفطرية.

## 1. Introduction:

Most species in the *Candida* genus are commensal healthful folks [1]. Candidiasis is a fungal infection that is brought on through way of *C. albicans*, a type of candida fungi that can lead to illnesses in both animals and humans, causing immune suppression or debilitating diseases. In chickens, they can cause infections in the upper digestive system or the skin, commonly affecting the comb. Crop mycosis is the most common type of candida-related disease in poultry. Long-term use of antibiotic medication disrupts the natural bacterial flora, allowing mycelial yeasts to infiltrate the mucosa and cause clinical illness. Crop candidiasis is clinically characterized by little mortality and morbidity, as well as nonspecific indications of sadness and stunted growth [2]. Candidiasis is an occasional disease caused by a yeast belonging to the genus *Candida*. The main species responsible for candidiasis in chickens is *C. albicans*, but it can also be caused by other types of *Candida*. There are multiple symptoms associated with *Candida*

infection [3]. Crop candidiasis is the most common illness caused by *Candida* in poultry. These infections are also referred to as crop mycosis, thrush, moniliasis, or sour crop [4]. There are approximately two hundred species of *Candida*, with very few of these species having harmful properties. *Candida albicans* is one such pathogenic species that can infect and cause diseases in various animals and humans. The infection typically occurs when the immune system of the host is weakened [5 and 6]. The pathogenesis and pathogenicity of *C. albicans* have been appreciably studied in contrast to different species, as it is the main reason for candidiasis in each animal and human. Aside from virulence factors, yeast has integrin-like molecules, proteases, and phospholipases that assist in joining to and infiltrating host tissues, inflicting disease. Furthermore, biofilm manufacturing and phenotypic trade are useful resources for the organism in evading the host's immunological responses [7 and 8]. In healthy animals with a properly functional immune system, phagocytes normally prevent infections. However, in people with weaker immune systems, *Candida* cells can adhere to mucosal cells and rapidly transform from yeast to hyphal. This larger shape makes it tough for phagocytic cells to destroy them. The invasion of host mucosal tissue by hyphae is aided by the abundance of phospholipase enzymes concentrated at the tips of the hyphae [9]. Recently, polymerase chain reaction (PCR) strategies have been broadly used in the diagnosis of chicken candidiasis [10]. The main aim of the study is to detect virulence factor genes in *Candida albicans* strains isolated from the oral cavities of local poultry in Mosul Province. Additionally, we will assess the antifungal susceptibility of these isolates.

## 2. Materials and Methods:

### 2.1 Sample Collection:

The research for this study was conducted from November 2023 to March 2024 at the biology department's laboratory at the University of Al-Hamdaniya's College Education for Pure Science, located in the Mosul Province. Seventy samples were collected from the mouths of local chickens in various locations in Bartella, Mosul province, between November 2023 to March 2024. Using sterile cotton swabs, the samples were collected, kept in a sterile environment, and then transmitted right away to a microbiology lab for processing and testing [11 and 12].

### 2.2 Isolation and identification of *Candida albicans*

Inoculate oral cavity samples on Sabouraud's Dextrose agar with 0.05 mg/ml chloramphenicol and incubate at 37°C for 24 hours to 1 week. After incubation, yeast identification was accomplished using both macroscopic and microscopic morphology tests

[13] This study utilized conventional PCR to identify *Candida albicans* at the molecular level by amplifying a segment of the 18S rRNA gene using a specific primer [11 and 14].

### 2.3. Molecular Detection Primers

Primer 3 plus online (Metallo-aminopeptidase, alkaline phosphatase, alpha-glucosidase, and sterol esterase and the NCBI-Genbank database were used to design the PCR primers for this investigation. While diagnostic primer is composed of a particular sense corresponding to the sequences of a partial gene of 18S rRNA by a reference [14]. These primers were supplied by the Korean Macrogen Company which are listed in Table 1.

**Table 1:** PCR identification gene primers with their nucleotide sequence and product size for *Candida albicans*

| Primers                |   | Sequence(5'-3')       | PCR product size bp |
|------------------------|---|-----------------------|---------------------|
| 18S rRNA gene          | F | GCCGCCAGAGGTCTAAACTTR | 415                 |
|                        | R | AGTTCAGCGGGTAGTCCTAC  |                     |
| Metallo-aminopeptidase | F | GCAACCACCCAAATGGAACC  | 538                 |
|                        | R | GATGGGCCAATTCGTGCATC  |                     |
| alkaline phosphatase   | F | GGGGCCACTGCATTTTCTTG  | 598                 |
|                        | R | CATTGTGGTGTGAAGCGTGG  |                     |
| alpha-glucosidase      | F | ATGCTACTCATGCCGATGGG  | 550                 |
|                        | R | GTATCAACGCCGGCCAATTC  |                     |
| sterol esterase        | F | TGTGCCTCGAGAACCATACG  | 489                 |
|                        | R | CTCTGGAGTCCACCTTGCAG  |                     |

### 2.4 Fungal DNA Extraction

Utilizing the Fungi/Yeast Genomic DNA Extraction Mini Kit, as described in the earlier publication [15]. The manufacturer's recommended procedure was followed to extract fungal genomic DNA from the isolates.

### 2.5 PCR amplification

The PCR master mix reaction was previously coordinated using the Maxime PCR premix reagent i-Taq protocol. Once formulated as per the manufacturer's guidelines, the master mix comprised three microliters of template DNA, twenty microliters of nuclease-free water, and one microliter each of forward and reverse primers (10 pmol). Table 2 outlines the previous configuration of the PCR workstation for 30 cycles. Following one hour of agarose gel electrophoresis at 100V, the PCR products underwent visualization and photography of the DNA bands using a gel documentation system.

**Table 2:** The PCR program settings for conducting tests on *Candida albicans*.

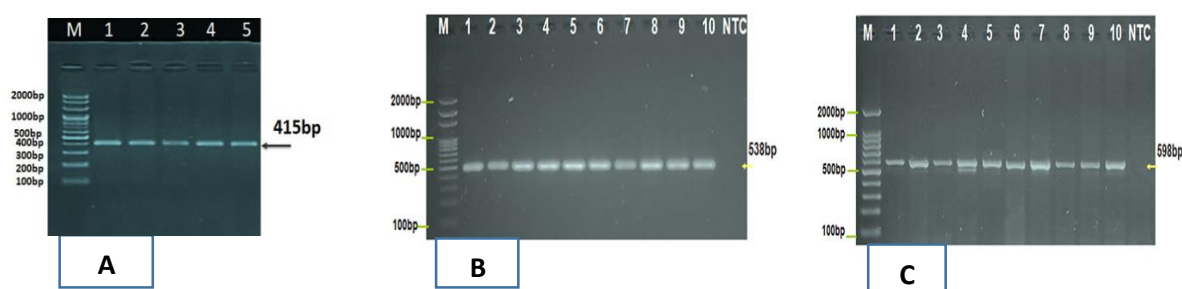
| Step                 | <i>Candida albicans</i> |                        |                      |                   |                 | Time      |          |
|----------------------|-------------------------|------------------------|----------------------|-------------------|-----------------|-----------|----------|
|                      | 18S rRNA gene           | Metallo-aminopeptidase | alkaline phosphatase | alpha-glucosidase | sterol esterase |           |          |
| Initial denaturation | 95°C                    | 95°C                   | 95°C                 | 95°C              | 95°C            | 2 min     | 30 Cycle |
| Denaturation         | 95°C                    | 95°C                   | 95°C                 | 95°C              | 95°C            | 30 sec    |          |
| Annealing            | 57.0                    | 64.9°C                 | 64.9°C               | 64.8°C            | 64.8°C          | 30 sec    |          |
| Extension            | 72°C                    | 72°C                   | 72°C                 | 72°C              | 72°C            | 50-60 sec |          |
| Final Extension      | 72°C                    | 72°C                   | 72°C                 | 72°C              | 72°C            | 5 min     |          |
| Hold                 | 4°C                     | 4°C                    | 4°C                  | 4°C               | 4°C             | 10 min    |          |

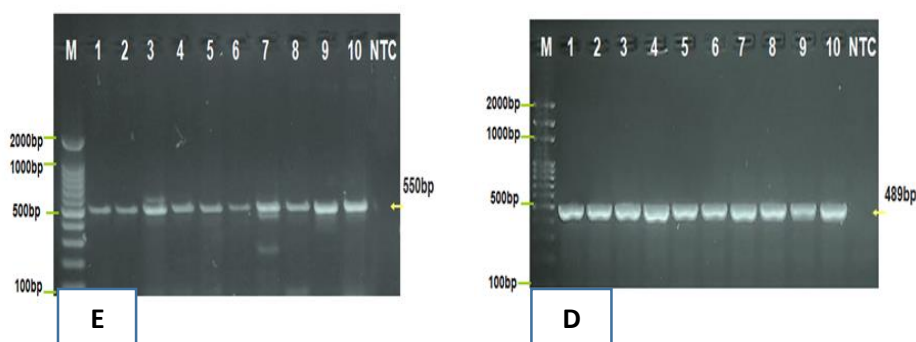
## 2.6 Susceptibility for Some Antifungal Drugs

*Candida albicans* isolates subjected to susceptibility testing by standard methods for the antifungal test have been used in this study [15, 16]. A standard reference procedure has been described by the National Committee for Clinical Laboratory Standards [17]. Antifungal susceptibility was determined by using 4 antifungal discs (Amphotericin B, Fluconazole, Ketoconazole, and Itraconazole) according to guidelines recommended by the [16] corresponding to the drugs considered routine testing and reporting on yeast.

## 3. Results:

50 (71%) *Candida* isolates out of 70 oral cavity samples were obtained from chicken with inflammation based on cultural, morphological, and molecular identification carried out the highest percentage was *C. albicans* 50/70 (71%). Based on molecular techniques, regarding the results of PCR amplification of the 18S rRNA gene for identification of *C. albicans*, this gene was present in 50 samples. Metallo-aminopeptidase gene was present in 50/50 (100%) and alkaline phosphatase sequence gene was present in 50/50 (100%). while alpha-glucosidase was present in 30/50 (60%) and sterol esterase genes 25/50 (50%), produced distinct bands corresponding to molecular sizes approximately 415 bp, 538 bp, 598 bp, 550 bp, and 489 bp which exhibited 18S rRNA gene, Metallo-aminopeptidase, alkaline phosphatase, alpha-glucosidase, and sterol esterase genes, respectively. Show **Figure 1**.

**Figure 1. a.:** the analysis of PCR products of pathogenic *Candida albicans*



**Figure 1. b.:** the analysis of PCR products of pathogenic *Candida albicans*

**Figure 1** Agarose gel electrophoresis image showing the analysis of PCR products of pathogenic *Candida albicans*. The marker ladder ranges from 2000 to 100 base pairs, A- lanes 1-10 (415 bp) displayed the 18S rRNA gene, B- Lanes 1-10 displayed the metallo-aminopeptidase gene (538bp), C- Lanes 1 to 10 display the alkaline phosphatase gene (598 bp), D- Lanes 1-10 display the alpha glucosidase gene (550 bp) and E- Lanes 1-10 display the sterol esterase gene (489 bp).

#### 4. Antifungal Profile for *Candida albicans*

Antifungal profile of *Candida albicans* isolates from the oral cavity of chicken according to antibiotic disc diffusion method. The sensitivity test was applied to all 50 isolates of *Candida albicans*. These isolates were tested for their sensitivity to Amphotericin B, Fluconazole, Ketoconazole, and Itraconazole. The results were interpreted by measuring the inhibition zones around the disc and compared with breakpoints interpreted according to the manufacturer's instructions (DHN PAN Krakow, Poland). Zones diameters of  $\geq 18$  mm indicated the susceptibility (S), and that of  $\leq 14$  mm indicated the resistance (R) to each fluconazole, ketoconazole, and itraconazole. The zones S were  $\geq 16$  mm and R  $\geq 12$  mm, for amphotericin B. These zones were translated in terms of sensitivity (S) and resistance (R), **Table 3**.

**Table 3:** Antifungal susceptibility and resistance profile for *Candida albicans*

| Type of antifungal | <i>Candida albicans</i> (N=50) |     |    |    |
|--------------------|--------------------------------|-----|----|----|
|                    | S                              | %   | R  | %  |
| Fluconazole        | 40                             | 80  | 10 | 20 |
| Amphotericin B     | 30                             | 60  | 20 | 40 |
| Ketoconazole       | 50                             | 100 | -  | -  |
| Itraconazole       | 50                             | 100 | -  | -  |

R= Resistance, S= Sensitive

## 5. Discussion:

Using sterile cotton swabs, samples were taken from the oral cavity, transported in a sterile medium, and then quickly moved to a microbiology lab for isolation and lab tests [18]. Although cases of candidiasis are rare, when appropriate control methods are abandoned, outbreaks can arise. Fungal infections are mostly caused by *Candida albicans*, and it is also the most often isolated etiological agent in cases of candidiasis. It can infiltrate host tissues and result in devastating infections in the right conditions. Because it suppresses the immune system or causes debilitating diseases, *C. albicans*, a kind of candida fungal, can cause sickness in both people and animals [19]. Numerous forms of candidiasis, including atrophic and hyperplastic candidiasis, pseudomembranous and erythematous candidiasis, and chronic mucocutaneous candidiasis syndromes, are caused by the *Candida* species. Many studies have demonstrated the pervasiveness of *Candida* species notably *Candida albicans*—in the environment. Many studies have highlighted the widespread presence of *Candida* species, notably *Candida albicans*, in the environment [20]. A 71% percentage of *Candida albicans* isolates were obtained through microscopical and diagnostic analysis of 70 samples per oral cavity chicken, with different outcomes. [21] who observed 13 (60%) of *C. albicans* isolates from the oral cavity of local chicken. The result is higher than study in the Egypt [22]. The presence of *Candida albicans* in test samples is 22.2% (n=8/36) from crop swabs. Regional variations caused by geographic location and environmental parameters including humidity, soil type, temperature, and bird species in the area could cause this variation [23]. The recent research results have confirmed the presence of the Metallo-aminopeptidase gene in 50 out of 50 cases (100%), and the identical ratio of the alkaline phosphatase gene. These genes are essential for the action of enzymes, which are involved in the conversion of *Candida* from a benign commensal organism to a pathogenic one, influenced by environmental factors that trigger the production of various virulence factors. The interplay between host factors and *Candida*-specific variables ultimately leads to the development of oral candidosis [24].

## 6. Conclusion:

The study concludes that *Candida albicans* is a major causative agent of thrush in local chicken, oral cavity isolates formed the highest percentage. Metallo-aminopeptidase gene the more virulence factors in the studied isolates. In

conclusion, the isolates were tested for highly Sensitive Ketoconazole, and Itraconazole. We recommend adding these antibiotics to poultry feed as a protection against fungi.

## 7. Recommendation:

Based on the previous discovery, the following suggestions have been proposed: To reduce bird exposure to fungal infection, precise management techniques, strict biosecurity, good disease detection, and suitable preventive measures must be implemented on a timely basis. To minimize exposure to airborne spores or reduce spore ingestion, environmental changes are required. Appropriate management approaches must be developed for dealing with mycotic illness.

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**Conflict of Interest:** The authors have disclosed that they do not have any conflicts of interest.

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