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Phenotypic and genotypic characterization of *Candida albicans* isolated from chicken

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ABSTRACT

Candidiasis is an occasional opportunistic yeast disease of importance in poultry has been reported to be in intestinal infection. The present study was directed mainly to throw lights on the isolation of *C. albicans* species from chicken and the identification of the isolates by phenotypic and genotypic methods. A total of 100 swabs samples collected from a crop of chicken from different localities in Cairo and Giza Governorates. The samples were investigated for mycological examination. The most frequent yeast species isolated from such swabs were Candida, Saccharomyces, Torulopsis and Rhodotorula species. The incidence of Candida species was (n=36/60, 60%), and the incidence of *Candida albicans* was (n=8/36, 22.2%) from crop swab. Early identification of yeast to the genus and species level was necessary for effective antifungal therapy and can also facilitate control such pathogens infections.

1. INTRODUCTION

Candida albicans (*C. albicans*) is the most common fungal pathogen in most clinical settings and the morphological flexibility plays a crucial role in several aspects of infection and host recognition (Swidergall, 2019). Candida organisms can be transmitted from infected parents to their nestlings through crop milk (Mugale et al., 2015). Candida albicans cause 95% of infections and the predominant pathogen. Early and accurate antifungal therapy would improve clinical outcomes. Antifungal susceptibility profiles differ substantially among species. Rapid and accurate identification of yeasts and yeasts-like isolates enables determining an effective antifungal therapeutic strategy (Diekema et al., 2012). Compare phenotypic systems for *C. albicans* identification Rap ID Yeast Identification panel and chromogenic media with multiplex PCR. All of the isolated yeasts 253 were tested for germ tube formation and then submitted to a multiplex PCR protocol tested in previous studies and to nine phonotypical commercial methods together with the reference ATCC strains. Comparison was limited to the ability of the tests to identify *C. albicans*. Although with differences in discriminatory power, the methods tested showed overall acceptable levels of sensitivity and specificity respect to the multiplex PCR, therefore, all could be useful for *C. albicans* identification where molecular differentiation is not available (Liguori et al., 2010).

The objective of this study was directed mainly to throw lights firstly on the isolation of *C. albicans* species of chicken and secondly to the identification of the mentioned isolates by phenotypic and genotypic methods.

2. MATERIAL AND METHODS

2.1. Collection of Samples:

A total of 100 random of chicken crop swabs were collected from different localities in Cairo and Giza Governorates. The collected samples were kept in sterile polyethylene bags and preserved in an ice box then transferred to the laboratory under complete aseptic condition without undue delay to be mycologically examined.

2.2. Isolation of yeast (Cruickshank et al., 1975).

2.2.1. Swab samples

The chicken crop swabs were inoculated into Sabouraud dextrose broth with chloramphenicol for 24-48 hours, and then transferred to acidify Malt extract agar plates. After inoculation the plates were incubated at 37 °C for 48 hours.

2.3. Identification of yeast isolates (Lodder and Krger-Van Rij, 1970)

2.3.1. Phenotypic methods of identification:

Phenotypic methods were done according to the morphological examination of colonies, biochemical test and Rap ID yeast plus system for identification.

*2.3.1.1. Macroscopically***:** by observation of the rate and pattern of growth, size, consistency and surface color of the isolated colonies (Finegold and Martin, 1982).

*2.3.1.2. Microscopically***:** by direct microscopic examination of the colonies using lacto phenol cotton blue

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(Himedia-India), Gram's stain (Cruick shank et al., 1975) and on Rice agar (Rieth, 1963), Germ tube test (Koneman *et al*., 1992).

2.3.2. Biochemical tests:

2.3.2.1. Sugar fermentation (Cruickshank, et al. 1975)

Isolated yeasts were inoculated into test tube containing 10 ml of 1% peptone, 2% sugar (glucose, galactose, maltose, sucrose and lactose). Bromocresol purple as indicator, Durham's tubes and incubated at 37 °C for 3-7 days. Change the colour into the yellow colour was considered as positive reaction.

2.3.2.2. Sugar assimilation tests (Lodder and Kreger Van Rij, 1970)

A nitrogen base medium devoid of any source of sugar was melted, cooled to 45 °C and then poured into sterile Petri dishes containing 2 ml of heavy yeast suspension and thoroughly mixed. After solidification, dishes were left to dry for one hour. Previously prepared and sterilized paper discs were soaked in a 20 % solution of sugar (glucose, galactose, maltose, sucrose and Lactose) and placed into the agar surface, then incubated at 25°C for 2-4 days. Growth of yeast around any disc considered as indication of sugar assimilation.

2.3.2.3. Nitrate assimilation tests (Terrence, 1971)

The nitrate assimilation medium was cooled to 45 °C then poured into sterile Petri dished containing 2 ml saline suspension of the suspected yeast isolate. After solidification, aseptically place discs containing 5% sterile potassium nitrate solution and peptone on the medium surface using a sterile forceps. Then the plates were incubated for 2-4 days at 37 °C. Check for growth around the peptone disc. A record result of growth around the potassium nitrate disc growth is a positive result.

2.3.2.4. Ureas test (Cruickshank, et al. 1975)

Christensen's urea agar slopes were inoculated with the yeasts and incubated at 25 °C for 2-4 days. Positive ureas production was indicated by the change of the yellow colour of the phenol red into pink colour.

2.3.3. RapID Yeast Plus System (Balows et al., 1991):

It uses enzyme technology to reduce the time-to-results to 4 hrs. The advantages: it provides a definitive answer to a clinically significant bacterial identification faster.

2.3.4. Extraction of genomic DNA from Candida albicans isolates: (Young and Do-Hyun, 2000)

DNA extraction from isolated using DNeasy plant Mini kit Qiagen Genomic as described by manufacturer manual of Qiagen, Germany. Cat. No.69104. Total chromosomal DNA extracted from *Candida albicans* strains were subjected to PCR with oligonucleotide primers identical to a bp fragment Oligonucleotide primer used in PCR reactions were synthesized by Chromogenic Company, (South Korea).

Forward LH1 AGC CACAAC AAC AAC AAC AAC TCT
Reverse LH2 TTGAGA AGG ATC TTT CCA TTG ATG.

3. RESULTS

The samples collected from chicken crop 100 swab were investigated for yeast species contamination and the result showed that (*n*= 60/100, 60%) were positive for yeast contamination.

The incidence of yeast genera isolated from chicken crop swabs showed that Candida spp. (*n*= 36/60, 60%) was the most frequent yeast species followed by Saccharomyces spp. (*n*= 11/ 60, 18.4%), Torulopsis spp. (*n*= 10/60, 16.6%), Rhodotorula spp. (*n*= 3/60, 5%).

Based on the morphology on SDA, fermentation, assimilation of sugar, culture on rice meal and germ tube formation for identification of isolated Candida species from all examined samples, and found *C. parapsilosis* was the most common yeast isolate from chicken crop swabs (*n*= 11/36, 30.5%) followed by *C. tropicalis* 10 (27.8%) , *C. albicans* (*n*= 8/36, 22.2%) , *C. guilliermondii* (*n*= 5/36, 13.9%), *C. pseudotropicalis* (*n*= 2/36, 5.6%) (Table 1).

Based on the morphology of SDA, culture on rice meal media and germ tube formation and Rap ID Yeast Plus System for identification of isolated *Candida albicans.*

Regarding the results tabulated in Table (2) shows the incidence of *Candida albicans* isolated from examining samples it seems was (*n*= 8/36, 22.2%) from crop swab.

Table 1 Incidences of yeast species from chicken sample:

Type of yeast species	Chicken Crop swabs	
	No.	%
Candida spp	36	60%
Saccharomyces spp	11	18.4%
Torulopsis spp	10	16.6%
Rhodotorula spp	3	5%
Total	60	100%

Table 2 Incidence of candida species from chicken sample.

Type of candida species	Crop swabs	
	No. of +ve	%
C. albicans	8	22.2%
C. guilliermondii	5	13.9%
C. parapsilosis	11	30.5%
C. tropicalis	10	27.8%
C. pesudotropicalis	2	5.6%
Total	36	100%

3.1. Microscopic examination of *Candida albicans* colonies

Colonies taken from Sabouraud-Dextrose Agar appeared as round to oval cells (Figure 1a)

3.2. Direct Microscopy on rice agar media

On rice meal agar a spherical thick wall Chlamydia spore, appeared to cluster of blastospores (Figure 1b).

3.3. Microscopic examination of Candida parapsilosis colonies.

Colonies taken from rice meal Agar appeared as elongated budding, short branches. (Figure 1c)

3.4. Microscopic examination of *Candida guilliermondii* colonies.
Colonies taken from rice meal agar appeared as clusters of small blastospores along the pseudo hyphae and particularly at septal points. Pseudo hyphae were short and few in number. The yeast cell divides by budding (Figure 1d).

3.5. Germ tube formation by *Candida albicans* in test tube.
The germ tube test of *C. albicans* using human serum revealed that all the isolates were germ tube producer (Figure 1e). The germination was tested after half an hour and 3 hours at 37 °C after incubation.

3.6. Identification by Rap ID Yeast plus System:
Identification of Rap ID Yeast system for *Candida albicans* isolates (Figure 1f).

3.7. Polymerase chain reaction:
Two *Candida albicans* isolates from chicken crop swab were examined by molecular methods polymerase chain reaction for confirmation of phenotypic identification.

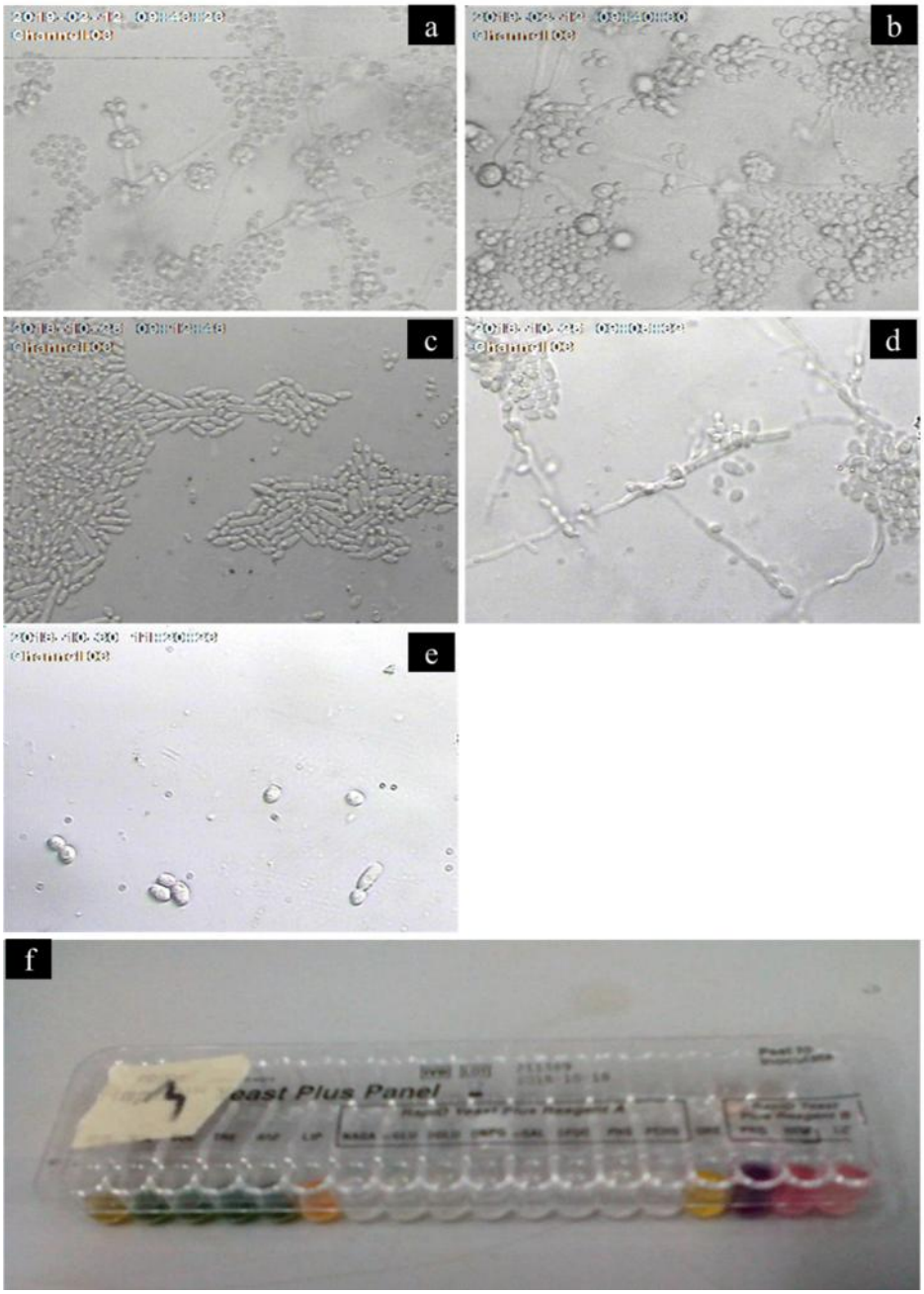

Figure 1 Microscopic and biochemical investigation of Candida. (a) Microscopy of *Candida albicans.* (b) Microscopy on rice meal agar media, chlamydospores and pseudo hyphae. (c) Microscopy of *Candida parapsilosis.* (d) Microscopy of *Candida guilliermondii* (e) Microscopy Germ tube test by using human serum after incubation 3hr. at 37 °C. (f) Rap ID Yeast system for *Candida albicans.*

Using of oligonucleotide primer for molecular identification of medical important yeasts. PCR for *LH1* and *LH2* regions confirmed the selected isolates as *candida albicans* which found at 344 bp on a 1.5 % agarose gel (Figure 2).

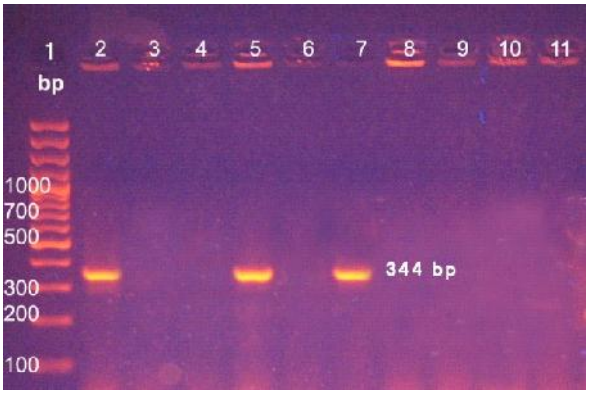

Figure 2 Agarose gel electrophoresis for *LH1*&*LH2* region gene of *C. albicans* isolated from chicken crop. Lane 1: Marker (GeneRuler100 bp DNA ladder, Thermo scientific™), Lane 2: Positive control for *C. albicans* (KX668262), Lane 3: negative control A. flavus (MF094441), Lanes 5, 7: Positive for *LH1*&*LH2* region *gene of C. albicans* with 344 bp amplicons, lanes 4, 6, 8-11: negative *LH1*&*LH2* region.

4. DISCUSSION

The incidence of yeast genera isolated from chicken crop swabs showed that Candida spp. (*n*= 36/60, 60%) was the most frequent yeast species isolated followed by Saccharomyces spp. (*n*= 11/ 60, 18.4%), Torulopsis spp. (*n*= 10/60, 16.6%), Rhodotorula spp. (*n*= 3/60, 5%) and this result was in agreement with that of Makhlouf (2003), Also, it agreed with El-Saadany (2014), who isolated 6 yeast genera from examined chicken samples and found that Candida species was the predominant yeast genera isolated from examined samples (30.28%), followed by Torulopsis spp. (26.15%). Candida albicans represented (22.72%) of the total isolated candida from swab samples 46 (92.0%). The incidence of yeast genera isolated from chicken swabs examined samples showed that Candida (31.43%) was the most frequent yeast species isolated. samples followed by Torulopsis (25.71%), Rhodotorula (21.43%), Trichosporon (10%), Cryptococcus (7.14%) and Saccharomyces (4.29%).

In the present study, the incidence of candida species isolated from the chicken crop results showed that Candida spp isolates (*n*=36, 60%) which agreed with Abd El-Tawab et al. (2015), who isolated Candida species isolated from chicken crop samples (*n*=42, 52.5%), Also Al-Temimay and Hassan (2016) isolated Candida spp. From poultry (*n*=62, 35.02%), Also Ismail et al. (2018), who identified of all isolates (n=30, with a total prevalence of 10.9%) belonged to Candida spp. and arranged as (*n*=20, 8.8%) from 227 chickens.

Based on the morphology on SDA, fermentation, assimilation of sugar, culture on rice meal and germ tube formation for identification of isolated Candida species from all examined samples, and found *C. parapsilosis* was the most common yeast isolate from chicken crop swabs (*n*= 11/36, 30.5%) followed by *C. tropicalis* 10 (27.8%), *C. albicans* (*n*= 8/36, 22.2%), *C. guilliermondii* (*n*= 5/36, 13.9%), *C. pseudotropicalis* (*n*= 2/36, 5.6%).

The obtained results revealed that all *C. albicans* colonies were creamy, past, soft and smooth on SDA at 37 ºC and microscopically by round to oval cells, appear purple with Gram stain and presence of budding cells. The above findings concerning the morphology of *C. albicans* which came in agreement with Refai et al. (1969), Jungerman and Schwartzman, (1972) and Abou-Elmagd et al. (2011), who differentiated between these different candida species depending on the morphological and cultural characters on Sabouraud dextrose agar.

Based on the morphology of SDA, culture on rice meal media and germ tube formation and Rap ID Yeast Plus System for identification of isolated *C. albicans*, the incidence of *C. albicans* isolated from examining samples was (*n*= 8/36, 22.2%) from chicken crop swab.

Using of oligonucleotide primer for molecular identification of medically important yeasts. PCR for LH1&LH2 region confirmed the selected isolates as *C. albicans* which was found at 344 bp on a 1.5 % agarose gel (Figure 2). PCR primers (LH1 and LH2), are based on sequence of the gene encoding the integrin like protein alpha-INT1p from *C. albicans*. The *C. albicans* gene INT1 is similar to vertebrate leukocyte integrin (Sabeeh et al., 2013; Singh and Raksha, 2013). Primer LH1 is located between nucleotides 401 and 424 on the sequence, while primer LH2 is located between 721 and 744 nucleotide sequences. The PCR assay showed that the primers (LH1 and LH2) were *C. albicans* specific. Oligonucleotide primers amplifying a 344 bp fragment on the integrin-like protein alpha-INT1p gene (alpha INT1) of *C. albicans* was synthesized for screening of *C. albicans* from clinical samples by the polymerase chain reaction (PCR).

5. CONCULSION

Early identification of yeast to the genus and species level is necessary for an effective diagnosis and can also facilitate control of infections. Attention must be paid toward accurate identification of Candida albicans as a cause of disease in chicken.

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