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Genetic variety among *Candida albicans* isolated from chicken and human clinical sample in Cairo Governorate, Egypt

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Abstract: *Candida albicans* was significant hazard for human and poultry throughout the world. This study is designed to investigate the prevalence of different members of *Candida spp.* in chicken and human and genetic characterization of isolated *C. albicans.* Two hundred swab samples (n=200) were collected from chicken crops (*n=100*), human vaginal swab (*n=50*) and human nasal swabs (*n=50*) between August 2018 to February 2019 from different localities in Cairo Governorate, Egypt. The swabs were subjected to *Candida* isolation and identification by using direct microscopy, conventional culture methods and Rap ID Yeast plus System®. Furthermore, they identified by sequencing of *αINT1* gene and RAPD PCR to investigate the genetic relatedness of *C. albicans* strains recovered from chicken and human origins. From the examined chicken crop swab (8/36, 22.2%) were positive of *C. albicans* isolation. In addition, (5/25, 20%) were positive of *C. albicans* isolation from the examined human vaginal swabs. Moreover, the examined human nasal swab (0/8) was negative for *C. albicans*. While, two *C. albicans* strains (one from chicken origin and one from human origin) were subjected to *αINT1* gene sequence analysis, which revealed that *C. albicans* from chicken origin were genetically distinct from that of the human origin. Nucleotide sequence analysis showed the rate of similarity greater than (97.15 %) for the entire alpha-INT1p (LH1 and LH2) gene sequence from 2 strains of *C. albicans*. While, amino acid sequence analysis showed the rate of similarity greater than (93.27%) for the entirealpha-INT1p (LH1 and LH2) gene sequence from *C. albicans* strains. Additionally, RAPD PCR clarified the heterogeneity among human and chicken *C. albicans* isolates. This study is of outmost importance regarding the role of molecular biology technique in detection the sources of *C. albicans* which help for effective diagnosis and differentiation between chicken origin and human origin.
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Keyword: *C. albicans, PCR, RAPD PCR*, *α*INT1 gene, chicken.

1. Introduction

Candida albicans is an opportunistic environmental pathogen that causes poultry and human candidiasis throughout the world. It is acquired through ingestion and becomes part of the flora of the avian alimentary tract that will predispose to infection under any immunosuppressive factors such as steroid usage, subclinical malnutrition, viral infection or other stressors (Velasco, 2000 and Jordan, et al., 2009). Chickens affected exhibit unsatisfactory growth, stunted appearance, rough feathers. However, when Candidiasis arises as a secondary infection, the signs of the predisposing disease predominate on the clinical signs (Asfaw and Dawit., 2017). In human, *C. albicans* infect about 138 million cases annually causing recurrent Vulvovaginal candidiasis (VVC) with symptoms of itching, burning and painful sensation, ruddiness of the vulva and vaginal mucosa, and discharge. It is also known to be a common cause of hospital-acquired systemic infections with mortality rates of up to 50% (Swidergall, 2019). Hence, the Early identification of *C. albicans* is crucial to provide appropriate treatment and control programs. Identification of *C. albicans* was based mainly on the morphological, biochemical and culture characters. Recently, molecular techniques were introduced as an alternative tool that allows rapid and accurate diagnosis as Real time PCR (Loëffler et al., 2000), Taq Man-based PCR Assay (Maaroufi et al., 2003), RAPD PCR (Shehata et al., 2008). Mention that the genotypic definitions of *Candida species* are molecular biological techniques allows beneficial alternative method. They were applied the PCR-RFLP size of about 376-930 bp and utilize global primers

amplified ITS region. PCR amplicons were the generated bans work in with to the foreseeable size. (El-Diasty et al., 2017). Nevertheless, most studies were concerned with human isolates with less consideration for poultry isolates. (Liu et al., 2018) conducted a molecular study to monitor the molecular epidemiology and the genetic relationship of *C. albicans* isolates from poultry.

The present study was designed to determine the prevalence of different *Candida spp*. in chicken and human with monitoring the genetic relatedness of *C. albicans* isolates by using *αINT*1 gene sequence analysis and RAPD PCR.

2. Material and Methods

2.1. Samples

Two hundred swabs samples (n=200) were collected from chicken crop (*n=100*), human vagina (*n=50*) and human nasal swabs (*n=50*). The sample werecollected from diverselocalities in Cairo Governorate, Egypt overa seven-month interval (August 2018–February 2019). The chosen human patients showed genital symptoms including (itching, white thick discharge that resembles cottage), respiratory symptoms (including cough, sneezing, and nasal discharge). Over the same period, 100 chicken crop swabs were collected from chicken suffering from poor appetite, delayed crop emptying, depression/ lethargy, white patches on the inside of their mouth, abnormal dropping.

2.2. Isolation of yeast

It was performed according to (Cruick Shank et al., 1975). The swab samples were inoculated into Sabouraud dextrose agar (Oxoid CM0041) with chloramphenicol (Biolife Italiana S.r.l. Viale Monza 272 REF 4240003) and incubated at 37^0C for 24-48 hours.

2.3. Morphological examination of yeast colonies

Direct microscopic examination of the isolated colonies was done using lactophenol cotton blue (Himedia-India), Gram's stain (Cruick Shank et al., 1975), Rice agar (Ajello.1981) and Germtubetest (Koneman et al., 1992).

2.4. Biochemical identification of Candida albicans

It was performed using Rap ID Yeast Plus System® (Thermo Fisher Scientific cat no. R8311007).

2.5. Molecular identification of Candida albicans isolates:

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

Candida *albicans* isolates were submitted for DNA extraction using DNeasy plant Mini kit (Qiagen, Germany, Cat. No. 69104) according to manufacturer's instructions.

2.5.2. PCR targeting aINT1 gene of Candida albicans:

Positive culture isolates were subjected for PCR targeting the species-specific aINT1 gene of *C. albicans* using forward primer *LH1*F 5' AGC CACAAC AAC AAC AAC AAC TCT 3' and reverse primer *LH2* R 5' TTGAGA AGG ATC TTT CCA TTG ATG 3' with amplicon size 344 bp (Young and Do-Hyun, 2000). PCR reaction was carried on S-1000 Thermal Cycler (Bio-RAD) in a total reaction volume of 50 µl containing 25 µL of Dream Taq Green PCR Master Mix (Thermo scientific™, K1081), 5 µL of target DNA ranging from 1 to 500 ng, and 2 µL of each primer (at 10 pmol/lL) with the addition of Nuclease free molecular biology grade water to 50 µL at thermal profile of 1 cycle of 93°C for 3 min; 30 cycles of 92°C for 1 min, 55°C for 1 min, 72°C for 1 min; and a final extension step at 72°C for 10 min. Amplicons were detected by electrophoresis on 1.5% agarose (Agarose; Sigma) stained by ethidium bromide and examined by transilluminator to observe the amplified PCR products.

2.5.3. Sequencing and Sequence Analysis of aINT1 gene of Candida albicans

The amplified fragments were purified using Gene Jet PCR purification kit (Thermo scientific™, Cat no. KO701) and sent to GATC biotech (GATC Biotech AG, Konstanz, Germany) for sequencing. The sequencing was conducted bi-directionally by using ABI 3730xl DNA analyser (Applied Bio systems™). Identification of homologies between nucleotide and amino acid sequences of *C. albicans* DNA sequences under investigation and other *Candida* published on GenBank was performed using BLAST 2.0 and PSI-BLAST search programs, (National Center for Biotechnology Information NCBI http://www.ncbi.nlm.nih.gov/). Nucleotide and amino acid sequences were aligned and compared to other *C. albicans* sequences published in GenBank using MegAlign (DNASTAR, Laser gene®, Version 7.1.0, USA). The phylogenetic trees were constructed using MegAlign for tree reconstruction of sequences by Neighbour-joining method based on Clustal W. Bootstrapping values were calculated using a random seeding value of 111 (Thompson et al., 1994). Clustal was used when end gaps were faced. Sequence divergence and identity percentage were calculated by MegAlign. The structural character of our protein sequence was identified with Protean (DNASTAR, Laser gene®, Version 7.1.0. USA). Human and chicken crop isolates were submitted to the GenBank Data base and given the following accession numbers: Human MK965917 (*C.albicans*-AYMAC1) and chicken MK965918 (*C.albicans*-AYMAC2).

2.5.4. The RAPD primer used in PCR: **(Shehata et al., 2008)**

RAPD PCR was performed by using GACA GACA GACA GACA primer. The reaction was carried on S-1000 Thermal Cycler (Bio-RAD) in a total reaction volume of 50 µl containing 25 µL of Dream Taq Green PCR Master Mix (Thermo scientific™, K1081), 5 µL of target DNA ranging from 1 to 500 ng, and 2 µL primer and Nuclease free molecular biology grade water up to 50 µL at thermal profile of39 cycles at 93ºC for 60Sec, 39 cycles of 50ºC for 60Sec, 39 cycles of 72ºC for 60sec and a final extension step at 72ºC for 7min.

3. Results and Discussion

Candida albicans is opportunistic yeast that colonizes on the skin as well as the mucous membrane of the genitals and intestinal mucosa of most healthy individuals (Weiner et al., 2019). There are several routes for transmission of this disease including ingestion of contaminated food or drinking water, contaminated environments such as contaminated litter, discharge sites for poultry operations, and areas contaminated with human rubbish (Barnes et al., 2003).

In this study, two hundred swab samples (n=200) collected from chicken crops (*n*=100), human vaginal swabs (*n*=50) and human nasal swabs (*n*=50) between August 2018 to February 2019 from different localities in Cairo city were submitted for Candida spp. The obtained results in chicken crop showed that Candida spp isolates were recovered from (*n*=36,60%). These findings were agreed with Abd-El Tawab et al. (2015) who isolated Candida species from chicken crop samples (*n*=42, 52.5%). AlsoAl-Temimay and Hasan, (2016), isolated Candida spp. from poultry (*n*=62, 35.02%), Also Radwan et al. (2018) recovered all fungal isolates (n=30, 10.9%) including Candida spp. at the rate of incidence as (*n*=20, 8.8%) from 227 chickens.

In the present work, based on the traditional mycological examination of the chicken crop swabs (n=100), *C. parapsilosis* was the most common isolated Candida spp. (*n*=11, 30.5%), followed by *C.tropicalis* (*n*=10, 27.8%), *C. albicans* (*n*=8, 22.2%), *C. guilliermondii* (*n*=5, 13.9%) and *C. pseudotropicalis* (*n*=2, 5.6%) (Table 1). These results agreed with the findings of Ali (2009) who isolated *Candida albicans* (*n*=68, 33.8%) from examined chicken crop swabs.

While, the mycological examination of the human vaginal swabs (n=50) showed that *C. parapsilosis* was the most common isolated *Candida spp.* (*n*=6, 24%) followed by *C. pseudotropicalis* (*n*=5, 20%), *C. tropicalis* (*n*=5, 20%), *C. albicans* (*n*=5, 20%) and *C. guilliermondii* (*n*=4, 16%). These results agreed with the finding of ElFeky et al. (2016) who isolated *Candida albicans* at rate of incidence (*n*=68 cases, 50.4%) from 125 patients vaginal swabs presented with a clinical picture suggestive of Vulvovaginal candidiasis (VVC).

On the other hand, Human vaginal swabs culturesyielded that *Candida albicans* was the dominant isolated species. Whereas, themycological examination of the human nasal swabs (n=50) revealed that *C. pseudotropicalis* was the most common recovered *Candida spp.* (*n*=3, 37%) followed by *C. tropicalis* (*n*=2, 25%), *C. guilliermondii* (*n*=2, 25%), *C. parapsilosis* (*n*=1, 12.5%) (Table1). These results agreed with that of Grant et al. (2016) who isolated the *C. tropicalis* (19%), and *C. parapsilosis* complex (13%). Also our results were agreed with (Muadcheingka and Tantivitayakul, 2015) who isolated *C. Tropicalis* (10.4%), *C. parapsilosis* (3.2%) and *C. guilliermondii* (0.4%).

In addition, *C. albicans* could not be isolated from human nasal swabs (table 1) which disagreed with the results of Andreas et al. (2005) who isolated *Candida albicans* (*n*= 37, 33%) from one hundred and fourteen samples of nasal mucus from 30 babies were examined. Also disagreed with results obtained by Muadcheingka and Tantivitayakul, (2015) who isolated *Candida* strains from 207 oral candidiasis patients. This *C. albicans* (61.6%) which was still the dominant species. They added that the proportion of mingled colonization with more than one Candida species was 18% from total cases.

Our results revealed that all *C. albicans* colonies were creamy, past, soft and smooth on SDA at 37 ^{0}C and microscopically by round to oval cells, appear purple with Gram stain and presence of budding cells. The above findings concerning the morphology of *Candida albicans*which come 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.

In the present study, the results of PCR targeting alpha-INT1 in isolated *C.albicans* showed that two isolates out of eightchicken crops isolate and two isolates out of five Human vaginal isolate were positive for detection the gene of alpha-INT1 *C. albicans* examined by molecular methods PCR for confirmation of phenotypic identification.

However, oneisolate of *C. albicans* was positive foralpha-INT1 of each human & chicken isolates which were selected randomly and submitted for sequencing of alpha-INT1. The obtained sequences were deposited at NCBI under accession no. chicken (MK965917) and accession no Human (MK965918). Nucleotide sequence analysis showed the rate of similarity greater than greater than (97.15 %) for the

entire alpha-INT1p (LH1 and LH2) gene sequence from two strains of *C. albicans*. While, amino acid sequence analysis showed the rate of similarity greater than greater than (93.27%) for the entirealpha-INT1p (LH1 and LH2) gene sequence from *C. albicans* strains. Our findings were come in agreement with that yielded by Osman et al. (2019) who reported that the homology of BLAST sequence analysis showed a high degree of similarity between the Egyptian isolates and two *C. albicans* isolates from humans in the United States (*C. albicans* SC5314 and *C. albicanis*-10261). They added that the rates of similarity were (96.3% to 98.4%) by NT sequence identity and (94.3% to 98.1%) by amino acid substitution identity. However, the degree of distinct divergence of (87.5–88.8%) and (84–86.3%) by identity for NT and amino acid sequences, respectively were observed when comparing *C. albicans* isolates with *C. dubliniensis;* a Candida spp. that is phylogenetically near to *C. albicans.*

The alpha-INT1p (LH1 and LH2) gene of these *C. albicans* strains was distinguished from those of the other species by the sex-base insertion (AGCCCC) of chicken (MK965917) at positions (N1, N2, N3, N196, N263, N308). While, in human, the ten-base insertion (MK965918) at positions (N, N2, N3, N196, N263, N308, N317, N318, N319 and N320) their distinctive nucleotide changes (AGCTTAACAA). These differences produced extra amino acids. A detailed comparison of the alpha-INT1p (LH1 and LH2) gene demonstrated that certain base variations could be used to differentiate *C. albicans* strains from chicken and *C. albicans* strains from Human. These differences comprise transitions at positions A.A1, A.A7, A.A30, A.A49, A.A57, A.A105, A.A106, A.A107, A.A108, and A. A109. Some of these variations resulted in (P, T, T, M, M, M, Q, H, Q, W) amino acid changes. Phylogenetic trees were constructed based on the alpha-INT1p (LH1 and LH2) gene and the predicted protein sequences, Figure (1).

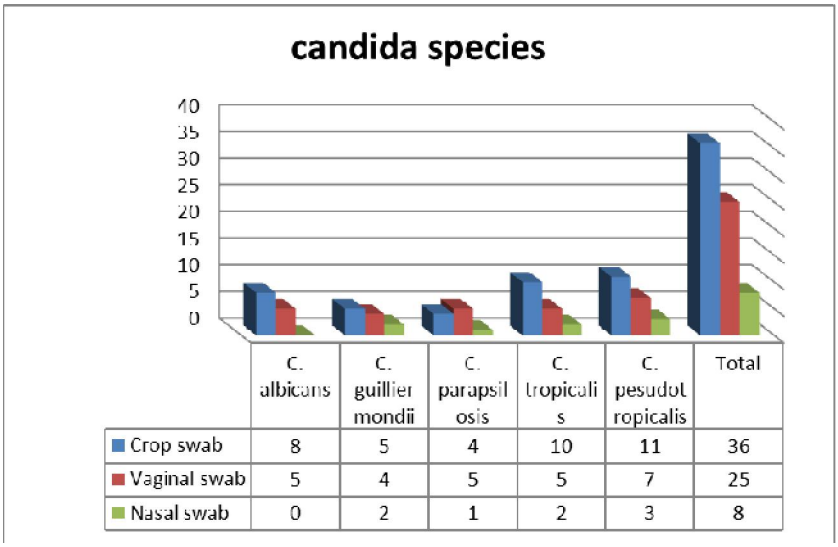
However, the phylogenetic analysis of nucleotides showed that chicken accession no. (MK965917) was isolated in different branches, away from Human accession no. (MK965918). While Phylogenetic analysis of amino acids showed that chicken accession no. (MK965917) is still in the near group of Human accession no. (MK965918), Figures (4,5).

The second PCR-based method used in this study utilized the short oligonucleotide (GACA) 4 as a primer for identification of *Candida albicans* isolates. Four isolates of *C. albicans* (two from chicken origin and two from human origin) were amplified with this simple, repetitive primer, and the numbers of the resulting PCR bands were reported. The two isolates of *C. albicans*recovered from human gave nearly the same pattern of 6 bright bands, (approximately 2349 bp, 2134 bp, 1981bp, 1610 bp, 749 bp and 649 bp) with strong band of 2134 bp, 2134 bp andone faint band of 649 bp. Whereas, the two *C. albicans* strains from chicken showed another profile of 4 bands, (2443bp, 1981bp, 1038bp and833bp). These results agreed with the obtained findings by Abou-Elmagd et al. (2011) who detected that the use of the RAPD-PCR for *C. albicans* strains isolated from chicken origins, using OPA-18 primer indicated the presence of 2 distinctive bands in all tested strains, which means that these bands are specific for *C. albicans* and might be used for diagnosis.

Table 1: prevalence of *Candida* species from chicken sample and Human sample.

Type of candida species	Crop swabs		Vaginal swabs		Nasal swabs	
	No. of +ve	%*	No. of +ve	%**	No. of +ve	%***
C. albicans	8	22.2%	5	20%	0	0
C. guilliermondii	5	13.9%	4	16%	2	25%
C. parapsilosis	11	30.5%	6	24%	1	12.5%
C. tropicalis	10	27.8%	5	20%	2	25%
C. pseudotropicalis	2	5.6%	5	20%	3	37.5%
Total	36	100%	25	100%	8	100%

*Percentage in ration to the total number of Crop swabs (*n* =36).
**Percentage in ration to the total number of Vaginal swabs (*n* =25).
***Percentage in ration to the total number of Nasal swabs (*n* =8).



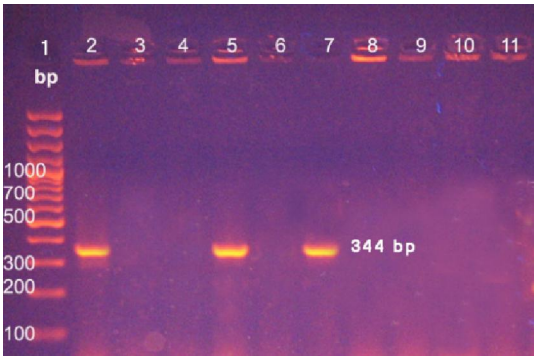


Fig (1): Agarose gel electrophoresis for *aINT1* gene of *C. albicans* isolated from chicken crop. Lane 1: Marker (GeneRuler100 bp DNA ladder, Thermo scientific™), Lane 2: Positive control for *C. albicans* (KX668262), Lane 3: negative control A. flavus (MF094441), Lanes 5, 7: Positive for *aINT1 gene of C. albicans* with 344 bp amplicons, lanes 4, 6, 8-11: negative *aINT1*.

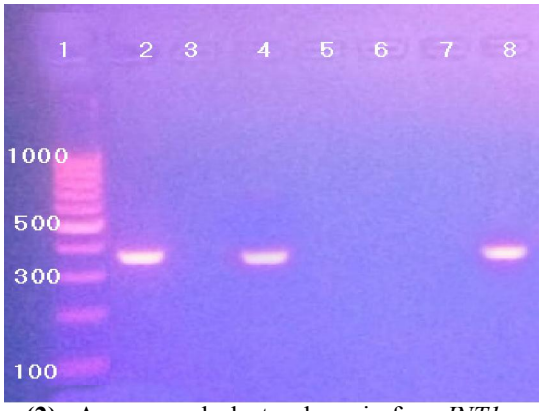


Fig (2): Agarose gel electrophoresis for *aINT1* gene of *C. albicans* isolated from chicken crop. Lane 1: Marker (GeneRuler100 bp DNA ladder, Thermo scientific™), Lane 2: Positive control for *C. albicans* (KX668262), Lane 3: negative control A. flavus (MF094441), Lanes 4, 8: Positive for *aINT1 gene of C. albicans* with 344 bp amplicons, lanes 5, 6, 7: negative *aINT1*.

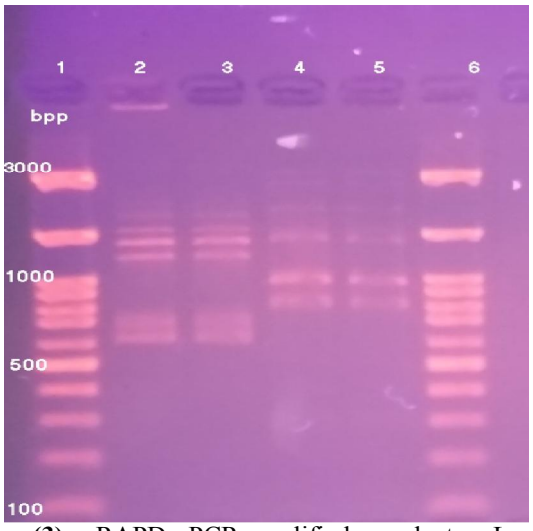


Fig (3): RAPD PCR-amplified products. Lanes contained the following: Lane1 and Lane 6: 100- 3000 bpDNA ladder, Lane2 and Lane3: which consisted of bright bands (approximately 2349 bp,2134 bp,1981bp,1610 bp,749 bp and 649 bp) human positive *C.albicans*, Lane 4 and Lane 5: consisted of bands, ranging from (2443 bp,1981bp,1038bp and 833bp) in size chicken positive *C. albicans*.

1-Percent Identity Matrix - created by Clustal 2.1

1:C.albicans-SC5314-GTH12-5A	100.0 100.0 97.15 97.72 96.38 97.47 97.12 97.15 97.15 97.15
2:C.albicans-SC5314-Int1p	100.0 100.0 97.15 97.72 96.38 97.47 97.12 97.15 97.15 97.15
3:C.albicans-TIMM-768	97.15 97.15 100.0 96.74 96.71 98.42 98.08 98.10 98.10 98.10
4:C.albicans-Cand-EWE.7-bov-EG2015	97.72 97.72 96.74 100.0 98.44 97.72 98.75 98.77 98.70 98.70
5:C.albicans-Cand-EWE.8-bov-EG2015	96.38 96.38 96.71 98.44 100.0 97.70 98.74 98.75 98.6898.68
6:C.albicans strain SOHILA1	**97.47 97.47 98.42 97.72 97.70 100.0 99.04 99.05 99.05 99.05**
7:C.albicans-Cand-Elsaadany	97.12 97.12 98.08 98.75 98.74 99.04 100.0 100.0 100.0 100.0
8:C.albicans strain SOHILA2	**97.15 97.15 98.10 98.77 98.75 99.05 100.0 100.0 100.0 100.0**
9:C.albicans-SC5314-P0	97.15 97.15 98.10 98.70 98.68 99.05 100.0 100.0 100.0 100.0
10:C.albicans-SC5314-GTH12-5B	97.15 97.15 98.10 98.70 98.68 99.05 100.0 100.0 100.0 100.0

1. Phylogenetic tree of nucleotide

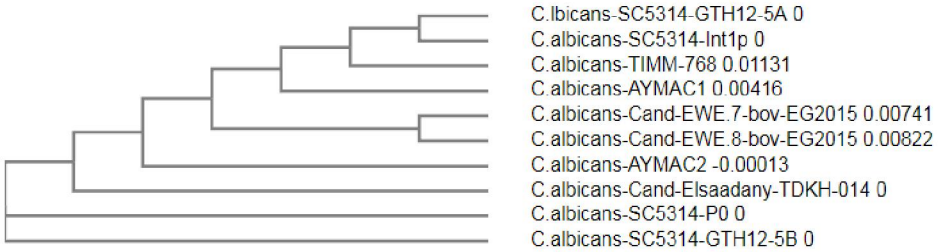


Fig. 4. Phylogenetic of nucleotide analysis of (*C.albicans*-AYMC1) chicken and (*C.albicans*-AYMC2) Human.

2. Percent Identity Matrix - created by Clustal2.1

1:C.albicans-SC5314-GTH12-5B 100.0	96.00 96.15 96.15 94.23 95.19 95.19 96.15 99.03 98.02
2:C.albicans-Cand-EWE.8-bov-EG2015	96.00 100.0 95.00 95.00 92.00 92.45 93.00 94.00 97.14 97.17
3:C. albicans-SC5314-GTH12-5	96.15 95.00 100.0 100.0 94.23 93.27 93.27 96.15 97.09 98.02
4: C. albicans-SC5314-Int1p	96.15 95.00 100.0 100.0 94.23 93.27 93.27 96.15 97.09 98.02
5: C.albicans strain SOHILA1	**94.23 92.00 94.23 94.23 100.0 99.04 99.04 94.23 95.15 94.06**
6: C.albicans strain SOHILA2	**95.19 92.45 93.27 93.27 99.04 100.0 100.0 93.27 95.37 94.39**
7: C. albicans-SC5314-P0	95.19 93.00 93.27 93.27 99.04 100.0 100.0 93.27 96.12 95.05
8: C. albicans-TIMM-768	96.15 94.00 96.15 96.15 94.23 93.27 93.27 100.0 97.09 96.04
9: C. albicans-Cand-Elsaadany	99.03 97.14 97.09 97.09 95.15 95.37 96.12 97.09 100.0 99.06
10:C.albicans-Cand-EWE.7-bov-EG2015	98.02 97.17 98.02 98.02 94.06 94.39 95.05 96.04 99.06100.0

2-Phylogenetic tree of amino acid

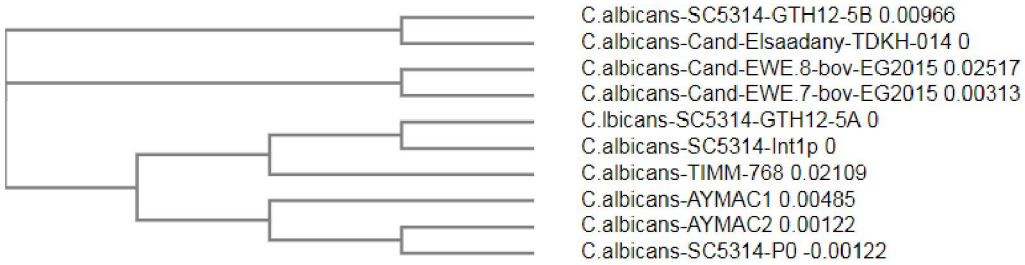


Fig. 5. Phylogenetic of amino acid analysis of (*C.albicans*-AYMC1) chicken and (*C.albicans*-AYMC2) Human.

4. Conclusion

This study can be concluded that the high incidence rates of *C. albicans* in chicken crop swabs and human vaginal swabs in different localities in Cairo city, Egypt. The Phylogenetic analysis of the aINT1 (LH 1 and LH 2) gene from four *C. albicans* strains (two from chicken origin and two from human origin) revealed that *C. albicans* from chicken origin were genetically distinct from that of the human origin. Additionally, RAPD PCR clarified the heterogeneity among human and chicken *C. albicans* isolates. Furthermore, the study recommends the use of the phylogenetic analysis of the aINT1 (LH 1 and LH 2) gene as a marker to study inter- and

intraspecies genetic relationships between *C. albicans* isolates from chicken origin and human origin. Further investigations are required to elucidate potential routes associated with the advanced molecular methods for detection of serious inter- and intraspecies genetic relationships because they are fast, sensitive.

5. Acknowledgement

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6. Conflict of Interest

The authors declare no conflict of interest.

Reference

1. Abd El Tawab A. Ashraf, Ahmed, A. A. Maarouf, Fatma. I. El-Hofy and Khalid, S.M. Ahmed (2015). Molecular characterization of some fungi isolated from broiler chicken farm. Benha veterinary medical Journal, Vol 29: 2:106 - 118. https://doi.org/10.21608/bvmj.2015.31682.
2. Abou-Elmagd S, Kotb H, Abdalla K, Refai M (2011). Prevalence of *Candida albicans* and *Cryptococcus neoformans* in Animals from Quena Governorate, with Special Reference to RAPD-PCR Patterns. Journal of American Science, Vol 7: 20-31.
3. Ajello, L. (1981). Laboratory Handbook of Medical Mycology. Archives of Dermatology, 117(7), 450. https://doi.org/10.1001/archderm.1981.01650070078043.
4. Ali. A, F., (2009). Studies on differences between virulence induced experimentally by *Candida albicans* isolated from different species. M. V. Sc. Thesis. Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Zagazig University.
5. Al-Temimay, I.A., Hasan, A.M., (2016). Isolation and identification of fungi from the droppings of some poultry and some detergents effect on some of them. Iraqi Journal of Science, Vol 57: 2634-2640.
6. Asfaw, M. and Dawit, D. (2017). Review on Major Fungal Disease of Poultry, British Journal of Poultry Sciences 6 (1): 16-25, 2017ISSN 1995-901X© IDOSI Publications.
7. Barnes, H., Vaillancourt, J., Gross, W. (2003). Collibacillosis. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE (eds), Disease of Poultry. Ames (Iowa State Press): pp 631-656.
8. Cruick Shank, R., Duguid, J.P., Marimion, B.P. and Swain, R.H. (1975). Medical Microbiology, the Practice of Medical Microbiology. Churchill Livingstone Limited, Edinburgh, London and New York, 12th Ed, Vol. 11: pp. 196-202. https://doi.org/10.1099/00222615-9-3-366a
9. El-Diasty, E.M., Ibrahim, E.-H., Abd, M., Khalafawy, E., Kamal, G., (2017). Isolation and Molecular Characterization of Medically Important Yeasts Isolated from Poultry Slaughterhouses and Workers. Pakistan Journal of Zoology,Vol. 49: 609-614. https://doi.org/10.17582/journal.pjz/2017.49.2.609.614.
10. ElFeky, D.S., Gohar, N.M., El-Seidi, E.A., Ezzat, M.M., AboElew, S.H., (2016). Species identification and antifungal susceptibility pattern of Candida isolates in cases of vulvovaginal candidiasis. Alexandria Journal of Medicine, Vol52: 269–277. https://doi.org/10.1016/j.ajme.2015.10.001.
11. Grant, M.L., Parajuli, S., Deleon-Gonsalves, R., Potula, R., Truant, A.L., (2016).Comparative evaluation of the BD Phoenix yeast ID panel and remel RapID yeast plus system for yeast identification. Canadian Journal of Infectious Diseases and Medical Microbiology, Vol 2016:pp1-4. https://doi.org/10.1155/2016/4094932.
12. Jordan, F., M. Pattison, P.F. Mcmullin, J.M. Bradbury and D.J. Alexander, 2009.Poultry Disease: Fungal disease, 6 ed. pp: 428-441.
13. Jungerman PF, Schwartzman RM (1972). Veterinary medical mycology. Veterinary medical mycology. Vol 1972:200.
14. Koneman, E.W.; Allen, S.D.; Janda, W.M.; Scheckenberger, P.C and Winn, W.C. (1992). Color Atlas and textbook of Diagnostic Microbiology. 4th Ed. J.B: p. 243–277. Lippincott Company. Philadelphia.
15. Lackner, A., Stammberger, H., Buzina, W., Freudenschuss, K., Panzitt, T., Schosteritsch, S., & Braun, H. (2005). Fungi: A Normal Content of Human Nasal Mucus. American Journal of Rhinology, 19(2), 125–129. https://doi.org/10.1177/194589240501900203.
16. Liu, J., Liu, H., Yan, J., Liu, N., Zhang, H., Zhao, C., Liu, Y., (2018). Molecular typing and genetic relatedness of 72 clinical *Candida albicans* isolate from poultry. Veterinary microbiology Vol 214: 36-43. https://doi.org/10.1016/j.vetmic.2017.11.030.
17. Loëffler, J., N. Henke, H. Hebart, D. Schmidt, L. Hagmeyer, U. Schumacher, and H. Einsele.2000.

Quantification of fungal DNA by using fluorescence resonance energy transfer and the Light Cycler system. J. Clin. Microbiol. 38:586–590. https://doi.org/10.1128/jcm.38.2.586-590.2000.

18. Maaroufi, Y., Heymans, C., De Bruyne, J.-M., Duchateau, V., Rodriguez-Villalobos, H., Aoun, M., & Crokaert, F. (2003). Rapid Detection of *Candida albicans* in Clinical Blood Samples by Using a TaqMan-Based PCR Assay. Journal of Clinical Microbiology, 41(7), 3293–3298. doi:10.1128/jcm.41.7.3293-3298.2003. https://doi.org/10.1128/jcm.41.7.3293-3298.2003.
19. Muadcheingka, T., Tantivitayakul, P., (2015). Distribution of *Candida albicans* and non-albicans Candida species in oral candidiasis patients: Correlation between cell surface hydrophobicity and biofilm forming activities. Archives of oral biology Vol60: 894-901. https://doi.org/10.1016/j.archoralbio.2015.03.002.
20. Osman Kamelia. Eman E. Abdeen. Walid S. Mousa. Walid Elmonir, Eman M. El Diasty and Ayman Elbehiry (2019). Genetic Diversity among *Candida albicans* Isolated from Humans and Cattle with Respiratory Distress in Egypt. Vector-borne and zoonotic diseases Volume 19: 199-206. https://doi.org/10.1089/vbz.2018.2321
21. Radwan A. Ismail, Ahmed H. Abed and Athar S. Abdallah (2018). Prevalence of fungal pathogens in broiler chickens and their environment. Vet. Med. Resear. 25 (2): 174-181. https://doi.org/10.21608/jvmr.2017.43316.
22. Refai M, Gobba A, Rieth H (1969). Monograph on yeasts. Diagnosis, diseases, and treatment. Veterinary Medical Journal, Giza, Vol 16: 255-316.
23. Rieth, H. (1963). Hefepilze als Kranlerreger, bei Mench and Tier. Springer, Berlin Guttingen Heidelberg.
24. Saleh, H.A., Moawad, A.A., El-Hariri, M., Refai, M.K., (2011). Prevalence of yeasts in human, animals and soil sample at El-Fayoum Governorate in Egypt. Int J Microbiol Res. Vol2: 233-239.
25. Shehata AS, Mukherjee PK, Aboulatta HN, El Akhras AI, Abbadi SH, Ghannoum MA (2008). Single-step PCR using (GACA) 4 primer: utility for rapid identification of dermatophyte species and strains. Journal of clinical microbiology. Vol *46*(8): 2641-2645. https://doi.org/10.1128/jcm.00697-08.
26. Swidergall, M., (2019). *Candida albicans* at Host Barrier Sites: Pattern Recognition Receptors and Beyond. Pathogens8: pp1- 40. https://doi.org/10.3390/pathogens8010040.
27. Thompson D, Higgins G, Gibson J, Clustal W.)1994. (Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nuc Acids Res. Vol 22:4673–4680. https://doi.org/10.1093/nar/22.22.4673.
28. Velasco, M. C. (2000). Candidiasis and cryptococcosis in birds. Seminars in Avian and Exotic Pet Medicine, 9(2), 75–81. doi:10.1053/ax.2000.4620. https://doi.org/10.1053/ax.2000.4620.
29. Weiner A, Orange F, Lacas‐Gervais S, Rechav K, Ghugtyal V, Bassilana M, Arkowitz RA (2019). On‐site secretary vesicle delivery drives filamentous growth in the fungal pathogen *Candida albicans*. Cellular microbiology. Vol 21: e12963. https://doi.org/10.1111/cmi.12963.
30. Young, H. L. and Do-Hyun, L. (2000). Rapid PCR Method for Detecting *Candida albicans* Using Primers Derived from the Integrin-like Protein Gene INT1of *Candida albicans* The Journal of Microbiology, vol 38(2):105-108.

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