Kufa Journal for Veterinary Medical Sciences Vol.4 No.1 (2013) 105-110

Kufa Journal for Veterinary Medical Sciences
www.vet.kufauniv.com

Genotyping of *Candida albicans* Isolated from broilers by 25S rDNA Analysis

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Abstract:

Candida albicans one of the most fungi which isolated from clinical samples that cause many problems to humans, animals and birds. Candidiasis is an opportunistic endogenous mycosis in that perturbance of the microflora or other debilitation of the host. So, this study come to focused on genotyping and antifungal susceptibility profile to *Candida albicans* isolated from broilers.

Ten isolates of *Candida albicans* were obtained from crop of broilers, samples was collected from poultry diseases lab. in Najaf veterinary hospital of different cases of enteritis broilers. All isolates were identified morphologically and biochemically by Hicandida identification kit and genotyped according to 25s rDNA of transposable intron I region of chromosome R.

The results showed that all isolates belonged to the genotype A with amplification product (450 bp). Antifungal sensitivity profile by disc diffusion method showed no resistant to nystatin, ketoconazole and micanazole while three isolates (30%) showed resistant to fluconazole.

Key: *Candida albicans*, genotyping, 25S rDNA, PCR.

التصنيف الجيني للمبيضات البيضاء المعزولة من دجاج فروج اللحم باعتماد تحليل, 25S rDNA

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الخلاصة:

تعتبر المبيضات البيضاء من أكثر الفطريات عزلا من العينات السريرية والتي تسبب الكثير من المشاكل للأنسان والحيوان والطيور على حد سواء .

أن داء المبيضات من الأمراض الأنتهازية والتي ترتبط بالمضيف بدرجة كبيرة وقد جاءت هذه الدراسة للتعرف على الأصناف الجينية وحساسيتها للمجموعة من المضادات الفطرية لعزلات معزولة من دجاج اللحم.

تم أستحصال عشر عزلات من المبيضات البيضاء من قانصات دجاج اللحم، جمعت العزلات من مختبر أمراض الدواجن في المستشفى البيطري في النجف الأشرف من حالات مختلفة من الفروج المصاب بحالات اسهال. جميع العزلات شخصت على أساس الشكل والتفاعلات الكيمياء الحيوية باستخدام العدد الخاصة ومن ثم صنفت جينيا بالاعتماد على 25S rDNA, PCR . أظهرت النتائج ان جميع العزلات تحت الصنف الجيني حيث حجم المنتج تفاعل البلمرة المتكرر قاعدة نايتروجينية .الحساسية للمضادات الفطرية بطريقة الأنتشار بالأقراص اظهرت عدم وجود مقاومة للنستاتين والكليتوكونازول والميكازول بينما ثلاث عزلات (30 %) كانت مقاومة للفلوكونازول .

Introduction:

Candida sp. are part of the microflora of healthy digestive system of humans, animals and birds. The term thrash is applied to *Candidia* infection of the upper digestive tract. Thrash has been observed in chickens, turkey, gees, pigeon, Guainía fowl, pheasant, quell, peacock and lovebirds (1, 2, 3.) The occurrence of avian candidiasis is sporadic, but outbreaks resulted in mortality of up to 20% in young turkeys (4).

However, the disease is generally asymptomatic and diagnosis occurs postmortem. The most frequent lesion is found in the crop and consists of plaques that resemble curdled milk and adhere lightly to the mucosa. In adult birds, candidiasis has a chronic course and causes thickening of the crop wall, on which a yellowish necrotic material accumulates. (5)

Immune dysfunction can allow *C. albicans* to switch from a commensal to a pathogenic organism capable of infecting a variety of tissues and causing a possibly fatal systemic disease.6

The first major reported outbreak resulted in mortality of up to 20% in young turkeys (5), and another report described the loss of 10,000 chicks due to the Candida infection(7). A recent outbreak resulted in 40% mortality in a flock of 6week-old turkey (8) .

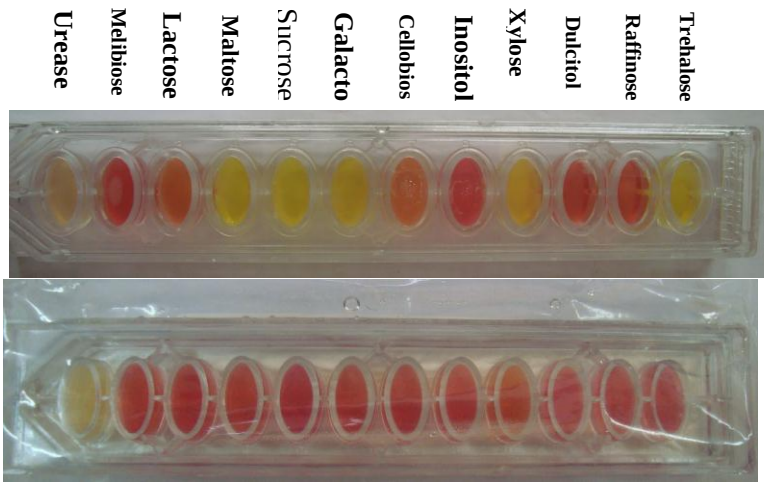
Genotyping and molecular characterization of *C. albicans* is important for epidemiological studies and for this purpose McCullough (10) developed a polymerase chain reaction (PCR) method using a primer pair designed to span V3 region in chromosome R, that includes the site of the transposable group I intron of the 25S rRNA gene. This method has been shown to be able to classify *C. albicans* strains into five genotypes on the basis of the amplified PCR product size: genotype A (450 bp), genotype B (840 bp), genotype C (450 and 840 bp), genotype D (1,080 bp) and genotype E (1,400 bp product) (10, 11, 12).

In Iraq, genotype A *C. albicans* was previously detected from turkey (Samaka, et al., 2012) and genotype A, B and C from humans (Samaka et al., 2013)

The aim of our study is to investigate the genotyping of *C. albicans* which were isolated from broiler and antifungal susceptibility profiles for these isolates.

Materials and Methods:

Clinical Isolates: Ten *C. albicans* isolates obtained from twenty-tow crop swaps from cases of broiler enteritis. All isolates were identified on the basis of colony morphological characteristics on Sabouraud's dextrose agar (SDA), Candida-CHROMagar media (CHROM agar microbiology, France), germ tube production in fresh human serum, chlamydospors production on Corn Meal-Tween-80(CM-Tween80) agar, and final identification done by using HiCandida Identification Kit (HiMedia, India) according to the manufacturer's instructions figure – 1



Figuer – 1, the first one Hicandida kit after incubation with *candida* (red or pink +ve; yellow –ve except the first well is opposite of others.) The second one Hicandida kit before inoculation with *candida* colonies.

DNA extraction: Genomic DNA was extracted using by the DNA-Pure Yeast Genomic Kit (bioWorld, USA) according manufacturer's instructions. Extracted DNA was precipitated by absolute isopropanol and washed with ethanol 70% and then transferred to a sterile eppendorf tubes and stored at -20°C prior to PCR.

Primers:

The primer pairs CA-INT-L (ATAAGGGAAGTCGGCAAAATAGAT CCGTAA) and CA-INT-R (CCTTGGCTGTGGTTTCGCTAGATAG TAGAT) were used to amplify 25S rRNA in chromosome R of *C. albicans* as described by McCullough.(10)

Amplification reactions were performed in 25µl final volume containing 12.5 mastermix (promega, USA), 1.25µl (25 pmol) each of the primers and 5µl DNA template and complete the volume by PCR grade water. The reaction mixtures were subjected to the following thermal cycling parameters in a TECHNE TC-300 (Bibby Scientific,USA): 94°C for 3 min followed by 30 cycles of 94°C for 1 min, 55 °C for 1 min, 72°C for 2.5 min and a final extension at 72°C for 10 min following the last cycle. All reaction products were characterized by electrophoresis on 1.5% agarose-ethidium bromide gel in 1X TBE buffer at 100 V for 60 min. and visualized in Gel documentation system (Vision-SCIE-PLAS. UK) and data analyzed by Gene tool analysis software (SynGen, UK).

Antifungal susceptibility testing:

Antifungal susceptibility to the following antifungals nystatin, fluconazole, miconazole and Ketoconazole (HiMedia,India)were done by using disc diffusion method on Muller-Hinton-Bromothymol blue agar (MHB) (13), and read the result as diameter of visible inhibitory growth zone around the antifungal disc on the media. Figure-3 and according to (CLSI,2002)

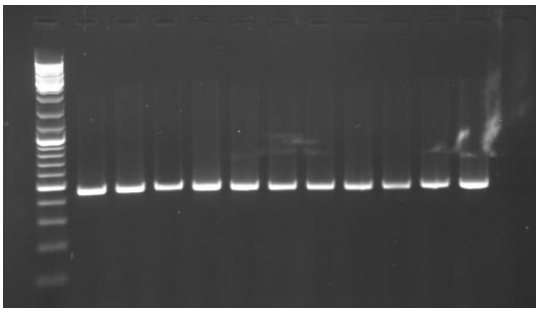


Fig 3: PCR genotyping with primer CA-INT; lane M,100bp ladder; lane CP, stander strain (control strain)* (genotype A) as control positive; other lanes represent *Candida albicans* genotype A (450bp).

Results and Discussion:

All *Candida albicans* isolates grow as green colony on Candida- CHROOMagar media, all isolates produce germ tube in fresh human sera and produce chlamydospores on CM-Tween 80 media.

Polymerase Chain Reaction amplification results showed single amplification product (450bp) for all isolates and this categorize all isolatesas genotype A *C. albicans* (Figure 3), this agrees with previous studies that identified *Candida albicans* genotype A as predominant in clinical samples.[11,12,13,14,15,16,17] . The results agree with Samaka et al. (2012) who detected genotype A as only genotype in *C. albicans* isolated from turkeys in Iraq, and also agree with Samaka et al. (2013) which observed genotype A as predominant genotype in *C. albicans* isolated from humans in Iraq. All the 10 isolates of *Candida albicans* were sensitive to miconazole, ketoconazole and nystatin (Table - 1). Three (30%) isolates were resistant to Fluconazole with inhibition zone ≤ 5mm, while 2(20%) isolates were sensitive-dose depended (SDD) with inhibition zone ranged (5-8mm).

Table 1: Antifungal susceptibility test to *C. albicans* isolated in this study by disc diffusion method.

antifungals	DDT for *C. albicans* (ATCC10231)	No. of isolates with DDT			%S	%R
		S	SDD	R		
Nystatin	21-25mm	10	-	-	100%	0%
Miconazole	19-22mm	10	-	-	100%	0%
Ketoconazole	25-28mm	10	-	-	100%	0%
Fluconazole	25-28mm	5	2	3	50%	30%

DDT=disc diffusion test
S=sensitive
R=resistant
SDD=susceptible dose dependent
* obtained from samaka 2012

No clear data available about antifungal sensitivity to *Candida albicans* isolated from animals, but the results of this study agree with (Samaka et al., 2012) whose found that there is no resistance to ketoconazole in isolates collected from turkeys but data obtained from this study found that three isolates were resist to fluconazole and this disagree with (Samaka et al., 2012) who confirms the scarcity of resistance of *C. albicans* isolated from clinical samples to antifungal. (19, 20, 21), we believe that rarity to antifungal exposure and short life period of broilers included in this study prevented antifungal resistant to develop and this opinion strongly supported by (22, 23).

Figure-4: Sensitivity test for candida isolates show resistance to Fluconazole and sensitive to nystatin, miconazole and ketoconazole.

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