

Genotyping and antifungal susceptibility profile of *Candida albicans* isolated from neonatal thrush infections in Iraq

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الخلاصة

يعد التهاب باطن الفم thrush بخميرة المبيضات البيضاء *C. albicans* من المشاكل الصحية المهمة في وحدات العناية المركزة للاطفال الخدج في كل دول العالم وفي مستشفياتنا ايضا لذلك هذه الدراسة سلطنا الضوء على التنميط الجيني والحساسية الدوائية للمضادات الفطرية ل 22 عزلة من خميرة المبيضات البيضاء المعزولة من اطفال مصابين في مستشفى الزهراء التعليمي في محافظة النجف فحصت 33 حالة مصابة للفترة من شباط الى اب / 2010 اذ عزلت 25 عزلة وبنسبة 75.75 % من خميرة *C. albicans* فقط 22 عزلة (66.6%) تم تشخيصها على انها *C. albicans* و3 عزلات اخرى 9% تعود الى انواع اخرى من الخمائر. اما التخطيط الجيني لمنطقة الانترون المتنقلة لعزلات *C. albicans* اظهر ان 21 (95.45%) من العزلات تعود للنمط الجيني نوع A وعزلة واحدة (4.54%) تعود للنمط الجيني B. وكانت نتائج فحص الحساسية الدوائية عدم ظهور مقاومة لمضاد الامفوتريسيبي- B بينما 3 عزلات اظهرت مقاومة عرضية cross- resistans لمضاد الفلوكانازول والاتراكونزول والكيوتوكانازول ، بينما عزلة واحدة (4.54) اظهرت مقاومة عرضية لمضادي الفلوكونازول والكيوتوكانازول.

Abstract

Thrush was an important problem in Neonatal Intensive Care Units (NICUs) in all the world and also in our hospitals. So, this study come to focused on genotyping and antifungal susceptibility to the 22 isolates of *Candida albicans* obtained from thrush babies in (NICU) in Al-Zahra educational Hospital, an-Najaf governorate, Iraq.

From February to August 2010, 33 cases of thrush were tested. A total of 25(75.75%) *Candida* isolates were obtained, only 22(66.6%) isolates were identified as *Candida albicans* and the others 3(9%) isolates were identified as *Candida* other than *albicans*.

The genotyping of the transposable intron region of *C. albicans* strains showed that 21(95.45%) isolates belonged to the genotype A and 1 (4.54%) isolates belonged to the genotype B. Antifungal sensitivity test show no resistant to Amphotericin-B, while 3(13.63%) isolates showed cross resistant to fluconazole, itraconazole and ketoconazole, while one(4.54%) isolates showed cross resistant to fluconazole and ketoconazole.

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Introduction

Candida is one of the most common nosocomial infections in the neonate intensive care units worldwide, increasingly important problem that may be associated with both morbidity and mortality^{1,2,3} and *Candida albicans* consider the most pathogenic member of the genus *Candida* and the predominant causative agent of candidiasis and neonatal infections^{4,5}

The incidence of infections due to *Candida* in NICU increased markedly in past few years and mainly related with the gestational age and birth weight^{6,7} This has been attributed to the relative immunodeficiencies in the preterms, high prevalence of hand carriage of *Candida* in health care workers^{8,9}, ability of *Candida* to survive on environmental surfaces¹⁰ and colonization of maternal vagina¹¹, colonization of the infant occurs early in life and this is affected by a variety of common practices in the neonatal intensive care unit (NICU).

Molecular typing of an infectious agent is important for epidemiological studies and for the development of appropriate infection control strategies¹² and for this purpose McCullough¹³ developed a polymerase chain reaction (PCR) based method using a primer pair designed to span the region 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 four genotypes on the basis of the amplified PCR product length: genotype A (~450 bp product), genotype B (~840 bp product), genotype C (~450 and ~840 bp products), genotype D (~1,080 bp product) and Most recently Tamura¹⁴ described the presence of a novel genotype (genotype E) with base pair (~1,400 bp), and Bii¹⁵ report AB genotype with base pair (~550bp).

Candida albicans isolated from NICU patients may exhibit a trend of decreasing susceptibility to antifungal agents, attributable to several factors that include the increased use of antifungal drugs. While such a tendency has been well described among patients with hematological malignancies and other immunocompromising conditions¹⁶, this issue has not been sufficiently addressed among neonates over time¹⁷.

The aim of our study was to investigate the genotyping of *Candida albicans* which were isolated from thrush neonate and antifungal susceptibility profiles for this isolates. This is the first study which investigated the genotyping of *Candida albicans* isolated from the thrush cases in NICU in Iraq.

Materials and Methods

Clinical Isolates

The study was performed on 22 *C. albicans* isolates from 25 total *Candida* isolates obtained from 33 thrush babies admitted to the neonatal intensive care units (NICU), in Al-Zahraa educational Hospital, an-Najaf governorate, IRAQ, from April to August 2010.

Candida albicans isolates were identified on the basis of their cultural and morphological characteristics on Sabouraud's dextrose agar (SDA), germ tube production, light green colony on Candida CHROMagar (CHROMagar microbiology, Paris, France), chlamydospore production on Corn-Meal Tween 80 agar and grow at 43C. The carbohydrate assimilation patterns of all the isolates were tested, using HiCandida Identification Kit (HiMedia Laboratories Pvt. Limited, India) according to the manufacturer's procedure.

Antifungal susceptibility testing:

Minimum inhibitory concentration for following antifungal Amphotericin-B, Fluconazole, Itraconazole and Ketoconazole were done by using HiComb MIC technique (HiMedia lab. Pvt. limited, India) on Muller-Hinton-Bromothymol blue agar(MHB)¹⁹ antifungal strips put on agar surface after spreading the inoculum by non toxic sterile cotton stick and incubated at 37°C for 24 hours, and read the result as minimum antifungal concentration causing visible growth inhibition, and quality control was ensured by testing a standard strain of *Candida albicans* (ATCC 10231) with each test.

DNA extraction

All *C. albicans* isolates were subcultured twice on SDA and incubated at 37°C for approximately 48–72 h prior to molecular analysis. A single colony of *C. albicans* was suspended in 3 ml of yeast extract-peptone-glucose (YPD) medium for 48 hours at 30°C with agitation. Genomic DNA was extracted using the DNA-Pure Yeast Genomic Kit (bioWorld, USA) according to manufacture instructions. Extracted DNA was transferred to a sterile Eppendorf tubes and stored at -20°C prior to PCR.

Primers:

The primer pairs used to detect the 25S rRNA were CA-INT-L (5-ATA AGG GAA GTC GGC AAA ATA CCG TAA-3) and CA-INT-R (5-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3) as described by McCullough¹³, All primers were synthesized by (BioCorp Co., Canada).

Amplification reactions were performed in 25 µl final volume containing 12.5 Robust Hotstart Readymix (Kappabiosystem, South Africa), 25 pmol each of the primers and 5 µl 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, UK): 94°C for 3 min followed by 30 cycles of 94°C for 30 sec., 60°C for 15 sec., 72°C for 1 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 gels in 1X TBE (Tris-borate-EDTA) buffer at 100 V for 60 min and data analyzed by gene tool analysis software (SCIE-PLAS, UK). During each run, molecular grade water was included randomly as negative controls and *C. albicans* (ATCC 10231) as reference strain in the study.

Results

Twenty five *Candida* isolates were obtained from 33 neonates showed clinical signs of thrush during study period, only 22 (66.6%) isolates diagnosed as *C. albicans*, and the other three (9%) diagnosed as *Candida* other than *C. albicans*, Eight (57.14%) isolates obtained

from male babies and other 14 (73.68%) isolates from females without statistical significant differences(table 1).

Table 1:distribution of isolates according to gender.

Gender	Positive(%)	Negative(%)	Total samples(%)	P
Male	8(57.14)	6(42.85)	14(42.42)	$p>0.01$
Female	14(73.68)	5(26.31)	19(57.57)	
Total	22(66.66)	11(33.34)	33(100)	

Most isolates obtained from babies at 2^{ed} week of age 11(50%) and for less frequency at 1st week of age 8(36.36%) and the cases decreased markedly in the weeks follows($p<0.01$). (table 2) .

Table 2:distribution of isolates according to age.

Age(day)	Male	female	Total(%)	p
1-7	1	7	8(36.36)	$p<0.01$
8-14	6	5	11(50)	
15-21	0	1	1(4.54)	
22-30	1	1	2(9.09)	

Polymerase Chain Reaction amplification results with the primer CA-INT show 21(95.45%) isolates as genotype A with a single amplification product (~450bp) and only one(4.54%) isolate classified as genotype B with single amplified product about (~850bp) and no isolates under genotype C or D or unusual type were detected (Figure 3).

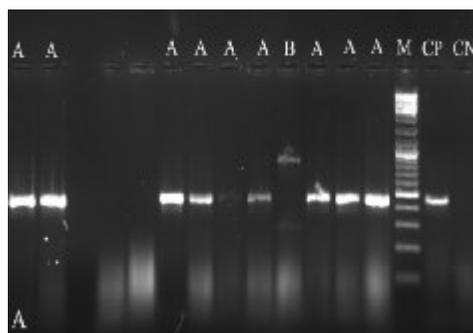
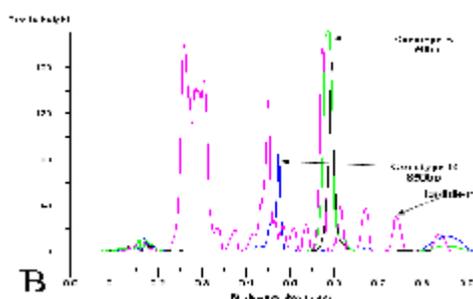


Fig.1: A) PCR genotyping with primer CA-INT; lane M,100bp ladder; lane CP, standard strain ATCC 10321(genotype A);lane CN, PCR grade water as control negative; lane B, *Candida albicans* genotype B, other lanes represent *Candida albicans* genotype A.

B)gene tool analysis for picture show genotype A(~450bp), genotype B(~850bp) and 100bp ladder as standard scale.

All the 22 *Candida albicans* isolates were sensitive to Amphotericin-B, while 4(18.18%) isolates were resistant to ketoconazole and itraconazole respectively and 5(22.72%)isolates resistant to fluconazole (table 3).

Table 3: MIC for Amphotericin B, ketoconazole, fluconazole and itraconazole.

Antifungal	MIC range(µg)	S	SDD	R	SDD%	R%
Fluconazole	0.256-0.5	16	1	5	4.5	22.72
Itraconazole	0.125-0.25	17	1	4	4.5	18.18
Ketoconazole	0.032-0.016	18	0	4	0	18.18
Amphoteric-B	0.064	21	1	0	4.5	0

S: sensitive, SDD: susceptible dose depended, R: resistance.

Three isolates(13.63%) show cross resistance between fluconazole, itraconazole and ketoconazole, and one(4.54%) isolates show cross resistance between fluconazole and ketoconazole and one (4.54%) isolate resisted to fluconazole and another one (4.54%) for itraconazole only.

Discussion

This study consider as a first report of genotypic analysis of *Candida albicans* isolated from neonate intensive care unit (NICU) in Iraq.

The result showed that *C. albicans* the most common isolate from clinical samples and these agree with previous studies^{1,20,21,22} and there is no significant differences in infection between male and female in group of study and these disagree with²¹ And agree with²³, there is no strong evidence indicate presence a relation between thrush cases and gender and this need more clarify and studies.

The results agrees with previous studies that *C albicans* genotype A predominant in clinical samples, while genotype B less frequency and this very close to previous studies^{14,24,25,26,27,28}

All strains were susceptible to Amphotericin-B and no resistant were detected for this antifungal and this agree with most previous studies that resistance to Amphotericin-B to be rare^{21,29,30} but resistant to fluconazole ,itraconazole and ketoconazole were detected in high percentage of resistant to clinical isolates, These results are similar to

those reported by a few investigators²¹ and different from those of some others^{20,29,31} and also we observed cross resistant between azoles which detected previously^{29,32}

We conclude that an increasing rate of *C. albicans* resistance to antifungal agents may be due to the frequent use of these agents in the prophylaxis of fungal infections and random wide abuse of azole in our society may develop such type of resistant and the emerging resistance to these agents give rise to concerns about their future clinical usefulness.

Also, this study show increasing in thrush cases at 2ed week of age in baby hospitalized in NICU, more than 1st week of age, neonate may become colonized with *C albicans* at birth²⁰ either from the mothers or during their stay in the NICU and these clarify by American national epidemiology of mycoses survey¹, colonization of *C albicans* in (NICU) as reported by bad hygiene condition in the hospital may be explain the increase frequency of *Candida* colonization at 2ed week of age after stay in NICU, and then fail at 3^{ed} and 4th weeks due to treatment, and this opinion strongly supported by³²

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