Microbiological and Molecular study On *Candida species* Isolated From Catheterized urine specimen In Ramadi general Teaching Hospital

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ABSTRACT

Background:_ A Catheter-associated with candidiasis infection is the most common nosocomial infection and the objective of this work is to isolate and identify *Candida species* from catheterized patients by ordinary culture and PCR.

Objective: To study the isolation and identification of Candida species from catheterized patients by culture media and polymerase chain reaction(PCR).

Methods: One hundred and thirty five *Candida species* isolates were obtained from urine culture of catheterized specimens from male and female patients , During the period between October 2011 to April 2012 , attending AL-Ramadi general teaching Hospital. A quantitative urine culture for isolation and identification of *Candida species* was. The isolation of *Candida species* was done out on selective media with antibiotics is Sabouraud Dextrose Agar. The identification of *Candida species* was based upon a combination of morphological and biochemical criteria as germ tube test and API 20 candida. Molecular study of *Candida species* was done using polymerase chain reaction (PCR).

Results: Out of the one hundred and thirty five catheterized urine examined .*Candida spp.* was isolated from in 92 samples. The isolated of *Candida spp.* were recorded 26(40.0%) *C.albicans* among female patients and 20(36.4 %) among male patients .positive candidiasis was detected among diabetic patients (28.6%) from female and (20.0%) from male .Also the candidiasis was detected among patients under antibiotic treatment was (20.3%) from female patients and (32.1%) from male patients.Polymerase chain reaction (PCR) results showed that out of 27cultured specimens , (18) were positive for *C. albicans* (66.7%) , and out of 9 specimens ,(7) were positive for *C.glabrata* (77.8%) while out of 4specimens ,(2) were positive for *C.parapsilosis* (50.0%) .Statistical analysis using chi – square test was applied in this work.

Conclusion: The three species of *Candida. albicans* , *Candida .glabrata* .& *Candida .parapsilosis* are important causes of UTI in patients under catheterization in Ramadi teaching hospital and they could be one of causes nosocomial infection .

Key words: UTI, *Candida albicans*, Candidiasis

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Candida is a genus of yeasts. Many species are harmless commensals or endosymbionts of hosts including humans, or harmless species in the wrong location, can cause disease. *Candida albicans* can cause infections (candidiasis or thrush) in humans, especially in immunocompromised patients[1] . Many species are found in gut flora, including *C. albicans* in mammalian hosts, whereas others live as endosymbionts in insect hosts[2] .Systemic infections of the bloodstream and major organs, particularly in immunocompromised patients[3] .Antibiotics promote yeast infections, including gastrointestinal *Candida* overgrowth, and penetration of the GI mucosa [4]. Many people are under the impression that only women get genital yeast infections. Regardless of gender, prolonged antibiotic use increases your risk of a yeast infection. Also, men and women with diabetes or impaired immune systems, such as those with HIV, are more susceptible to yeast infections[5]. Among *Candida species, C. albicans*, which is a normal constituent of the human flora, a commensal of the skin and the gastrointestinal and genitourinary tracts, is responsible for the majority of *Candida* bloodstream infections (candidemia). Yet, there is an increasing incidence of infections caused by *C. glabrata* and *C. rugosa*, which could be because they are frequently less susceptible to the currently used azole antifungals[6] .

Fungal nosocomial infections: Several fungi have become more common in nosocomial infections with a rate reported as 3.8 per 1,000 hospital patients The most common are Candida (mostly *Candida albicans*), *Aspergillus, Fusarium, Trichosporon, and Malassezia*. Candidiasis remains the most common type of nosocomial fungal infection, particularly in the immunocompromised..Catheter - associated urinary tract infection is the most common nosocomial infection, Up to half of the patients requiring an indwelling urethral catheter for 5 days or longer will develop bacteriuria or candiduria [7].

Methods

The samples were collected from patients attending to Ramadi general and teaching Hospital clinic. Through the period from October 2011 to April 2012, the patients were subjected to a careful clinical examination, catheterized urine was taken to detect the prevalence of *Candida species* by using various microbiological methods. A total of 135 patients with catheterized were included in this study. They were divided into 89 female and 46 male patients. The specimen were inculcated on blood agar (Mast), and Sabouraud Dextrose Agar (Mast) ,incubated at30˚C for4-7days . The specimens smeared on to glass slide ,heated ,fixed ,and can be stained by Gram stain .These slides were examined for presence of Gram positive

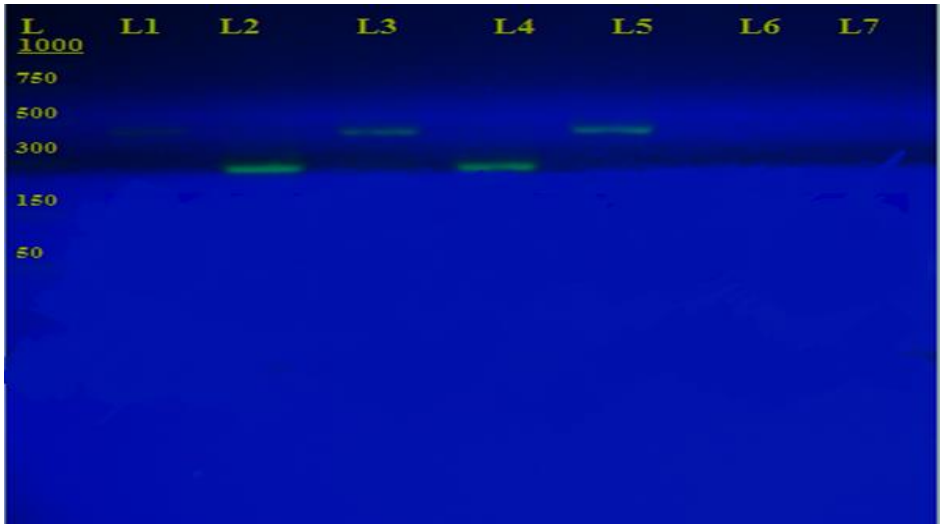

Figure (1): Multiplex Polymerase Chain Reaction performed with genomic DNA from clinical isolates of *Candida albicans* showed by Lanes (L1) and (L4) and *Candida glabrata* indicated by Lanes(L1), (L3) and (L5), L : bench Top DNA marker

yeast cell[8] ..Identification of *Candida species* has been identified by Germ tube test and API 20 (Biomerieux company) candida [9].The identification of these fungi is based upon a combination of morphological and biochemical criteria.Morphology was primarily used to establish the genera, whereas biochemical assimilations have used to differentiate than other species [10].

Polymerase Chain Reaction (PCR):A multiplex PCR method was developed to identify simultaneously multiple fungal pathogens in a single reaction. Six sets of species-specific primers were designed from the internal transcribed spacer (ITS) regions, ITS1 and ITS2, of the rRNA gene to identify *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* [11] Multiplex PCR. A total of three species were tested in there multiplex PCR panel.Each reaction tube contained three pairs of primers that were designed and commixed to produce amplicons sufficiently different in size and migration to identify three fungal species. Each PCR reaction tube contained 1ul of each primers (CGL1 and CGL2,CALB1 and CALB2 and CPA1 and CPA2).OF single-primer-pair PCRs;10ul of DNA template then added deionize distilled water to reach to the final total volum as equal to 20 ul [12] Table 1 showed All primers were supplied by Promega company as a lyophilized product of different Pico moles concentrations and re-suspension using deionised water to reach a final concentration for 5 Pico moles /1μ of suspension was achieved by using the following equipment :

CI x V1= C2 x V2

. **Table (1): Primer pairs designed to amplify DNA specifically from the listed species of pathogenic fungi (Promega company).**

F:forward R:reverse

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Primer name	Sequence (5-3)	Specificity
CALB1	**F:**TTT ATC AAC TTG TCA CAC CAG A R:AAA TAG TGA AAC AGT GTG GTC T	*Candida albicans*
CALB2	**F:**ATC CCG CCT TAC CAC TAC CG R:TAG GGC GGA ATG GTG ATG GC	
CGL1	**F:**TTA TCA CAC GAC TCG ACA CT R:AAT AGT GTG CTG AGC TGT GA	*Candida glabrata*
CGL2	**F:**CCC ACA TAC TGA TAT GGC CTA CAA R:GGG TGT ATG ACT ATA CCG GAT GTT	
CPA1	**F:**TTG GTA GGC CTT CTA TAT GGG R:AAC CAT CCG GAA GAT ATACCC	*Candida parapsilosis*
CPA3	**F:**GCC AGA GAT TAA ACT CAA CCA A R:CGG TCT CTA ATT TGA GTT GGT T	

Result: Out of the 135 cultured catheterized urine specimens were, 89 female and 46 male patients and 92 patients had candidiasis as the following 52 female and 40 male patients .*C.albicans* and other *Candida species* isolated from catheterized urine specimen among female patents were 26 (40.0%) *C.albicans* , 10 (15.4%) *C. tropicalis*, 9 (13.8%) *C.glabrata*, 4(6.2%) *C. parapsilosis*, and *C.famata* 3 (4.6%).While *C.albicans* and other *Candida species* isolated from catheterized urine specimen among male patents were 20 (36.4%) *C. albicans* &*C. tropicalis* 9(16.4), *C. parapsilosis* 2 (3.6%) in table 2.

Table 2: The frequency of C. albicans and other Candida species among female and male patients

Types of isolates	Female		Male	
	No.of isolates	%	No.of isolates	%
C. albicans	26	40.0	20	36.4
C. tropicalis	10	15.4	9	16.4
C. glabrata	9	13.8	9	16.4
C. parapsilosis	4	6.2	2	3.6
C.famata	3	4.6	-	-
Totat	52		40	

Table 3 shows the age distribution of candidiadsis. Different age groups were subjected in this study ranging from (20 -89) years old and the age patients were classified into seven groups and it was found the percentage of age groups were 21.3%, 20.2%, 19.1%, 16.9%, 9.0%, 10.1% and 3.4% respectively among female patients with the incidence of *C. albicans* infection in first group than other groups at age 20-29 years old. While the percentage of age groups among male patients were 26.1%, 8.7 %,4.3%,19.6%,21.7%,13.0 %, and 6.5% respectively and showed the frequency of *C. albicans* at age 20-29 and 60-69 years old.

Table 3: Distribution of age groups among female and male patients

Age group	Female		Male	
	No.	%	No.	%
20-29	19	21.3	12	26.1
30-39	18	20.2	4	8.7
40-49	17	19.1	2	4.3
50-59	15	16.9	9	19.6
60-69	8	9.0	10	21.7
70-79	9	10.1	6	13.0
80-89	3	3.4	3	6.5
Total	89	100.0	46	100.0

X^2= 28.95 ($p < 0.01$)

Table(4.) illustrated the relationship between *C. albicans* and diabetic patients. Out of the 21 had candidiasis 21 (22.8%) out of 92 were female patients with age groups were 9.5%,9.5%,8.4%,14.3%,28.6%,23.8%,and9.5%respectiv ely and the high incidence of candidiasis at age 60-69 years old and out of the 10 male patients had candidiasis ,10(10.8%) were diabetic male with age groups were10.0% to 20.0% and the incidence of *C. albicans* infection at age 50-59 years old among male diabetic patients.

Table 4: The relationship between C. albicans and diabetic patients

Age group	Female		Male	
	No.	%	No.	%
20-29	2	9.5	1	١٠.٠
30-39	2	9.5	1	10.0
40-49	1	4.8	1	10.0
50-59	3	14.3	2	20.0
60-69	6	28.6	2	20.0
70-79	5	23.8	2	20.0
80-89	2	9.5	1	10.0
Total	21	100.0	10	100.0

In female: X^2= 22.41 ($p < 0.01$),
In male : X^2= 0.40 ($p >0.05$)

Table 4 reviled the diabetic patients according to sex and age. Table(5). Showed the relationship between *C. albicans* and antibiotic uses .It was found 59(64.1%) out 92 patients among female patients with age groups were 3.4%,15.3%,20.3%,13.6%,16.9%,18.6%, and 11.9 %. and the high incidence of *C. albicans* was 20.3% at age (40- 49) years old and 28 (30,4 %) out of 92 patients showed the relationship between *C. albicans* and antibiotic uses among male patients were 3.6%,7.1%,10.7%,32.1%, 32.1%, 7.1%,and 7.1%with high incidence of *C. albicans* and antibiotic uses at age 50-59 years old among male patients.

Table 5 :The relationship between C. albicans and long dose of antibiotic uses

Age group	Female		Male	
	No.	%	No.	%

20-29	2	3.4	1	3.6
30-39	9	15.3	2	7.1
40-49	12	20.3	3	10.7
50-59	8	13.6	9	32.1
60-69	10	16.9	9	32.1
70-79	11	18.6	2	7.1
80-89	7	11.9	2	7.1
Total	59	100.0	28	100.0

In female :X^2= 13.68 ($p < 0.05$)
In male :X^2= 86.96 ($p < 0.01$)

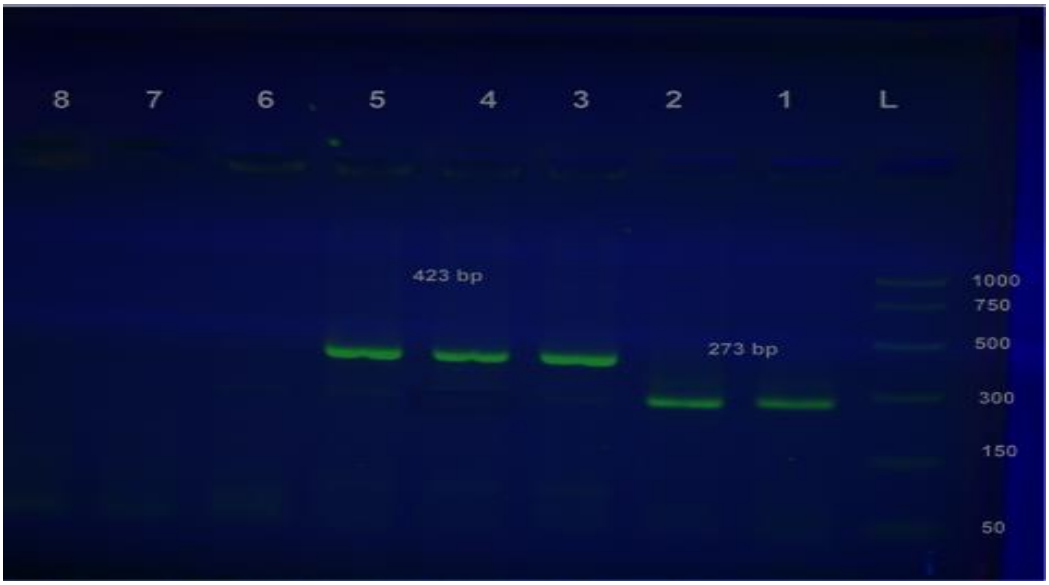
This table showed the relationship between antibiotics uses and *C. albicans* patients.
Polymerase chain reaction (PCR) results showed that out of 27 (18) were positive for *C. albicans* at (66.7%) , while 27(9) at (33.3%) was negative ,while out of 9 (7) were positive for *C.glabrata* at (77.8%) and out of 9 (2) at (22.8%) was negative , and out of 4(2) were positive for *C.parapsilosis* at (50.0%) and negative at same percentage (table 6) .

Discussion.: Out of the 135 examined catheterized urine specimens were , 92 had candidiasis among male and female patients .The percentage of candiasis among female patients was (56.5%) and (43.4%) among male patients. Candida species are part of the patients' endogenous bowel flora but they can also be acquired by cross contamination from other patients or hospital personnel or by exposure to contaminated solutions or non-sterile equipment(13). Also, use of catheter should be limited to carefully selected patients so as to reduce the size of population at risk. One host factor that predisposes to catheter associated UTI is advanced age The mean age of patients in this study is 20-29 years old. This finding agrees with a previous study (Oni et al, 2003) (14). Our work studied the relationship between candidiasis and diabetic patients . Patients with diabetes mellitus have increased susceptibility to certain mycotic infections. In this article describes the predisposing factors of fungal infections. It is generally known that patients with diabetes

Table5: PCR result of Candida spp.

Candida spp.		PCR		Total
		Negative	positive	
C.albicans	No.	9	18	27
	%	33.3%	66.7 %	100.0 %
C.glabrata	No.	2	7	9
	%	22.2%	77.8 %	100.0 %
C.parapsilosis	No.	2	2	4
	%	50.0%	50.0 %	100.0 %
total	No.	13	27	40
	%	32.5%	67.5 %	100.0 %

mellitus are more prone to fungal infections and that the course of the disease may be more severe. This occurs in some mycoses e.g. candidiasis, which are more frequent in diabetes. This work studied the relationship between candidiasis and antibiotic uses . Antibiotic candidiasis can result from overuse or over-presciption of broad-spectrum antibiotics[15]. Consequently, it is now rare for such antibiotics to be prescribed for extended periods. The apparent effect of the antibiotic is to reduce the commensal bacterial flora in the gastrointestinal tract, resulting in an environment conducive to the propagation of existing candida in the absence of any major competition. Logic would suggest that the probiotic should be taken as long as possible before and after an antibiotic dose, to avoid destruction by the antibiotic[16]. This finding agrees with Other authorsAl-Hashime,2000[17] and Ahmid, 2003[18] whom studied the relationship between candidiasis and diabetic and antibiotic uses .



Figure(2): Multiplex PolymeraseChain Reaction performed with genomic DNA shows amplicons from each of five individual clinical isolates of *candida glabrata* (lanesL3-L5), *candida albicans*(lanes L1&L2), LaneL, Bench top.

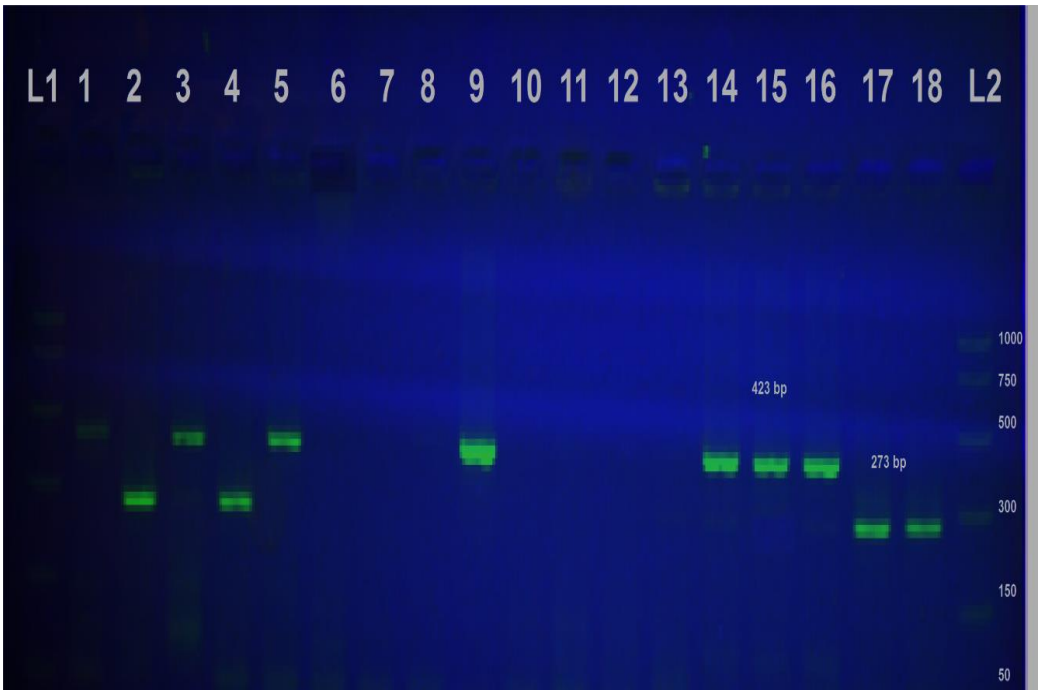


Figure (3): Multiplex Polymerase Chain Reaction performed with genomic DNA from clinical isolates of each species. Multiplex A-G-P Polymerase Chain Reaction,*Candida glabrata* DNA presented by **Lanes**1, 3, 5, 9, 14, 15 and 16. *Candidaparapsilosis* DNA presented by lanes 2 and 4. *Candida albicans* DNA

Our study was investigated the use of newer techniques of polymerase chain reaction (PCR) to identify *candida albicans* in samples obtained from growth culture . Choosing of this project for the present research might be due to importance of PCR for detection Pathogenic yeast , such PCR amplification uses primers designed to target Internal Transcribed Spacer (ITS) . During 1990th, majority of previous researches were conducted on detection of *Candida species* by PCR, and they shows lacks of sensitivity when the test is performed with blood or serum specimens[19]. In recent years, several DNA-based molecular identification methods have been established which make use of the variable domains of the 18S or 28S rRNA gene Since the variability of 18S and 28S rRNA genes is limited, it can be difficult to differentiate between species[20] .The ITS region, located

between the 18S and 28S rRNA genes, is more promising for species discrimination because of its higher variability [21]. In GenBank, some species cannot be distinguished from others of the same genus by the ITS sequence. It is not always obvious whether failure of discrimination is a result of mislabeling, of close relationship, or of erroneous taxonomic separation. The definition of a species in mycology is complicated [22]One species may have several names given by different mycologists or due to reassignment of a species based on sequence analysis. Today, morphological characteristics have to be supported by molecular analysis before definition of a new species is approved[23]. This study concluded *Candida species* are isolated from Urinary Catheterized patients , and the diagnosis *Candida species* by microbiological and PCR is specific and sensitive test for the identification of *Candida species .*

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