

Prevalence of Non-Albicans Candida in Samples Isolated From Human, Animals and Poultry and Methods of Identification With Special Reference to Antifungal Sensitivity Test.

Khafagy A.R.¹, Ibrahim H.M.¹, Taha M.² and Eidaroos N.H.¹

¹ Dept. of Microbiology, faculty of Veterinary Medicine, Suez Canal University, ² Dept. of Microbiology, faculty of Veterinary Medicine, Zagazig University,

Abstract

During the last two decades, the frequency of fungal infections increased with serious infections. Among these fungi, Candida species was the predominant yeast infection of human and animals. The predominant Candida spp. isolated from human and animals were *C.albicans* but, the incidence of NAC Candida has been increased. In this study, a total of 205 samples were collected from different human and animal sources. The isolates were identified by phenotypic, commercial biochemical API 20 C AUX kit and RFLP PCR and it was found that the RFLP PCR was the rapid, accurate and cost effective method of NAC identification. The most effective antifungal drug was amphotericin B and some isolates show resistance to common antifungal drugs as fluconazole.

Key words: NAC, RFLP PCR, antifungal sensitivity test.

Introduction

During the last 2 decades, the frequency of fungal infections increased that cause serious infections. Among the fungi, Candida species are opportunistic agent, which normally inhabitant in the intestinal tract, vaginal and oral cavity of human and animals (*Pal et al., 2015*).

Twenty years ago, the predominant Candida spp. that isolated from oral or systemic candidiasis was *C.albicans* with percentage of 80%. Although *C. albicans* continues to be the most frequently isolated

species, the number of infections caused by non-albicans species (NCAC) has increased significantly over the last 2 decades (*Pincus et al., 2007*) such as *C.glabrata*, *C.tropicalis*, *C.parapsi* Ilosis, *C.krusei*, *C.guilliermondii*, *C.lusitaniae*, *C.kefyr*, *C.famata*, and *C.rugosa*. NCAC species accounted for 10%–40% of all systemic candidiasis from 1970 to 1990, and this proportion reached 35%–65% in the last 2 decades (*Pal et al., 2015*).

There are many factors that increase the frequency of NAC infections as severe illness and

immunosuppression, young or old age, the increase using of broad spectrum antibiotics and empirical use of antimycotic drugs are reported to be associated with this change. The clinical signs of the infections caused by NAC species are indistinguishable, and several NAC spp. are inherently resistant or acquire resistance, or both, to commonly used antifungal drugs (**Deorukhkar *et al.*, 2014**)

Allam and Salem (2012), studied the prevalence of *Candida* species isolated from sputum, urine and oral human candidiasis and found that *C.albicans* was the most frequently *Candida* spp. isolated from every specimen type with percentage of 44.2% and other *Candida* species isolated with lower percentage from each samples including *C.glabrata*, *C.krusei*, *C.kefyr*, *C.tropicalis* and *C.stillatoidea* with a percentage of 65.8% from total samples. *C. albicans* was the most common species associated with vulvovaginitis (71.4% in pregnant women versus 64.7 % in non pregnant women), followed by *C. glabrata*, *C.tropicalis*, *C.krusei* and *C.kefy* (**Emam *et al.*, 2012**), However **Us and Cengiz, (2006)** reported a lower percentage of *C. albicans* species (53.2%) in pregnant patients.

Ghazaly (2001) studied the prevalence of yeast from mastitic milk and diarrheic calves where *C.albicans* isolated from milk with a percentage of 36% while NAC with percentage of 64%. From

diarrheic calves, *C.albicans* were isolated in low percentage 18.18% and NAC by 81.82%.

Saleh *et al.* (2011) studied the prevalence of yeast spp. From different animals from different sources where the mycological examination revealed that the yeast isolates were representatives for only 3 species *Candida albicans* (110 isolates), *Cryptococcus neoformans* (20 isolates) and *Rhodotorula rubra* (66 isolates). The most frequently isolated yeast species were *Candida* species other than *C. albicans* from animal vaginal discharges [24.6%], followed by *C. albicans* [15.6 %], *R. rubra* [7.5 %] and *C. neoformans* [2.24 %], while, the most frequently isolated yeast species from animal nasal discharges were *R. rubra* [17.8 %], *C. albicans* [16.2 %], *Candida* spp. other than *C. albicans* [11.7 %] and *C. neoformans* [1.67 %].

The present work was aimed to study the prevalence of NAC from human, animals and poultry samples and methods of identification and susceptibility of the isolated species to antifungal agents.

Material and Methods

A total of 205 samples were collected from human, animals and poultry. The numbers, types, condition of cases and sources are shown in table 1.

All samples firstly isolated on SDA media then subcultured for

morphological examination on RAT80 and CHROM Candida agar (Conda, France) for microscopic examination of fungal elements and macroscopic examinations of the color of the colony of different Candida species respectively. Twenty three samples that were identified by phenotypic methods were examined using API 20 C AUX kit for biochemical identification (Biomérieux, France).

Genotypic identification of *Candida* species (RFLP PCR).

Genomic DNA of 27 *Candida* isolates were extracted by Blood-Animal-Plant

DNA preparation kit (Jena Bioscience). Fungal specific universal primer pairs were used to amplify internal transcribed spacer 1(ITS1)-5.8S rDNA-ITS2 regions in all yeast isolates according to (Mohammadi *et al.*, 2013), ITS-1(5`-TCCGTAGGT GAACCTGCGG-3`) and ITS-4(5`-TCCTCCGCTTATTCATATGC-3`). The amplification reaction was performed in a final volume of 25 µl containing 12.5 µl master mix (Fermentase), 1 µl of forward (ITS-1) and reverse primer (ITS-4), 2 µl of template DNA and the volume made up to 25 µl with sterile nuclease-free water. The reaction

mix was kept in the thermocycler (Eppendorf). The program was performed as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 56 °C for 30 sec, extension at 72 °C for 30 sec and final extension at 72 °C for 5 min for 35 cycles.

RFLP was performed by using *MspI* enzyme according to company instructions (Thermo Scientific) by 2 µl of enzyme buffer, 1 Units of *Msp I* enzyme and 10 µl of PCR product were added in a 200-µl PCR tube and the volume was made up to 30 µl with nuclease-free water. The reaction mix was incubated at 37°C for 5 min. PCR products and RFLP products were electrophoresed in 1.5% and 2.0% agarose gel respectively, stained with Ethidium bromide (0.5 µg/ml) and visualized under UV light and photographed. *Candida* species that didn't identified by RFLP PCR were subjected for sequencing of the PCR products by 3500 genetic analyzer (Applied Biosystem, USA). Antifungal susceptibility testing by Disk diffusion (DD) technique by using fluconazole, ketoconazole, clotrimazole, amphotericin B, itraconazole and nystatin for *Candida* isolates.

Table 1: *The numbers, types, condition of cases and sources of samples.*

Type of samples	No.	Condition of cases	Type of samples	No.	Condition of cases
I Human	68	VVC	II- Animals		
1- Vaginal swabs	21	Oral thrush	A- Cows	90	
2- Oral swabs	12	Respiratory disorder	1- Milk samples	49	Mastitis
3- Sputum samples	15	UTI	2- Vaginal swabs	24	Reproductive disorders
4- Urine samples	20		3- Rectal swabs	17	Diarrhea
			B- Dogs		
			Ear swabs	28	Otitis externa
			III- Poultry	19	Crop mycosis

UTI: Urinary tract infection, VVC: Vulvovaginal candidiasis.

Results

A total of 205 samples were collected from human, animals and poultry that give 143 positive culture (69.8%). Numbers and percentage of positive yeast cultures from human, animals and poultry is shown in table 2 .

All yeast isolates were subcultured on RAT80 and CHROM Candida agar for morphological examination. Table (3) shows the numbers and percentage of Candida species that identified as Candida species by morphological methods which able to identify medically important species as *C.albicans*, *C.tropicalis*, *C.krusei*, *C.glabrata* and *C.parapsilosis* according to the color of the colonies on CHROM agar (fig.1) and fungal elements on RAT80 (fig. 3, 4, 5)

With morphological examination, it was found that the predominant Candida species isolated from sputum, urine, VVC and oral candidiasis was *C.albicans* (79.6%) while NAC represent 20.4%. Morphological methods were able

to identify all NAC from human isolates.

Concerning to cows samples, *C.albicans* was isolated with low percent 10.8% while NAC were isolated with high percentage 89.2%. Morphological methods were able to identify *C.albicans*, *C.tropicalis*, *C.krusei*, *C.glabrata* and *C.parapsilosis* while 12 isolates couldn't be identified to the species level.

Swabs of dog's otitis externa revealed 12 *Candida* isolates where *C.albicans* was isolated with 16.7% while NAC isolated with percentage of 83.3%.

Crop mycosis of poultry revealed 19 *Candida* isolates and *C.albicans* was isolated in high percentage 63.2% while NAC were 36.8%.

Biochemical identifications.

Twenty two representative *Candida* species were further identified by API 20 C AUX kit. The API 20 C AUX kit was able to identify 13 examined NAC to the species level, 3 isolates that identified with morphological method as *Candida* spp. identified by API as

Cryptococcus laurentii, 6 examined isolates gave unacceptable profile.

Molecular methods

Twenty four representative isolates that were identified by phenotypic methods and API 20 C AUX were subjected for further identification by RFLP PCR (fig, 6 to 13)

Table 4, shows isolates that identified with phenotypic methods, API 20 C AUX and RFLP PCR.

RFLP PCR proved to be the accurate, rapid and simple method of NAC where, the 3 NAC that identified by API as *Cryptococcus laurentii* were identified as *C.kefyr* by RFLP PCR, one *Candida* spp. was identified by API as *C.rugosa* and identified as *C.krusei* with RFLP PCR, 4 isolates that gave unacceptable profile with API kit were found to be mixed isolates that clearly identified by RFLP PCR (fig. 12,13). Two isolates didn't identified by both API and RFLP PCR.

Six isolates that had different results with API and RFLP PCR were

subjected for sequencing for confirmation of the results (table 5) The sequencing proved that the results of RFLP PCR were accurate as the 3 isolates that identified with morphological method as *Candida* spp. identified by API as *Cryptococcus laurentii* were proved to be *C.kefyr*, the other isolate that identified as *C.rugosa* by API was proved to be *C.krusei* which was the same result of RFLP PCR. *C.pelliculosa* was truly identified by API in comparing with sequencing and RFLP PCR couldn't identify it.

Antifungal sensitivity test.

Concerning to antifungal sensitivity test, all isolates showed sensitivity to amphotricin B and clotrimazole except *C.pelliculosa* that showed resistance against clotrimazole. *C.parapsillosis* and *C.glabrata* were sensitive to all tested antifungal discs. *C.albicans* showed resistance to fluconazole and ketoconazole, *C.kefyr* were resistant to itraconazole and ketoconazole and *C.inconspicua* showed resistance against fluconazole.

Table (2): Numbers and percentage of positive yeast cultures from human, animals and poultry.

Type of samples	Numbers of samples	Numbers and percents of positive culture		Numbers of isolates
A- Human samples				
1- Vaginal swabs	21	15	71.4%	16 (1 MC)
2- Oral swabs	12	7	58.3%	8(1 MC)
3- Sputum samples	15	15	100%	15
4- Urine samples	20	20	100%	20
B- Animals samples				
I- Cows 1- Vaginal swabs	24	23	95.8%	25 (2MC)
2- Rectal swabs	17	14	82.4%	16 (2MC)
3- Milk samples	49	12	24.5%	12
II- Dog's ear swabs	28	22	79%	24(2MC)
C- Poultry's crop swabs	19	15	79%	19 (4MC)
Total	205	143	69.8%	155

MC: Mixed culture

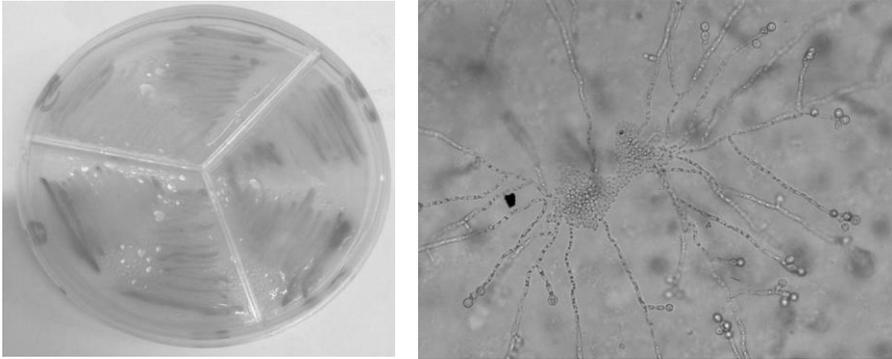


Fig.(1): CHROM Candida agar: *C.albicans* (green), *C.krusei* (purple), *C.glabrata* and *C.parapsilosis* (pink)

Fig.2: *C.albicans* on RAT shows blastospores, pseudohyphae & chlamydoconidia

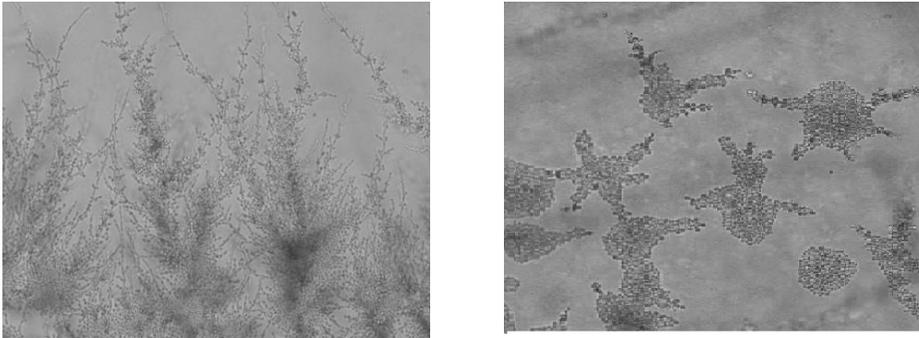


Fig.3: *C.parapsilosis* on RAT show branched pseudohyphae that carry abundant of blastospores.

Fig.4: Candida species on RAT show primitive pseudohyphae and blastospores.

Table (3): Numbers & percentage of *Candida* spp. identified by phenotypic methods.

	Identification	No.	%		Identification	No.	%	
I- Human	<i>C.albicans</i>	47	79.6	II- A-Cows	<i>C.albicans</i>	4	10.8	
	<i>C.parapsilosis</i>	5			<i>C.parapsilosis</i>	3		8.1
	<i>C.glabrata</i>	5			<i>C.glabrata</i>	5		13.5
	<i>C.krusei</i>	1			<i>C.krusei</i>	11		29.7
	<i>C.tropicalis</i>	1			<i>C.tropicalis</i>	1		2.7
					1	<i>Candida</i> spp.		12
		59			37			
III- Poultry	<i>C.albicans</i>	12	63.2	B-Dogs	<i>C.albicans</i>	2	16.7	
	<i>C.glabrata</i>	2			<i>C.albicans</i>	3		25
	<i>C.parapsilosis</i>	3			<i>C.parapsilosis</i>	7		58.3
	<i>C.krusei</i>	2			<i>Candida</i> spp.			
		2						
		19			12			

Table (3): Identification of yeast isolates by phenotypic, biochemical & molecular methods

No.	Phenotypic identification	Api 20 C AUX	RFLP PCR
1	<i>C.albicans</i>	<i>C.albicans</i>	<i>C.albicans</i>
2	<i>C.glabrata</i>	<i>C.glabrata</i>	<i>C.glabrata</i>
3	Candida spp.	<i>C.laurentii</i>	<i>C.kefyr</i>
4	Candida spp.	<i>C.krusei</i>	<i>C.krusei</i>
5	Candida spp.	<i>C.laurentii</i>	<i>C.kefyr</i>
6	Candida spp.	<i>C.norvegenesis</i>	<i>C.norvegenesis</i>
7	Candida spp.	<i>C.rugosa</i>	<i>C.rugosa</i>
8	Candida spp.	<i>C.krusei</i>	<i>C.krusei</i>
9	Candida spp.	<i>C.pelliculosa</i>	Not identified
10	Candida spp.	<i>C.rugosa</i>	<i>c.krusei</i>
11	Candida spp.	<i>C.krusei</i>	<i>C.krusei</i>
12	Candida spp.	<i>C.krusei</i>	<i>C.krusei</i>
13	Candida spp.	<i>C.krusei</i>	<i>C.krusei</i>
14	Candida spp.	Not identified	Not identified
15	Candida spp.	<i>Cryp.laurentii</i>	<i>C.kefyr</i>
16	Candida spp.	<i>C.inconspicua</i>	<i>C.parapsillosa</i>
17	<i>C.parapsillosis</i>	<i>C.parapsillosis</i>	<i>C.parapsillosis</i>
18	Candida spp.	Not identified	Not identified
19	Candida spp.	Not identified	Mixed (<i>C.glabrata</i> & <i>C.kefyr</i>)
20	Candida spp.	Not identified	Mixed (<i>C.glabrata</i> & <i>C.kefyr</i>)
21	Candida spp.	Not identified	Mixed (<i>C.glabrata</i> & <i>C.albicans</i>)
22	Candida spp.	Not identified	Mixed (<i>C.albicans</i> & unidentified yeast)

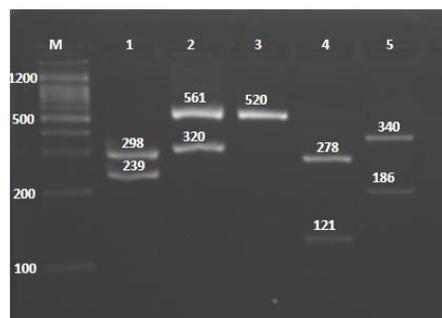
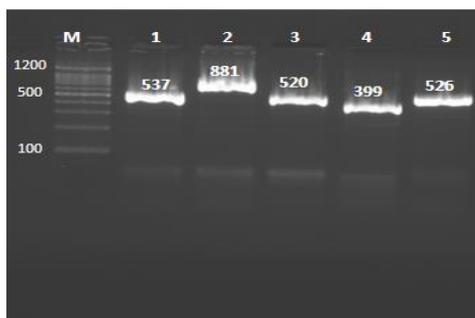


Fig. (6): PCR product of Candida spp. lane M, 100 pb ladder, lane 1, *C.albicans*, lane 2, *C.glabrata*, lane 3, *C.parapsillosis*, lane 4, *C.rugosa*, lane 5, *C.tropicalis*.

Fig. (7): RFLP-PCR product of Candida spp. lane M, 100 pb ladder, lane 1, *C.albicans*, lane 2, *C.glabrata*, lane 3, *C.parapsillosis*, lane 4, *C.rugosa*, lane 5, *C.tropicalis*.

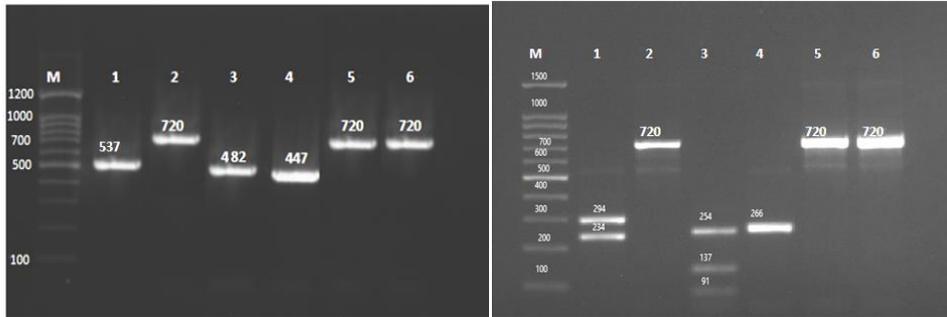
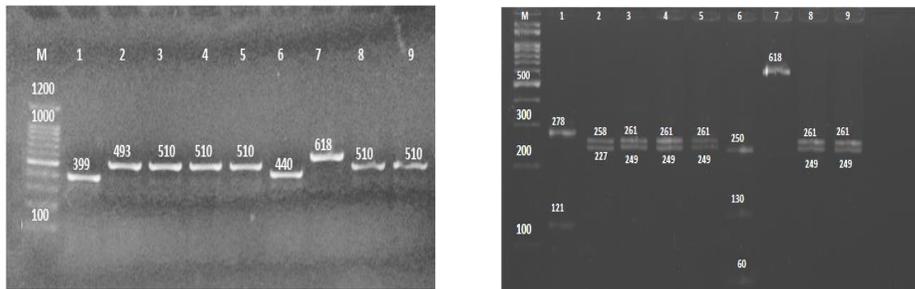


Fig (8): PCR product of *Candida* spp. lane M, 100 pb ladder, lane 1, *C. albicans*, lane 2,5,6 *C. kefyr*, lane 3, *P. manchurica*, lane 4, *C. inconspicua*.

Fig (9): RFLP-PCR product of *Candida* spp. lane M, 100 pb ladder, lane 1, *C. albicans*, lane 2,5,6 *C. kefyr*, lane 3, *P. manchurica*, lane 4, *C. inconspicua*.

Fig. (10): PCR product of *Candida* spp. lane M, 100 pb ladder, lane 1, *C. rugosa*, lane 2, *C. norvogenensis*, lane 3,4,5,8,9 *C. krusei*, lane 6, *C. ethanolica* lane 7, *C. pelliculosa*



(*P. anomala*).

Fig.(11): RFLP-PCR product of *Candida* spp. lane M, 100 pb ladder, lane 1, *C. rugosa*, lane 2, *C. norvogenensis*, lane 3,4,5,8,9 *C. inconspicua*, lane 6, *C. ethanolica*, lane 8, *C. pelliculosa*.

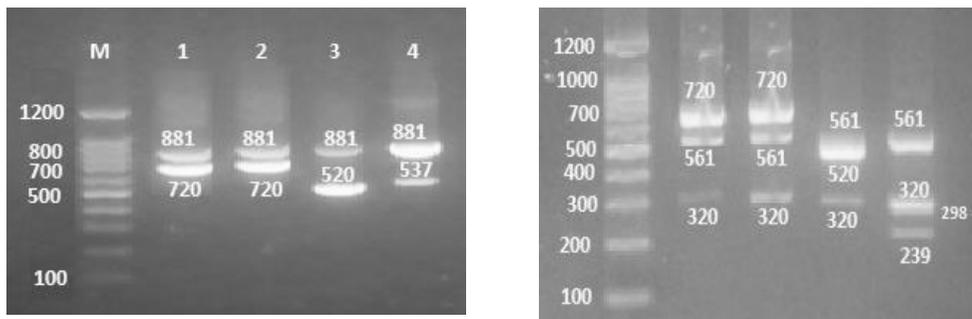


Fig. (12): PCR product of mixed *Candida* spp. lane M, 100 pb ladder, lane 1,2, *C. glabrata* and *C. kefyr*, lane 3, *C. glabrata* and *C. parapsilosis*, lane 4, *C. albicans* and *C. glabrata*.

Fig. (13): RFLP-PCR product of mixed *Candida* spp. lane M, 100 pb ladder, lane 1,2, *C. glabrata* and *C. kefyr*, lane 3, *C. glabrata* and *C. parapsilosis*, lane 4, *C. albicans* and *C. glabrata*.

Table (4): Correlation between identification by API kits, RFLP-PCR and sequencing for 6 isolates.

No. of isolates	API identification	RFLP-PCR identification	Sequencing of DNA
3	<i>Cryptococcus laurentii</i>	<i>C.kefyr</i>	<i>Kluyveromyces marxianus</i> (the perfect state of <i>C.kefyr</i>)
1	<i>C.pelliculosa</i>	Not identified	<i>Pichia anomala</i> (the perfect state of <i>C.pelliculosa</i>)
1	Not identified	Not identified	<i>C.ethanolica</i>
1	<i>C.rugosa</i>	<i>C.krusei</i>	<i>Pichia kudriavzevii</i> (the perfect state of <i>C.krusei</i>)

Discussion:

The number of infections caused by non-albicans *Candida* species (NAC) has increased significantly over the last 2 decades such as *C.glabrata*, *C.tropicalis*, *C. .krusei*, *C.parapsilosis* , *C.guilliermondii* , *C.lusitaniae*, *C.kefyr*, *C.famata*, and *C.rugosa*. The clinical manifestations of infections caused by diferent member of NAC spp. are usually indistinguishable, but several NAC spp. are inherently resistant or acquire resistance, or both, to commonly used antifungal drugs So, rapid and accurate identification is important for detection of susceptible antifungal drugs.

The present work aimed to study the prevalence of NAC from candidiasis or *Caandida* infections from human, animals and poultry and study the different

The predominant *Candida* species that isolated from human was *C.albicans* (79.6%) while NAC were 20.4%, these results are in line with (*Allam and Salem, 2012*) who isolated *C.albicans* as the

predominant *Candida* spp. from sputum, urine and oral candidiasis as *C.albicans* has virulence factors more than NAC that aid in establishment of the infection as such as germ tube formation, adherence (biofilm formation), dimorphism, phenotypic switching, toxins and hydrolytic enzymes (*Calderone and Fonzi, 2001*). The widespread abuse of antifungal drugs, use of topical dose or single oral and long term of oral azole lead to increase in the incidence of NAC yeast infection that are relatively non pathogenic (*Neerja et al., 2006*).

Concerning to cow samples, *C.krusei* was the predominant isolate (29.7%) , *C.albicans* isolated with low perecentage (10.8%), these results are in agreement with (*Tartor, 2013*) who isolated *C.krusei* as the predominant isolate. Some yeast species considered with other potentially pathogenic microorganisms which may cause diarrhea under certain conditions but are not considered primary enteric pathogens. Conditions

produced in the digestive tract by the diarrhea favor the proliferation of the yeast. Antibacterial treatment of such cases, might be not only unnecessary but counterproductive, resulting in the intensification of the microbial imbalance in favor of the mycotic flora in the GIT. In this study, yeasts isolated from diarrheic calves that not respond to antibiotic treatment with a percentage of 82.35% where *C.albicans* (12.5%) , *C.glabrata* (31.25%), *C.krusei* (18.75%) and *Candida* spp. (31.25%) that involve 3 *C.kefyr*, 1 *C.pelliculosa* and 1 *C.ethanolica*. These results is in line with (**Elad *et al.*, 1998**) who isolated *C.glabrata* as the predominant cause of calves diarrhea and also isolated *C.kefyr*, *C.krusei*, *C.rugosa* and another NAC species from diarrheic calves.

The reproductive tracts of different animals are the major reservoir of yeasts such as *C. albicans* and *C. neoformans*. In this study, 15 *Candida* species were revealed from vaginal swabs where, *C.krusei* (5) was the predominant isolate (35.7%) while *C.albicans* (2) (14.3%), and totally NAC were (85.7%) from the vagina of cows with vaginitis and reproductive disorders which were *C.parapsilosis*, *C.tropicalis*, *C.glabrata*, *C.kefyr* and *C.inconspicua* . These results sre in agreement with **Saleh *et al.* (2011)** who found that the most frequently isolated yeast species were *Candida* species other than *C. albicans* (NAC) from animal vaginal

discharges [24.6%], followed by *C. albicans* [15.6 %].

Candida species are often responsible for mastitis. This phenomena is due to frequent use of antibiotics in the treatment and in the dry period. Antibiotic therapy lead to perturbation in udder homeostasis, inhibition of T cells and neutrophil activity and inconsequence to stimulation of yeast growth (**Kano *et al.*, 2001**). It is well established that the yeasts of the genus *Candida*, for example, are capable of utilizing antibiotics as source of nitrogen like penicillin and ampicillin . The fungal invasion may be facilitated by injury to the udder's epithelium due to uptake of large doses of antibiotics that cause reduction in the vitamin A which are responsible for the health of epithelium (**Noris *et al.*, 2007**). In this study, *C.albicans* didn't isolated from mastitic milk and *C.krusei* was the predominant. *C.parapsilosis*, *C.rugosa*, *C.glabrata*, *C.inconspicua* and *C.norvogenesis* also isolated. This result is in agreement with (**Czernomysy-Furowicz *et al.*, 2008**).

As regard to crop mycosis, *C.albicans* (63.15%) was the most *Candida* species isolated followed by *C.parapsilosis*, *C.glabrata* and *C.krusei* isolated from crop thrush. This results are in line with (**Tartor, 2013**) and (**Keymer 1982**) who concluded that the specific instance of avian crop candidiasis is either primary or secondary mostly due to

C.albicans, *C.krusei* and *C.tropicalis*. (Kano et al., 2001) also isolated *C.parapsilosis* from crop mucosa in cockatiel.

In this study, *Candida* species were identified by phenotypic methods, API 20 C AUX biochemical kit and molecular methods.

Phenotypic methods include subculture of isolates on RAT80 and CHROM agar. This method was able to identify *C.albicans* from all samples from human, animals and poultry and other medically important *Candida* species including *C.tropicalis*, *C.parapsilosis*, *C.glabrata* and *C.krusei*.

Concerning to RAT 80, more than one species have the same elements as *C.krusei* and *C.rugosa* show primitive hyphae while *C.glabrata* and *C.inconspicua* characterized by blastospores only. By CHROM agar, some *Candida* spp. that have the same micromorphology on RAT 80 can be identified as *C.krusei* give purple color while *C.rugosa* had pink color, *C.glabrata* gave pink colonies with white borders while *C.inconspicua* had pink colored colonies. So, identification by morphological methods should be dependent on the 2 media and this time consuming and in the same time, this method couldn't identify all NAC to the species level accurately. So, accurate identification of all NAC couldn't be performed by morphological methods.

Comparing between API 20 C AUX kit and RFLP PCR, API can identify 56.5% of examined isolates that confirmed with PCR, 3 *Candida* spp. were identified by API as *Cryptococcus laurentii* were identified by PCR as *C.kefyr*, 4 tested isolates couldn't be identified by API and it found to be mixed isolates with PCR. There were 2 species that couldn't identify by API and RFLP PCR and 1 *C.krusei* identified as *C.rugosa* by API and identified by PCR as *C.krusei*.

Isolates that didn't identified and samples that had different results with API and RFLP PCR were sequenced and it was found that the 3 isolates that identified by API as *Cryptococcus laurentii*, identified by sequencing as *Kluyveromyces marxianus* (the perfect state of *C.kefyr*), one isolate identified as *C.rugosa* by API and identified by PCR as *C.krusei* was identified by sequencing as *Pichia kudriavzevii* (the perfect state of *C.krusei*), one isolate identified as *C.pelliculosa* by API and identified by sequencing as *P.anomala* (the perfect state of *C.pelliculosa*), 2 species couldn't identified by both API and RFLP PCR that identified by sequencing as *C.ethanolica* and *P.mandchurica*. *Kluyveromyces marxianus*, *Pichia kudriavzevii* and *C.ethanolica* not present in API database and mixed cultures didn't involved in the API database, so, with reference to the database, API 20 C AUX was able to identify 100% of examined

Candida species. This in agreement with (Guñdes *et al.*, 2001).

RFLP PCR could identify all *Candida* species accurately to the species level except 2 species that couldn't be identified because of no previous work (to my knowledge) used ITS1 and ITS4 for amplification of these species.

RFLP-PCR method proved to be simple, cost-effective and rapid method for differentiation between all *Candida* species that is applicable in clinical laboratories.

In this study, all examined isolates were sensitive to amphotricin B as amphotricin B has fungicidal effect against all *Candida* spp. Amphotricin B interact with fungi membrane ergosterols to produce an aggregate that forms a transmembrane channel, allowing the cytoplasmic contents to leak out and subsequent fungal cell death but amphotricin B has nephrotoxic effect so, it is not used as first choice or first line of treatment. (Hung, 2008)

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المخلص العربي

خلال العقدين الماضيين، ارتفعت وتيرة الالتهابات الفطرية مع الإصابات الخطيرة. ومن بين هذه الفطريات، كانت المبيضات أكثر أنواع الخمائر المعزولة من الإنسان والحيوان. أكثر أنواع المبيضات التي عزلت هي الكانديدا البيكانز ولكن في السنوات الاخيره زادت نسبة العدوى بانواع الكانديدا غير الالبيكانز. العديد من هذه الخمائر تقاوم مضادات الفطريات ولذلك تحديد نوع مضاد الفطريات الاكثر تأثيرا مهم جدا للتحكم في عدوى المبيضات. في هذه الدراسة، تم تجميع عينات من الانسان والحيوان والطيور وتم التعرف عليها بواسطة الطرق الظاهرية والطرق الكيمائية التجارية واختبار البلمرة المتسلسل الذي يعقبه تقطيع للامبليكون وقد وجد ان اختبار البلمره المتسلسل الذي يعقبه تقطيع للامبليكون بواسطة الانزيمات اكثر الطرق دقة وحساسية واسرعها في التصنيف. وقد وجد ان اكثر انواع مضادات الفطريات تأثيرا هي الامفوتريسين بي. هناك بعض انواع المبيضات اظهرت مقاومة لاكثر انواع مضادات الفطريات استخداما مثل الفلوكونازول .