Damanhour Journal of Veterinary Sciences 11 (2), (2024) 8-14

Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

E-ISSN 2636-3003 | ISSN 2636-3011

Candidiasis in Mastitic Milk and Humans in El-Beheira Province, Egypt: Isolation, Molecular Characterization, and Antifungal Susceptibility

Asmaa, M. Badran[1], Mousa, A. Ayoub[1], Nabil, M. Bekear[2] and Sabah, I. Shaaban[1, *]

[1]Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Damanhour University, Egypt
[2]Department of Internal Medicine, Infectious Diseases, Faculty of Veterinary Medicine, Damanhour University, Egypt

Abstract: The current work was carried out in El-Beheira Province, Egypt to estimate the frequency of *Candida* species occurrence in mastitic milk of cattle and buffalo as well as oral swabs from humans besides detection of some virulence factors and determine the antifungal susceptibility of the obtained *Candida* strains. A total of 350 raw milk samples were randomly collected from clinically mastitic bovine milk. Also, 140 oral swabs were obtained from patients attending external clinics of El-Delingat Hospital to be examined for the presence of *Candida*. The recorded results showed a high frequency of *Candida* spp. (36.9%) among the examined milk samples. *Candida* spp. isolated from cow milk (39.9%) was higher than that isolated from buffalo milk (29.3%). Also, the total prevalence of Candidiasis in human samples was 62.9% and the highest prevalence in relation to age groups was noticed in the age group > 2 - < 16 years (65.5%) and finally, the age group >40 years (58.8%). The identified *Candida* spp. in human samples were *C. albicans* (37.5%), *C. glabrata* (17.1%), *C. krusei* (14.8%), *C. parapsilosis* (13.6%), *C. tropicalis* (11.4%), *C. guilliermondii* (4.5%) *and C. kefyr* (1.1%). It was observed that the highest prevalence of *Candida* species was among artificially fed infants (77.8%) and the lowest prevalence was detected in breastfed infants (53.3%). Based on the recorded results, *Candida* species constitute a major threat to both dairy animals and humans. Also, improperly heated milk constitutes a potential source of infection for humans so efficient pasteurization must be performed to avoid this risk.

Keywords: Candida, Milk; Human, Isolation; Virulence genes; Antifungal susceptibility

*Correspondence: Sabah, I. Shaaban
Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Damanhour University, Egypt
Email: sabah_ibrahim@vetmed.dmu.edu.eg
P ISSN: 2636-3003
EISSN: 2636-3011
DOI: 10.21608/DJVS.2023.208559.1115
Received: May 03, 2023; Received: May 10, 2023; Accepted: May 10, 2023; Published: March 6, 2024
Editor-in-Chief:
Prof Dr/Ali H. El-Far (ali.elfar@damanhour.edu.eg)

1. Introduction

Mastitis is one of the most common pathologies in dairy animals. Despite the low incidence of mycotic mastitis compared to other mastitic agents, during the last decade, it has significantly increased and has been frequently recorded as the causal agent of mastitis in ruminants (Yanuartono et al., 2019).

Candida species are found normally around dairy cattle environments like milking machines, farm , treatment instruments, floors, hay, feeding...etc., and they are also found naturally on the skin (udder and teats) of dairy animals in low habitats *Candida* spp. is the most commonly isolated pathogen in cases of mycotic mastitis in cattle (Radostitis, 1995) and this may constitute a zoonotic hazard through contamination of milk with these yeasts and the possibility of transmission to consumers when consumed raw or even processed form (Hasan and Yassein, 2018).

The prevalence of bovine mycotic mastitis has been steadily rising in Egypt and that's for a number of reasons, including the country's hot climate, the predominance of small-scale and household cattle rearing systems that are frequently linked to unhygienic milking practices, the overuse and abuse of antibiotics, the misdiagnosis and subsequent delay of specific antifungal therapy (Abd El-Razik et al., 2011).

Candida species, opportunistic pathogens, are a major cause of morbidity and mortality worldwide and thus represents a serious threat to public health (Pappas et al., 2016). Further, *Candida* species can cause vaginitis, oral candidiasis, cutaneous candidiasis, candidemia, and systemic infections (Wächtler et al., 2012). Candidemia is the most frequent hospital infection accounting for up to 15% of bloodstream infections, and *Candida* species are the main causative agents in 50–70% of systemic fungal infections (Barchiesi et al., 2016).

However, *Candida* isolates can become pathogenic in instances of host weakness, as they affect around 10% of elderly people in poor health. *Candida albicans* is the most common species associated with infection, as it represents more than 80% of fungi isolated from the oral cavity (Lewis and Williams, 2017).

The intensity of the *Candida albicans* infection is correlated with the virulence factors of the pathogen, which are responsible for the location and stage of infection, type of infection, and the nature of the host response. Once the contact has occurred, enzymes enhance the adherence by damaging cell membranes and releasing the extracellular proteins, then permitting the *C. albicans* to enter the host causing infection (Lahkar et al., 2017). Some of these virulence factors are pseudohyphae formation (Germ tube) for adherence, phenotyping, switching, proteinase, and phospholipase production for invasion. When a phospholipase is present, phospholipid hydrolysis results in cell membrane disintegration, while proteinase causes host protein breakdown by hydrolyzing peptide bonds. These virulence factors are associated with the pathogenicity of *C. albicans* (Udayalaxmi et al., 2014).

The non-Candida albicans Candida (NCAC) spp., Previously considered non-pathogenic or mildly virulent, but now they are recognized as a leading cause of morbidity and mortality in immunocompromised individuals (Pfaller and Diekema, 2007).

Not every anti-mycotic agent succeeded in the treatment of *Candida* species. Before the 1980s, the use of antifungal medicines was constrained but has expanded since. In actuality, the surge in fungal illnesses over the past ten years has served as a guide for the creation of antifungal medications. Since the use of antifungal agents has increased recently, endogenous fungal flora has been suppressed, and more resistant strains have been discovered as a result of the inhibition of vulnerable strains (Koç, 2003).

So, the objective of the current work is to determine the prevalence of *Candida* species in mastitic milk of cattle and buffalo as well as oral swabs from humans besides the

identification of some virulence factors and determine the antifungal susceptibility of the obtained isolates of *Candida.*

2. Materials and Methods

2.1. Ethical approval

Ethical approval of the current research was approved by the Ethics Committee of Scientific Research, Faculty of Veterinary Medicine, Damanhour University, Egypt. Issued Approval Number: DMU/VetMed-2023/006.

2.2. Sampling

2.2.1. Milk samples

A total of 350 raw milk samples were randomly collected from cattle and buffalo with clinical mastitis who had not responded to antibiotic treatment from January to December 2022 in EL-Beheira province. The Udder, teats orifice, and milkers' hands were perfectly washed with water and soap and disinfected with 70% ethyl alcohol before the collection of milk samples. Twenty-five ml of milk were gathered in clean, sterile tubes after the initial streams of milk were discarded and then placed in an ice box.

2.2.2. Human oral swabs

One hundred and forty oral swabs were obtained from patients attending external clinics and the ICU (Intensive Care Unit) of El Delingat Public Hospital with different age groups and health statuses. Each sample was collected in sterile, dry, disposable swabs, and labeled with respective data then kept in the ice box.

All samples were transported as soon as possible under complete aseptic conditions to the laboratory of the Animal Hygiene and Zoonoses Department, Faculty of veterinary medicine, Damanhur University, for processing, isolation, and identification of *Candida*.

2.3. Isolation and identification of Candida spp.

2.3.1. Isolation of Candida spp.

About 1 ml of each milk sample was inoculated primarily into tubes of 9 ml of Sabaroud dextrose broth and then incubated at 37 ˚C for 24 hours. Then subcultured on Sabaroud dextrose agar (SDA) plates and incubated for 24 – 37 hours at 25-37 ˚C, suspected white creamy colonies were picked up and kept on slope agar for biochemical identification. Swabs were cultured on Sabaroud dextrose agar (SDA) plates and incubated for 24-37 hours at 25-37°C, suspected white creamy colonies were picked up and kept on slope agar for biochemical identification (Ogba et al., 2013).

2.3.2. Identification of Candida spp.

- Microscopic identification. (Bhavan et al., 2010 and Ogba et al., 2013
- Cultural characters on Chrome agar candida medium (CAC) (Bhavan et al., 2010 and Ogba et al., 2013).
- Biochemical identification (Yarrow, 1996).
- Germ tubes test (Ellis et al., 2007).

2.3.3. Detection of some virulent factors in the recovered isolates

- Phospholipase Activity (Price and Wilkinson, 1982).
- Assessment of Hemolytic Activity (Malcok et al., 2009).
- Aspartyl protease (proteolytic) Activity (Ruchel et al., 1982).
- Esterase activity (Aktas et al., 2002).

2.3.4. Determination of Antifungal susceptibility of Candida spp.

The agar-based E-test (Biomeriux, France) method was used to test the yeast isolates' susceptibility with Roswell Park Memorial Institute (RPMI) medium which was buffered to pH 7.0 with HCl acid and poured into Petri dish plates. The plates were inoculated by streaking a sterile swab across the surface of the agar in four different directions after dipping it into the inoculum suspension that had been adjusted to the turbidity of a 0.5 McFarland standard (106 cells/ ml). Before putting on the E-test strips, the plates were allowed to dry at room temperature for 15 minutes. After 24 and 48 hours at 35°C, the minimum inhibitory concentrations (MIC) endpoints were established. The point of complete inhibition for fluconazole, voriconazole, amphotericin B, and itraconazole was identified by the MIC, which was read as the drug concentration in that zone. The considerable inhibition slowed the growth by 80%. Itraconazole 1.0 micrograms per millilitre (10-15), voriconazole 8.0, amphotericin B > 1.0, and fluconazole 64 are the resistance breakpoints for antifungals. MIC50 and MIC90 (the MIC at which 50% and 90% of the isolates are inhibited) were also estimated (Badiee and Alborzi, 2011).

2.3.5. Molecular characterization of the recovered C. albicans strains

2.3.5.1. DNA Extraction

DNA extraction was done according to the technique recommended by Yamada et al., (2002). Accurately, to a 1.5 ml Eppendorf tube a fresh colony was transferred, and then 300 µl of lysis buffer containing (100 mM Tris pH 8, 10 mM pH 8, 100 mM NaCl, 1% SDS, Triton 2% X-100), 300 µl of phenol: chloroform (1:1) and 200 µl of glass beads, with a diameter of 1 mm were added, and the tube was vigorously shaken for 60 minutes. The sample was centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a clean tube and 400 µl of chloroform was added. After centrifuging as in the previous conditions, the aqueous phase was transferred to a clean tube and then 1 volume of cold isopropanol and 5 of 3M sodium acetate (pH: 5.2) were added and kept at -20 °C for 10 minutes. After that, the sample was washed with 70% ethanol. Then 30 µl distilled water was added and the sample was kept at -20 °C.

Table 1. Primer sequences for molecular characterization of *C. albicans*

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
ITS1 (F)	5′ TCCGTAGGTGAACCTGCGG ′3	500	Zimbeck et al (2010)
ITS4 (R)	5′ TCCTCCGCT TATTGATATGC ′3		

2.3.5.2. Amplification reaction of ITS gene (Internal transcript spacer)

DNA amplification was done in a thermal cycler using the primer sequences in **Table 1** using the following conditions: 95 ˚C for 5 min as the initial denaturation step, 5 minutes at 95 °C followed by 42 cycles of denaturation (95 °C for 1 minute), annealing (63 °C for 1 minute), and extension (72 °C for 1 minute). A final extension step (72 °C for 1 minute) was performed after the completion of the cycles. The PCR products were electrophoresed in 2% agarose gel stained with ethidium bromide solution (0.5 µg/ml), visualized under an ultraviolet transilluminator, and photographed. A 100 bp DNA ladder was used to measure the fragment size (**Figure 1**).

2.4. Statistical Analysis

The statistical analysis was done by the chi-square (χ^2) test using the statistical package for Social Sciences (SPSS 15.0). It was used to indicate whether the differences observed in the prevalence of *Candida* species among the different groups studied were statistically significant or not. Differences were recorded as significant whenever the probability was less than or equal to 0.05.

3. Results and Discussion

The isolation and identification of *Candida* spp. which may be associated with mycotic mastitis in cattle and buffalo in El-Beheira province were investigated in this study. As shown in **Table 2**, 129 (36.9%) isolates belonging to the genus *Candida* spp. were recovered from 350 examined cow and buffalo mastitic milk samples (39.9% in cattle and 28.3% in buffalo). This result was substantially identical to the one obtained by Sonmez and Erbas, (2019) (25%) and EL-Desouky et al., (2016) (27.3%) in clinically mastitic cattle in Egypt, while it is lower than that obtained by AL-abidy et al., (2019) who isolated C*andida* spp. from 46.4% of milk samples obtained from cows with mastitis and Ksouri et al., (2015) who isolated C*andida* spp. from 71.93 % of milk samples obtained from cows. These differences may be attributed to differences in

the number of the studied milk samples. Also, the presented data in **Table 2** exhibited that *Candida* spp. isolated from cow milk (39.9%) was higher than that isolated from buffalo milk (28.3%). This result was in accordance with Khalaf et al., (2021) who recorded that the prevalence of *Candida* spp. in milk samples of cows with mastitis was higher than that of buffalo's milk with mastitis (47.2% and 32.6%, respectively).

Species identification of *Candida* recovered from mastitic milk samples were presented in **Table 3** and assured the presence of *C. parapsilosis* (30.2%), *C. albicans* (18.6%), *C. tropicalis* (17.8%), *C. krusei* (16.3%), C. *zeylanoid* (12.4%) and *C. guilliermondii* (4.7%). This result agreed with Dworecka-Kaszak et al., (2012) who found that the predominant isolate of *Candida* spp. in mastitic milk was *C. parapsilosis* (45%) from C*andida* isolates while AL-abidy et al., (2019) recorded that the highest percentage among *Candida* spp. was *C. albicans* (51.7%) followed *C. parapasilosis* (12.9%). On the other hand, EL-Desouky et al., (2016) noticed that *C. tropicalis* and *C. guilliermondii* were the second most frequently isolated strains, and *C. krusei* and *C. kefyr* were less frequently isolated while *C. albicans* were predominant.

Phospholipase, proteinase, hemolysin, and esterase activities are thought to have essential roles in the pathogenesis of opportunistic fungi. **Table 4** showed that all non-*Candida albicans* isolates from milk samples were 100% positive to produce phospholipase while it was only 50% in *C. albicans*; hemolysin production was 51.3% in *C. parapasilosis* and 50% in both *C. albicans* and *C. zeylanoid* while in *C. tropicalis* 47.8 % and 33.3% in *C. krusei.* In addition, *C. albicans, C. guilliermondii,* and *C. zeylanoid* showed no production of proteinase while *C. tropicalis* was the highest production 47.8% followed by *C. krusei* and *candida parapasilosis* 33.3% and 25.6%, respectively. Esterase production was 100% in both *C. albicans* and *C. guilliermondii* and was 95.7% in *C. tropicalis,* 66.7% in *C. krusei,* 50% in *C. zeylanoid,* and 25% in *C. parapasilosis.* D'Eça Júnior et al., (2011) observed that *C. tropicalis* was the species with the highest positive isolates producing phospholipase and *C. parapsilosis* was the species with the lowest isolates for phospholipase production while regarding acid proteinase production, the tested *C. parapasilosis* isolates showed a higher number of producers, followed by *C. glabrata* and those exceeding the production of *C. albicans.* In vitro production of these virulence factors are strain-dependent and may differ according to the anatomical site infected or the yeasts involvement in a pathological process (Sacristán et al. 2011).

The anti-fungal susceptibility of *Candida* spp. recovered from mastitic milk samples was illustrated in **Table 5**. It was observed that all *Candida* isolates are sensitive to Amphotericin B, all non *C. albicans* are sensitive to Fluconazole, while *C. albicans* showed 50% resistance to it. All isolates of *C. krusei, C. guilliermondii,* and *C. zeylanoid* were sensitive to Voriconazole while 56.5% of *C. tropicalis*, 51.3% of *C. parapasilosis* and 50% of *C. albicans* were resistant to Voriconazole. Sensitivity to Itraconazole was 100% in *C. tropicalis* and resistant by 56.5%, 50%, 50%, and 25.7% in *C. parapsilosis, C. albicans, C. zeylanoid and C. parapasilosis,* respectively while C. *guilliermondii* showed 100 % resistance. The obvious sensitivity of the tested NCAC strains may be attributed to the uncommon use of anti-mycotic preparations in the treatment of bovine mastitis in Egypt. This came in agreement with Asfour et al. (2009) who found high sensitivity rates to the used anti-mycotic drugs in their study. Tanwani and Yadava (2006) recommended nystatin as intra-mammary, or amphotericin B given intravenously for the treatment of mycotic mastitis.

As shown in **Table 6**, the total prevalence of *Candida* spp. in human oral samples was 62.9% and the highest prevalence in relation to age groups was noticed in the age group > 2 - < 16 years (65.5%) followed by the age group <2 years (64.7%) and finally, the age group >40 years (58.8%). This result was similar to that of Junqeira et al., (2012) who found that 62% of human samples were positive for oral carriage of yeast. In addition, Buranarom et al., (2020) recorded oral candida colonization in 47.2% of the elderly population. Moreover, it disagreed with Anaele and Okafor, (2020) who observed that the age group ≥ 50 years had the highest frequency of occurrence of *Candida* (88.9%) while the age group 10-19 years had the least frequency (9.52%). In a review by Odd (1988) the highest reported frequencies were 71% of schoolchildren in the UK. Although one could suggest that these changes in frequency may be due to physiological changes related to age, the changes in environment and diet (breast-feeding versus formula milk feeding) may represent important factors.

The data recorded in **Table (7**) showed the identification of *Candida* spp. recovered from the examined human samples. The identified *Candida* spp. were *C. albicans* (37.5%), *C. glabrata* (17.1%), *C. krusei* (14.8%), *C. parapsilosis* (13.6%), *C. tropicalis* (11.4%), *C. guilliermondii* (4.5%) *and C. kefyr* (1.1%). A nearly similar result was obtained by Kumar et al., (2014) who found that *C. albicans* (51%) was the major candida species found in diabetic patients' oral cavities. Udayalaxmi et al (2014) found *C. albicans* the predominant isolate followed by *C.tropicalis*, *C.glabrata* and *C.krusei.* Lata et al, (2012) reported an increasing trend in occurrence of non- *C. albicans* over time.

The data recorded in **Table 8** showed the detected virulence factors in *Candida* spp. recovered from human oral samples. It was found that the highest phospholipase production was recorded in *C. albicans* (63.6%) followed by *C. parapasilosis* (58.3%), *C. krusei* (46.1%), *and C. glabrata* (13.3%) and other candida isolates did not show any phospholipase production. Hemolysin production was 78.8%, 46.2%, 42.6%, 30%, and 26.7% in *C. albicans, C. krusei, C. parapsilosis, C. tropicalis,* and *C. glabrata,* respectively, while *C. kefyr* and *C. guilliermondii* did not show any hemolysin production. *C. tropicalis* and *C. guilliermondii* showed 100% protease production followed by *C. glabrata* (80%), *C. krusei* (69.2%), and *C. albicans* (27.3%).

Esterase production was 100% in *C. kefyr,* 70% in *C. tropicalis,* 42.4% in *C. albicans* and 41.7% in *C. parapasilosis.* These results are nearly similar to the results obtained by Silva et al., (2018) that *C. glabrata* was the species with the highest proteinases production, followed by *C. krusei*. While in the phospholipase test, of the isolates that tested positive, *C. parapsilosis* showed the highest production. However, in the hemolysin test, all species analyzed had hemolytic potential, especially *C. parapsilosis* and *C. krusei* as major causes of grade IV hemolysis. The study of Rossoni et al. (2013) indicated the predominance of *C. albicans* in the production of hemolysin (100%), thus being an important virulence factor responsible for causing systemic infections.

Junqeira et al. (2012) observed phospholipase activity in all isolates of *C. albicans, C. dubliniensis, C. tropicalis,* and *C. krusei*. Proteinase activity was found in all isolates of *C. albicans* and *C. parapsilosis*. However, phospholipase and proteinase activity were negative in *C. glabrata*. Also, according to Costa et al. (2010), C. albicans produces large levels of proteinases and phospholipases while non-albicans Candida species often have low levels of these enzymes. In addition, Fatahinia et al. (2015) demonstrated a significantly different hemolytic activity of *Candida* species isolated from the oral mucosa of diabetes patients and healthy, nondiabetic controls. Moreover, Neufeld et al., (2015) studied 141 clinical *Candida* specimens in Brazil and found that *C. albicans* was the most frequently isolated species (45.4%), followed by *C. parapsilosis* (28.4%), *C. tropicalis* (14.2%), and *C. glabrata* (1.4%).

Detection of anti-fungal susceptibility of *Candida* spp. recovered from humans was tabulated in **Table 9**. It was found that *C. tropicalis and C. kefyr* showed 100% sensitivity to Voriconazole, Fluconazole, Amphotericin B, and Itraconazole while 9.1% of *C. albicans* were resistant to Voriconazole and Amphotericin B, 39.4% and 21.2% resistance to Itraconazole and Fluconazole, respectively. The recorded result was in harmony with that of Junqeira et al., (2012) who found that *C. albicans* and *C. glabrata* were resistant to Fluconazole while isolates of *C. parapsilosis* and *C. krusei* were resistant to Amphotericin B. Also, Hamza et al. (2008) found that among clinical oral yeasts from 292 HIV-infected patients in Tanzania analyzed, 5% showed resistance to Fluconazole, including isolates of *C. glabrata, C. tropicalis,* and *C. krusei*, and all isolates were susceptible to Amphotericin B.

Patients with candidemia who are less seriously ill and have not recently been exposed to azoles (A-III) are advised to take fluconazole, according to Pappas et al. (2009). Children are advised to follow the same treatment plan, with possible dosing regimen adjustments.

The effect of the type of feeding in infants on the prevalence of *Candida* was presented in **Table 10**. It was observed that the highest incidence of *Candida* species was in infants who depend on artificial milk feeding (77.8%) followed by infants fed both breast and artificial milk (70%) and the lowest prevalence was detected in breastfeeding infants (53.3%). This finding was supported by the finding of Jorge and Zollner, (2003) who recorded a prevalence of 34.5% for oral colonization by *Candida* yeasts in breastfed infants and 66.7% in formula feeding. Also, Amadio and Hahn (2011) detected *Candida* spp. in 33% of breastfed infants and 83% in both mixed feed and artificial feed infants. This may be due to the role of breastfeeding in raising the immunity status of infants.

Results in **Table 11** revealed that the highest incidence of *Candida* spp. was reported in patients with diabetes mellitus (66.7%) followed by ICU patients (63.6%), patients with other types of diseases (56.3%) and patients with gastritis and common cold (55.6% for each). A similar finding was obtained by Kumar et al., (2014) who found *C. albicans* in the oral cavity of diabetic patients (51%). NCAC species were 47%. *C. tropicalis* (15%) and *C. krusei* (14%) showed predominance over the other NCAC isolates. Also, Kumar et al., (2005) recorded a higher prevalence of candidiasis (83.67%) in patients with type 1 diabetes mellitus, 68.52% in patients with type 2 diabetes mellitus, and 27% in non-diabetic subjects. Finally, according to Jasim et al. (2016), oral samples had the highest prevalence of *Candida* spp. isolates (49.09%). These differences were due to different health status and immune response among different patients.

Techniques based on molecular biology have helped identify fungus species from clinical specimens. The commonly used PCR method for identifying *Candida* species has made it possible to separate *C. albicans* from *C. dubliniensis*, a morphologically similar species (Luo and Mitchell, 2002).

Table 2. Prevalence of *Candida* spp. in the examined mastitic milk samples

Sources of mastitic milk	No.	Positive	%	p-value
Cattle	258	103	39.9	0.047
Buffalo	92	26	28.3	
Total	**350**	**129**	**36.9**	

Table 3. Identification of *Candida* spp. recovered from the examined mastitic milk samples

Candida spp.	Milk samples						p-value
	Cattle		Buffaloes		Total		
	No.	%	No.	%	No.	%	
C. albicans	24	23.3	0	0.0	24	18.6	0.006
C. parapsilosis	34	33.0	5	12.2	39	30.2	0.172
C. krusei	11	10.7	10	38.5	21	16.3	0.001
C. tropicalis	23	22.3	0	0.0	23	17.8	0.008
C. guilliermondii	0	0.0	6	23.1	6	4.7	0.001
C. zeylanoid	11	10.7	5	12.2	16	12.4	0.237
Total	**103**	**79.8**	**26**	**20.2**	**129**	**100.0**	

Table 4. Detection of some virulence factors in Candida spp. recovered from mastitic milk

Candida spp.	No. of tested isolates	Virulence factors							
		Phopholipase		Hemolysin		Protease		Esterase	
		No.	%	No.	%	No.	%	No.	%
C. albicans	24	12	50.0	12	50.0	0	0.0	24	100.0
C. parapsilosis	39	39	100.0	20	51.3	10	25.6	10	25.6
C. krusei	21	21	100.0	7	33.3	7	33.3	14	66.7
C. tropicalis	23	23	100.0	11	47.8	11	47.8	22	95.7
C. guilliermondii	6	6	100.0	0	0.0	0	0.0	6	100.0
C. zeylanoid	16	16	100.0	8	50.0	0	0.0	8	50.0
Total	**129**	**117**	**90.7**	**58**	**45.0**	**28**	**21.7**	**84**	**65.1**

Table 5. Detection of anti-fungal sensitivity of *Candida* spp. recovered from mastitic milk

Candida spp.	No. of tested isolates	Anti-fungal agents							
		Voriconazole		Fluconazole		Amphotericin B		Itraconazole	
		S.	%	S.	%	S.	%	S.	%
C. albicans	24	12	50.0	12	50	24	100	12	50
C. parapsilosis	39	19	48.7	39	100	39	100	29	74.3
C. krusei	21	21	100	21	100	21	100	21	100
C. tropicalis	23	10	43.5	23	100	23	100	10	43.5
C. guilliermondii	6	6	100	6	100	6	100	0	0
C. zeylanoid	16	16	100	16	100	16	100	8	50

Table 6. Prevalence of *Candida* spp. in human oral samples in relation to age groups

Age groups	No.	Positive	%	p-value
Infants (≤ 2 years)	34	22	64.7	0.001
Children (> 2 - < 16 years)	55	36	65.5	
Adults (≥ 16 years)	51	30	58.8	
Total	**140**	**88**	**62.9**	

Table 7. Identification of *Candida* spp. recovered from the examined human oral samples

Candida spp.	Human samples								p-value
	Infants		Children		Adults		Total		
	No.	%	No.	%	No.	%	No.	%	
C. albicans	8	36.4	16	44.4	9	30.0	33	37.5	0.110
C. glabrata	4	18.2	4	11.1	7	23.3	15	17.1	0.117
C. parapsilosis	3	13.6	3	8.3	6	20.0	12	13.6	0.295
C. krusei	6	27.3	3	8.3	4	13.3	13	14.8	0.012
C. tropicalis	0	0.0	6	16.7	4	13.3	10	11.4	0.252
C. kefyr	1	4.5	0	0.0	0	0.0	1	1.1	0.060
C. guilliermondii	0	0.0	4	11.1	0	0.0	4	4.5	0.453
Total	**22**	**25.0**	**36**	**40.9**	**30**	**34.1**	**88**	**100**	

Table 8. Detection of some virulence factors in *Candida* spp. recovered from human oral samples

Candida spp.	No. of tested isolates	Virulence factors							
		Phospholipase		Hemolysin		Protease.		Esterase	
		No.	%	No.	%	No.	%	No.	%
C. albicans	33	21	63.6	26	78.8	9	27.3	14	42.4
C. glabrata	15	2	13.3	4	26.7	12	80.0	0	0.0
C. parapsilosis	12	7	58.3	3	42.6	0	0.0	5	41.7
C. krusei	13	6	46.1	6	46.2	9	69.2	0	0.0
C. tropicalis	10	0	0.0	3	30.0	10	100	7	70.0
C. kefyr	1	0	0.0	0	0.0	0	0.0	1	100
C. guilliermondii	4	0	0.0	0	0.0	4	100	0	0.0

Table 9. Detection of anti-fungal sensitivity of *Candida* spp. recovered from human oral samples

Candida spp.	No. of tested isolates	Anti-fungal agents							
		Voriconazole		Fluconazole		Amphotericin B		Itraconazole	
		Sensitive	%	Sensitive	%	Sensitive	%	Sensitive	%
C. albicans	33	30	90.9	26	78.8	30	90.9	20	60.6
C. glabrata	15	9	60.0	11	73.3	15	100	13	86.7
C. parapsilosis	12	8	66.7	10	76.9	12	100	10	76.9
C. krusei	13	11	84.6	13	100	13	100	11	84.6
C. tropicalis	10	10	100	10	100	10	100	5	50.0
C. kefyr	1	1	100	1	100	1	100	1	100
C. guilliermondii	4	4	100	4	100	4	100	4	100

Table 10. Prevalence of *Candida* spp. in Infants in relation to type of milk feeding

Type of feeding	No.	Positive	%	p-value
Breast feeding	15	8	53.3	0.439
Artificial feeding	9	7	77.8	
Mixed feeding	10	7	70.0	
Total	**34**	**22**	**64.7**	

Table 11. Prevalence of *Candida* spp. in adults in relation to the presence of other diseases

Disease conditions	No.	Positive	%	p-value
Diabetes mellitus	6	4	66.7	0.439
Gastritis	9	5	55.6	
Common cold	9	5	55.6	
ICU patients	11	7	63.6	
Others	16	9	56.3	
Total	**51**	**30**	**58.8**	

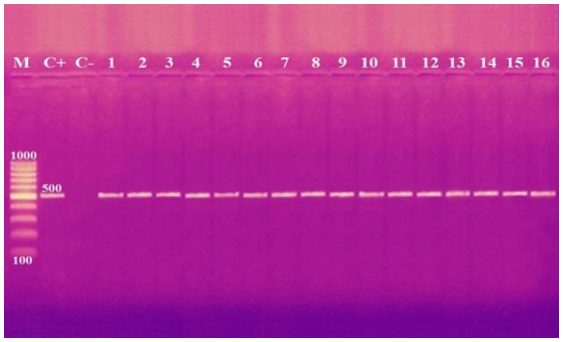

Figure 1. Agarose gel electrophoresis of PCR of species-specific gene (500 bp) for characterization of *C. albicans.*
Lane M: 100 bp ladder as molecular size DNA marker. **Lane C+:** Control positive. **Lane C-:** Control negative. **Lanes from 1 to 14:** *C. albicans* strains isolated from humans. **Lanes 15 and 16:** *C. albicans* isolated from milk.

4. Conclusion

Based on the recorded results, *Candida* species constitute a major health threat for both dairy animals and humans. Also, raw milk may be a potential source of infection for human consumers with candidiasis so, efficient pasteurization must be performed to avoid the risk of transmission of *candida* spp. Although *Candida* species are opportunistic pathogens, they can turn out to cause serious disease under certain conditions of the host, namely immunocompromised. Artificially fed infants showed the highest prevalence of candida isolates among infants due to their low immunity compared to other infants. In elders, the highest prevalence was among diabetic patients followed by ICU patients. *C. albicans* was the most frequently isolated species in the oral cavity of humans in different age groups, C. parapsilosis was the predominant isolate from mastitic milk samples followed by *C. albicans*. NCAC isolated from milk showed high production to phospholipase, hemolysin, and esterase which to their high virulence. Amphotericin B is considered the drug of choice as its sensitivity were 100% and 98.7% to *Candida* isolates from milk and human oral swabs, respectively.

Conflict of interests: There are no conflicts of interest stated by the authors.

5. References

Abd El-Razik K., Abd Elrahman K., Abd El-Moez S. and Danial E. (2011): New approach in diagnosis and treatment of bovine mycotic mastitis in Egypt. African Journal of Microbiology Research, 5: 5725-5732.

Aktas E., Yigit N. and Ayyildiz A. (2002): Esterase activity in various Candida species. Journal of International Medical Research, 30: 322- 324.

AL-abidy H, Khudaier B. Y. and Al-attraqchi A. A. (2019): Conventional and molecular detection of Candida albicans and Candida parapasilosis isolated from bovine mastitis in Basrah-Iraq. Biochemical and Cellular Archives 19: 3285- 3289.

Al-Rashedi, N. A. (2012): Evaluation of Candida albicans Diagnosis by using conventional PCR. AL-Qadisiya Journal of Science., 17 (1): 1- 6.

Amadio J.V.R., de S. and Hahn R.C. (2011): Candida spp in the oral cavity of infants and breasts of nursing mothers. Journal of Pediatric Infectious Diseases 6 (2011) 231–236 231 DOI 10.3233/JPI-2012-0329.

Anaele E.N. and Okafor J. I. (2020): The Influence of Age, Health Care, and Hygienic Habits on Candida Species Prevalence in the Human Oral Cavity and Genitourinary Tract. International Journal of TROPICAL DISEASE& Health41(11): 1-13,

Asfour H.A.E, El-Metwally A.E and Kotb M.H, (2009): Yeast as a cause of bovine mastitis and their Histopathological effect on the mammary gland tissues. Journal of Egyptian Veterinary Medicine Association 69: 41-72

Badiee P. and Alborzi A. (2011): Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five-year study. Iran. Journal of Microbiology., 3 (4): 183-188.

Barchiesi, F., Orsetti, E., Osimani, P., Catassi, C., Santelli, F., and Manso, E. (2016): Factors related to outcome of bloodstream infections due to Candida parapsilosis complex. BMC Infectious Disease, (16): 387-393

Bhavan P., Saravana R., Rajkumar S., Radhakrishnan C., Seenivasan E. and Kannan S. (2010): Culture and Identification of Candida Albicans from Vaginal Ulcer and Separation of Enolase on SDS-PAGE. International Journal of Biology, 2 (1): 84-89.

Buranarom N., Komin O. and Matangkasombut O. (2020): Hypo salivation, oral health, and Candida colonization in independent dentate elders. PLOS One. 2020; 15(11): e0242832.

Costa C.R., Passos X.S., Sousa L.K., Lucena A.P., Fernandes F.O. and Silva R.M. (2010): Differences in exo enzyme production and adherence ability of Candida spp. isolates from catheter, blood, and oral cavity.Revista do Instituto de Medicina Tropical de Sao Paulo . 2010; 52:139-43.

D'Eça Júnior A, Silva A.F., Rosa F.C., Monteiro S.G., Figueiredo P.D.M.S, and Monteiro C.D.A. (2011): In vitro differential activity of phospholipases and acid proteinases of clinical isolates of Candida. Revista da Sociedade Brasileira de Medicina Tropical. 44:334–8

Dworecka-Kaszak B., Krutkiewicz A., Szopa D., Kleczkowski M. and Biegańska M. (2012): High prevalence of Candida yeast in milk samples from cows suffering from mastitis in Poland. The Scientific World Journal Volume 2012: Article # 196347.

EL-Desouky I., Mohamed N., Khalaf D., Salama A., ELSify A., Ombarak R., EL-Effat S., Ballal M., and AL Shabrawy A. (2016): Candida Mastitis in Dairy Cattle with Molecular Detection of Candida albicans. Kafkas Universitesi Veteriner Fakultesi Dergisi 22 (3): 461-464, 2016. DOI: 10.9775/kvfd.2015.14843.

Ellis D., Davis S., Handke R. and Bartley R. (2007): Description of Medical Fungi" 2nd ed., Mycology Unit Women's and Children's Hospital North. Adelaide 5006 Australia book.

Fatahinia M., Poormohamadi F. and Mahmoudabadi A.Z. (2015): Comparative study of esterase and hemolytic activities in clinically important Candida species, isolated from oral cavity of diabetic and non-diabetic individuals. Jundishapur Journal of Microbiology. 2015;8: e20893.

Hamza O.J., Matee M.I., Moshi M.J., Simon E.N., Mugusi F. and Mikx F.H. (2008): Species distribution and in vitro antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiol. 2008; 8:135.

Hasan, K.A.M. and Yassein S.H.N. (2018): Prevalence and type of fungi in milk from goats with subclinical mastitis. Online Journal of Veterinary Research. 22(8): 669-674.

Jasim S.T., Flayyih M.T.and Hassan A.A. (2016): Isolation and identification of Candida spp. from different clinical specimens and study the virulence factors. World Journal of Pharmaceutical Sciences:121-137.

Junqeira J.C., Vilela S.F.G., Rossoni R.D., Baraosa J.O., Costa A.C.B.P., Rasteior V.M.C., Suleiman, J.M.A.H. and Jorge, A.O.C. (2012): Oral colonization by yeasts in HIV-positive patients in Brazil.Revista do Instituto de Medicina Tropical de São Paulo, 54(1): 17-24.

Khalaf D.D., Soliman M.M.H. and Mansour A.S., (2021): Conventional and molecular identification of mycotic mastitis caused by Candida in farm animals. International Journal of Veterinary Science 10(1): 64-68.

Koç N. (2003): Antifungal resistance in our country. 3. National Fungal Diseases and Clinical Micology Congress (27-30) Congress Book.Bornova, Izmir, Turkey, pp: 285-300.

Ksouri S., Djebir S., Hadef Y. and Benakhla A. (2015): Survey of bovine mycotic mastitis in different mammary gland statuses in two north-eastern regions of Algeria. Mycopathologia, 179, 327-331. DOI: 10.1007/ s11046-014-9845-2.

Kumar B., Padshetty N., Bai K.Y., Rao M. (2005): Prevalence of Candida in the oral cavity of diabetic subjects. Journal of the Association of Physicians of India. 2005; 53:599.

Kumar D. A., Muralidhar S., Biswas K., Banerjee U., Basir S. F., and Khan L. A. (2014): Species Diversity, Antifungal Susceptibility, and Virulence Attributes of Candida Colonising the Oral Cavities of Adult Diabetic Patients. Hindawi Publishing Corporation Journal of Mycology Volume 2014, Article ID 395041, 9 pages

Lahkar V., Saikia L., Patgiri S.J., Nath R. and Das P.P. (2017): Estimation of biofilm, proteinase & phospholipase production of the Candida species isolated from the oropharyngeal samples in HIV infected patients. Indian Journal of Medical Research., 145(5): 6

Lata RP, Jayshri DP, Palak B, Sanjay DR, Parul DS. Prevalence of *candida* infection and its antifungal susceptibility pattern in tertiary care hospital, Ahmedabad. National Journal of Medical Research. 2012; 2:439–41.

Lewis M.A.O. and Williams D.W. (2017): Diagnosis and management of oral candidosis. British Dental Journal 2017 Nov;223(9):675-81. doi: 10.1038/sj.bdj.2017.886.

Luo G. and Mitchell T.G. (2002): Rapid detection of pathogenic fungi directly from cultures by using multiplex PCR. Journal of Clinical Microbiology. Vol.40, No.8.

Malcok H., Aktas A., Nimet Y. and Yazgi H. (2009): Hemolytic activities of the Candida species in liquid medium. The Eurasian Journal of Medicine (EAJM), 41 (8): 95-98.

Neufeld P. M., Melhem Mde S., Szeszs M. W., Ribeiro M. D., Amorim L.and da Silva M. (2015): Nosocomial Candidiasis in Rio De Janeiro State: Distribution and Fluconazole Susceptibility Profile.Brazilian Journal of Microbiology. [publication Braz. Soc. Microbiology] 46, 477–484.

Odd FC (1988). *Candida and candidiasis*, 2nd edn. London: Bailliere Tindall, 1988; 3

Ogba, O., Lydia N., James E., Baki I. and Godwin D. (2013): Characterization of Candida Species Isolated from Cases of Lower Respiratory Tract Infection among HIV/AIDS Patients in Calabar, Nigeria. World Journal of AIDS, 2013, 3, 201-206 http://dx.doi.org/10.4236/wja.2013.33027.

Pappas P.G., Kauffman C.A.and Andes D. (2009): Infectious Diseases Society of America. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clinical Infectious Diseases. 2009; 48(5):503–535.

Pappas, P. G., Kauffman, C. A., Andes, D. R., Clancy, C. J., Marr, K. A., Ostrosky-Zeichner, L., et al. (2016): Clinical practice guideline for the management of candidiasis: 2016 update by the infectious diseases society of America. Clin. Infect. Dis. (62):, e1–e50.

Pfaller M.A.and Diekema D.J. (2007): Epidemiology of invasive candidiasis: a persistent public health problem. Clinical Microbiology Reviews 2007; 20(1):133–63.

Price M., Wilkinson I. and Gentry O. (1982): Plate method for detection of phospholipase activity in Candida albicans. Sabouraudia., 20 (1): 7-14. doi 10.1080/00362178285380031.

Radostitis O.M., Blood D.C. and Gay C.C. (1995): Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses. 10th edition. Elsevier Saunders, USA, 842.

Rossoni R.D., Barbosa J.O., Vilela S.F., Jorge A.O. and Junqueira J.C. (2013): Comparison of the hemolytic activity between C. albicans and non-albicans Candida species. Brazilian Oral Research2013 Nov-Dec;27(6):484-9.

Ruchel R., Tegeler R. and Trost M. (1982): A comparison of secretory proteinases from different strains of Candida albicans. Sabouraudia., 20 (3): 233-44.

Sacristán, B., Blanco, M.T., Galán-Ladero, M.A., Blanco, J., Pérez-Giraldo, C. and Gómez-Garca, A.C. (2010): Aspartyl proteinase, phospholipase, hemolytic activities and biofilm production of Candida albicans isolated from bronchial aspirates of ICU patients. Medical Mycology. Volume 49, Issue 1, January 2010, Pages 94-97

Santos R. C. and Marin J. M. (2005): Isolation of Candida species from mastitic bovine milk in Brazil. Mycopathologia 159, 251-253

Schmitt J.A. (1971): Epidemiological investigation of oral *C. albicans*. Review of Medical and Veterinary Mycology. 1971;10(1):218

Silva D.M., Souza T.C., Alencar C.F.C., Souza I.S., Bandeira M.F.C.L. and Fernandes O.C.C. (2019): Virulence factors of Candida species from the oral mucosa and prostheses of elderly people from a riverside community in the Amazon state, Brazil. Rev Odontol UNESP. 2019;48: e

Sonmez M. and G Erbas. (2017): Isolation and identification of Candida spp. from mastitic cattle milk and determination of antifungal susceptibilities. International Journal of Veterinary Science, 6(2): 104-107. www.ijvets.com

Tanwani S.K and Yadava R, (2006). Fungi responsible formastitis in animals. Agricultural Entomology. Public Health, April.

Udayalaxmi S.J. and D'Souza D. (2014): Comparison Between Virulence Factors of Candida albicans and Non-Albicans Species of Candida Isolated from Genitourinary Tract. Journal of Clinical and Diagnostic Research. 8(11): DC15-DC17.Yamada Y., Makimura K., Merhendi H., Ueda K. and Nishiyama, Y. (2002): Comparison of different methods for extraction of mitochondrial DNA from human pathogenic yeasts.The Journal of Infectious Diseases ., 55: 122-125.

Wächtler, B., Citiulo, F., Jablonowski, N., Förster, S., Dalle, F., Schaller, M., et al. (2012): Candida albicans-epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the infection process. PLoS One (7): e36952.

Yanuartono A.N., Indarjulianto S., Raharjo S., Purnamaningsih H. and Haribowo D.N. (2019): Review: mycotic mastitis in ruminants. Jurnal Ilmu Peternakan 29(2): 109 – 130.

Yarrow D. (1998): Methods for the isolation, maintenance and identification of yeasts. In The Yeasts, a Taxonomic Study (Ed. by C. P. Kurtzman, & J. W. Fell. Amsterdam: Elsevier), 4th ed., pp. 77–100.

Zimbeck A., Iqbal N., Ahlquist A. and Merhendi H. (2010): FKS mutations and elevated echinocandin MIC values among Candida isolates from U.S. population-based surveillance. Anti-microbe Agents Chem other., 54: 5042–5047.

Zollner M.S. and Jorge A.O. (2003): Candida spp. occurrence in oral cavities of breastfeed in infants and in their mothers' mouth and breasts. Pesqui Odontol Bras. 2003; 17(2):151-155.