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Molecular typing, virulence genes and potential public health implications of *Candida albicans* isolated from bovine milk

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Abstract

Yeast pathogens recently became an important cause of bovine mastitis worldwide. The prevalence of yeast pathogens in milk samples collected from chronic cases of mastitis as well as healthy cattle in Egypt was investigated. Genotyping and virulence gene detection in the isolated *Candida albicans* strains were also conducted. *C. albicans* was the predominant *Candida spp*. in mastitic milk samples (8%). Molecular genotyping revealed that all *C. albicans* isolates were of the *RPS* type 3. *HWP*1 and *PLB*1 were detected in all *C. albicans* isolates from mastitic cases, while *ALS*1 and *SAP*4 were isolated from 2 and 1 isolates of these cases, respectively. All virulent genes detected in animal isolates were previously associated with human clinical cases implicating its potential zoonotic risk. This is the first report of the *RPS* typing and virulence genes detection in *C. albicans* isolates from bovine milk samples in Egypt.

Key words: bovine milk, *Candida albicans,* molecular Typing, virulence genes, public health

Introduction

Mastitis is the most prevalent production disease in dairy herds worldwide. Mastitis is predominantly caused by bacterial pathogens but recently there was an emerging increase in the incidence of mycotic mastitis[1)]. In Egypt, there are many predisposing factors that may contribute to the continuous increase in the prevalence of bovine mycotic mastitis as relatively hot climate, predominant small-scale and household rearing systems of cattle that usually associated with unsanitary milking practices, the excessive and misuse of antibiotic therapy, the misdiagnosis and consequent delay of specific antifungal therapy[1,9)]. Candida albicans was prevalent in several cases of mycotic bovine mastitis worldwide[1,3,13)]. It was isolated form subclinical and healthy milk as well[13)]. In humans, *C. albicans* was associated with several cutaneous, mucosal and invasive manifestations especially in immuno-compromised patients[17)]. The ability of *C. albicans* to invade multiple organs of the host was attributed to wide range of virulence factors[17)]. In particular, adhesion and hydrolytic enzymes secretion appear to be critical for pathogenicity[10,17)]. Several studies

reported that genes encode for the Agglutinin-like Sequence (*ALS*) protein family and the Hyphal Wall Protein 1 (*HWP*1) in C. albicans are implicated in the process of adhesion to the host surfaces[17]. Secreted Aspartyl Proteinases (*SAP*) are encoded by the *SAP* gene family and they degrade many proteins of the host as albumin, hemoglobin, keratin, and secreted Immunoglobulin A[18]. Phospholipases, particularly Phospholipase B (*PLB*), are critical for tissue invasion[10]. This study aimed to estimate the *C. albicans* prevalence in mastitic and healthy bovine milk collected from Menofia governorate, Egypt. In addition, utilizing molecular tools for genotyping and virulence genes detection in *C. albicans* isolates with its relevant public health implications.

Material and Methods

Milk Sampling: During the period between 15 June to 20 of August of 2015, All the lactating cattle (n= 853) in 20 small-scale dairy farms reared under the intensive system of husbandry in the Menofia governorate of Egypt were examined for detection of mastitis by clinical examination of the udder and California Mastitis Test (CMT). Milk samples were collected from cows with chronic cases with no response to antibacterial chemotherapy for two or more weeks (n= 50). Another 20 milk samples were collected from healthy animals (no precipitate in CMT) in contact of the chosen mastitic cases. The milk samples were collected under aseptic condition from all quarters and pooled as one sample per animal.

Yeast culturing and identification: Each milk sample was inoculated on Tryptone Soy Agar (TSA) medium (LabM, UK) supplemented with 5% sheep blood agar and Sabouraud Dextrose Agar media (Oxoid, Germany) containing chloramphenicol (400mg/l). The inoculated plates were incubated at 37°C for 2 to 5 days. Only samples with unified infection by yeast were included in this study. Yeast identification was conducted according to Barnett *et al.*[2].

Molecular identification, typing and virulence genes detection of C. albicans isolates: DNA extraction from purified yeast culture was performed using the QIAamp DNA Mini kit (Qiagen, Germany) according to manufacturer'srecommendations. Identification of *C. albicans* isolates was conducted using *C. albicans*-specific primers amplifying a portion of the Internal Transcribed Spacer 2 (*ITS*2) gene[16]. Genotyping of the isolates was based on the Repetitive Sequence type (*RPS*)[14]. The virulence genes detected were *ALS*[16], *HWP*[16], *SAP*4[11] and *PLB*1[10].

Ethical approval: Ethical approval was obtained from a committee of Research, Publication and Ethics of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

Results

Yeast pathogens were detected in 21 of the examined milk samples (30%); 16 samples of mastitic milk (32%) and 5 samples of healthy milk (25%). Genera of detected yeasts were as follows; Genus *Candida* (18.6%), Genus *Rhodotorula* (5.7%) and genus *Geotrichum* (5.7%). Results in table 1 showed that *C. albicans* was the most isolated *Candida* spp. from mastitic milk samples (8%), while *C. parapsilosis* predominate in healthy milk samples (10%). *R. rubra* was recorded in 8% of mastitic milk samples, but none of *Rhodotorula spp*. was found in healthy milk. *G. candidum* was isolated from 6% and 5% of mastitic and healthy milk samples respectively. *C. albicans* isolates identification was confirmed using *C. albicans*-specific amplification of *ITS*2 gene and all of the isolates were of the *RPS* type 3 (Fig. 1). *HWP*1 and *PLB*1 genes were found in four of the C. *albicans* isolates (80% per each). The *ALS*1 gene was detected in two of the isolates (40%). Only one isolate contained the *SAP*4 gene in its genome (20%) (Table 2, Fig. 1). None of the virulence genes was detected in the *C. albicans* strain isolated from

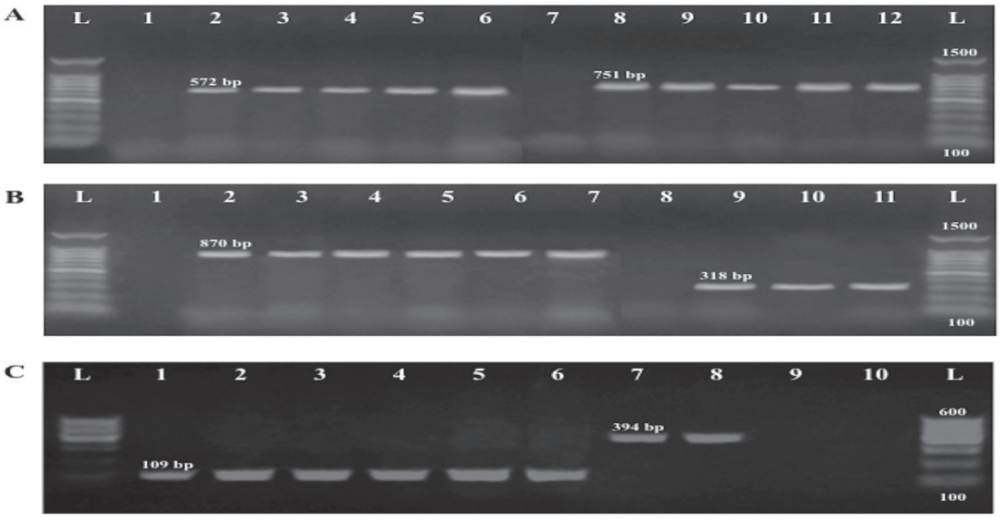

Fig. 1. Molecular identification, *RPS* typing and detection of virulence genes in the isolated *C. albicans*. A) Positive *HWP*1 (lane 3-6) and *PLB*1 (lane 9-12) genes in in CaM1-4 isolates, respectively. B) Positive of *RPS* type 3 (lane 3-7) in CaM1-4 and CaH1 isolates, respectively and positive *ALS*1 gene (lane 10, 11) in CaM1 and CaM4 isolates, respectively. C) Positive *ITS*2 gene (lane 2-6) in CaM1-4 and CaH1 isolates, respectively and *SAP*4 gene (lane 8) in CaM4 isolate. Blank Lanes: control negative. Bands with bp size: control positive. L: DNA ladder

healthy milk.

Discussion

Mycotic mastitis in cattle is a raising problem in Egypt and other countries as well. Yeast pathogens were detected in 30% of the milk samples collected in this study. These results nearly agree with previous report in Egypt[9] and India[13]. Yet a much lower prevalence was reported in Europe[8], which could be attributed to higher sanitary environment and lower ambient temperature in Europe. *C. albicans* was the predominant *Candida* spp. in the examined mastitic milk samples. This was in line with other reports in Egypt[9] and abroad[3,13] and highlights the importance of *C. albicans* pathogens in cases of bovine mycotic mastitis. All *C. albicans* isolates in this study were of the *RPS* type 3. This may indicate a common source of infection especially when all milk samples were collected from same geographical area (Menofia governorate of Egypt). The *RPS* type 3 *C. albicans* was the predominant type among patients from Japan[7] and Iran[14]. Detection of The *HWP*1 adhesion gene was higher than the *ALS*1 gene in *C. albicans* isolates from bovine milk samples. These results were in contrast with other reports, which showed higher percentages of *ALS*1 gene detection than that of *HWP*1 in *C. albicans* isolates from urine, blood, vaginal smears, wounds and respiratory tracts of human cases[6,11].

Table 1. Number (percentage) of yeast pathogens isolated from bovine milk samples

Samples	*C. albicans*	*C. parapsilosis*	*C. tropicalis*	*R. rubra*	*G. candidum*
Mastitic milk	4 (8)	3 (6)	2 (4)	4 (8)	3 (6)
Healthy milk	1 (5)	2 (10)	1 (5)	0 (0)	1 (5)
Total	5 (7.1)	5 (7.1)	3 (4.3)	4 (5.7)	4 (5.7)

Table 2. Number (percentage) of virulence genes detected in the isolated *C. albicans*.

Samples	Isolates	*ALS*1	*HPW*1	*PLB*1	*SAP*4
Mastitic milk	CaM1[a]	+	+	+	-
	CaM2	-	+	+	-
	CaM3	-	+	+	-
	CaM4	+	+	+	+
Healthy milk	CaH1[b]	-	-	-	-
Total		2 (40)	4 (80)	4 (80)	1 (20)

These results may imply difference in mechanism of adhesion by *C. albicans* based on the type of infected organs of the host. PLB1 gene was detected in all *C. albicans* isolates from the mastitic milk samples. PLB1 gene was also detected in all C. albicans isolates from Kareish cheese prepared from skimmed bovine milk in another study in Egypt[4]. This may indicate the importance and high frequency of PLB1 gene in the isolated *C. albicans* strains from bovine milk in Egypt. In human cases, higher expression of *PLB*1 gene usually associated with *C. albicans* isolates from blood and invasive diseases than isolates from superficial diseases and commensals[5]. The SAP4 gene was the lowest detected virulence gene in the isolated *C. albicans* in this study. Same result was recorded in *C. albicans* isolates from vulvovaginal candidiasis in human[11]. Interestingly, none of the virulence genes examined in this study were detected in the *C. albicans* isolate from healthy milk. Previous studies showed that *C. albicans* mutants that deprived either of the *ALS*1, *HWP*1, *SAP*4 or *PLB*1 genes were less adherent to the host epithelium and less virulent in animal models to a varying degree[5,17]. Therefore *C. albicans* strains naturally deprived these virulence genes may be less capable of inducing diseases. As a public health hazard, *C. albicans* remains the most prevalent fungal pathogen in humans. All *C. albicans* strains isolated from mastitic milk in this study possessed virulence genes that have been identified and expressed in various clinical cases of human[5,6,11], hence these milk isolates may have the potentials to induce diseases when transmitted to susceptible humans. A special attention must be given to milk as source of mycotic pathogens for human. Candidemia in hospitalized children were attributed to milk contamination in a previous study[12]. Moreover, oral thrush in milkers was associated with close contact with mastitic cattle in another study[15].

In conclusion, *C. albicans* is an important cause of mycotic mastitis in Egypt. This the first study exploring the RPS genotype and presence of *HWP*1, *ALS*1, *PLB*1 and *SAP*4 virulence genes in *C. albicans* isolates from bovine milk samples in Egypt. Understanding the virulence mechanism in animal isolates of *C. albicans* and identifying its possible infection sources may elucidate their zoonotic potentials and allows designing effective preventive measures against its public health risks.

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