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Original article

DETECTION OF *CANDIDA TROPICALIS* FROM CAMEL MASTITIS

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Summary

Esmaeel, S. A., S. F. H. Al-Abedi, M. A. Altaliby & Q. T. Al-Obaidi, 2025. Detection of *Candida tropicalis* from camel mastitis. *Bulg. J. Vet. Med.,* **28**, No 1, 154–163.

The present study was conducted to detect *Candida tropicalis* as a cause of camel mastitis (CM) using routine diagnostic tests including Sabouraud's dextrose agar (SDA) and API (analytical profile index) test strips, which were confirmed through amplification of the *18S rRNA* gene of *C. tropicalis* using conventional polymerase chain reaction (c-PCR) technique and to investigate of the virulence genes of *C. tropicalis* including secreted aspartyl proteinase (*SAPT4*) and agglutinin-like sequence (*ALST1*) genes using c-PCR. Between January 2023 and May 2023, seventy mastitis milk samples were obtained from camels in Wasit province, Iraq. Results indicated that *C. tropicalis* was identified in 15/70 (21.4%) and 10/15 (66.6%) of mastitis milk samples based on milk cultured in SDA and API 20C AUX, respectively. The positive samples for *C. tropicalis* were 15/15 (100%) via the c-PCR technique. The results also showed that *C. tropicalis* positive for virulence genes *SAPT4* and *ALST1* in camel mastitis samples were 12/15 (80%) and 10/15 (66.6%), respectively. This study concluded that *C. tropicalis* in camels is the most common cause of mycotic mastitis in Wasit province, Iraq. The presence of virulence factors *ALST1* and *SAPT* genes of *C. tropicalis* in camel milk samples could be considered as transmission vehicle of these pathogens.

Key words: camel mastitis, *Candida tropicalis*, c-PCR technique, routine diagnostic tests, virulence factors

INTRODUCTION

Camels are the most dominant animals on many continents, such as Asia and Africa with an important contribution to human life on desert land (Abdella *et al.,* 2014). The milk of camels is the main component of the diet and is regarded as a vital nutritional material for the people of this region (Seifu, 2023). Camel milk nutritional characteristics vary depending on its content, but is an excellent source of minerals such as calcium, salt, magnesium, iron, copper, with less sugar and cholesterol than cow milk. Furthermore, it contains vitamins, particularly ascorbic acid, antimicrobial compounds, and a high lactoferrin level (Seifu, 2022). The she-camel

is distinguished by ongoing lactation, regardless of difficult circumstances such as desert seasons, when milk production of other dairy animals ceases (Faraz *et al.,* 2021). Camel milk is commonly utilised in Russia to cure tuberculosis and other lung disorders, as well as in India to treat tuberculosis, dropsy, jaundice, and anaemia. It has been utilised as well to treat diabetes in the past (Agrawal *et al.,* 2005). Camel milk has been demonstrated to improve long-term glucose control and lower insulin doses in type 1 diabetes patients. Furthermore, garris, a customarily fermented camel milk product, is prescribed in Sudan to treat leishmaniasis, a protozoal infection of the gastrointestinal tract (Radwan & Mohammed, 2022). Traditional fermented products produced from camel milk include garris and koumiss (Sulieman *et al.,* 2006), Domiati cheese and fresh soft white cheese (Solanki & Hati, 2018), hard cheese (Konuspayeva & Faye, 2021), and ice cream. (Seifu, 2023). The camel milk viscosity of yoghurt did not change during the gelation process (Oselu *et al.,* 2022). The contents of immunoglobulin G, lactoferrin, lysozyme, lactoperoxidase, and secretory immunoglobulin A in camel milk are high. Furthermore, when compared to cow and buffalo milk, these antimicrobial components are substantially more abundant in camel milk and also more heat-stable (Swelum *et al.,* 2021).

Mastitis is regarded as a serious problem with a global impact on dairy cattle creating huge economic costs; further according to estimates, more than 25% of nursing camels suffer from she-camel mastitis (Abera *et al.,* 2010; Darwish, 2023), accounting for almost 70% of milk production losses (Alamin *et al.,* 2013). Mastitis can be described as inflammation of the parenchyma of the mammary gland caused by a combination of factors, including physical, chemical, and unusual presence of microorganisms in milk, as well as pathological changes in the glandular tissue of the udder, such as discoloration, the presence of clots, and a high level of white blood cells in the milk (Constable *et al.,* 2017). It can be caused by over 100 different pathogens, each with a unique mechanism of infecting animal and inducing different phases of the disease. The infection may be managed and minimised by applying control and management strategies, based on the farm environment, which influence the pathogens that may attack the animal and its capacity to resist them (Lyer *et al.,* 2014).

Mastitis is divided into two categories: subclinical mastitis, which has imperceptible indicators and requires indirect diagnosis, and clinical mastitis, with obvious symptoms that is easy to identify (Al-Dughaym *et al.,* 2015; Seligsohn *et al.,* 2021). It can be clinically diagnosed, also many field and laboratory tests, such as the California mastitis test, pH response, somatic cell count, and bacterial confirmation tests, are feasible using culture and molecular techniques (Kalla *et al.,* 2008). Traditionally, yeast identification was based on phenotypic assays, which are laborious and challenging and have poor reproducibility and precision due to yeast physiological and growth environment dependence (Fernandez-Espinar *et al.,* 2006; Lopandic *et al.,* 2006). However, advances in molecular biology, the simplicity, specificity, and reproducibility of techniques and results provided after a short period of time are the reason for increasing use of PCR assays for identification of *Candida* (Eghtedar Nejad *et al.,* 2020).

The documented investigations on the pathogenic factors linked to camel mastitis are fewer compared to bovine mastitis. Bacterial infections are the most common cause of mastitis in domestic animals, including camels: *Micrococcus spp., Streptococcus sp., Staphylococcus aureus* (Al-Majali *et al.,* 2007; Al-Juboori *et al.,* 2013), *Streptococcus agalactiae* (Al-Majali *et al.,* 2007), coagulase-negative staphylococci (Al-Majali *et al.,* 2008*), Pasteurella haemolytica, Escherichia coli* and *Staphylococcus epidermides* (Al-Majali *et al.,* 2007; Al-Juboori *et al.,* 2013), *Corynebacterium spp.* (Almaw & Molla, 2000), *Candida albicans* (Al-Abidy *et al.,*2019). The presence of yeast and yeast-like fungi alters dairy products and milk by producing extracellular cell enzymes such as proteinases and lipase, which have an impact on the quality and organoleptic qualities, and shorten product shelf life. The type of management used in dairy farms may have had an impact on the wide range of yeasts and yeast-like fungi (Krukowski *et al.,* 2006; Mir Khan & Selamoglu, 2020).

The study goals were to use existing diagnostic tests to identify *Candida tropicalis*, implicated as a cause of mycotic mastitis in camels using traditional diagnostic tests and to investigate *C. tropicalis* virulence genes (*SAPT4* and *ALST1*) using c-PCR technique.

MATERIALS AND METHODS

A total of 70 milk samples were gathered from she-camels with clinical or subclinical mastitis, as defined by the California mastitis test from January 2023 to May 2023 from several parts of Iraqi Wasit province. Mastitis was detected by abnormal milk production, signs of udder irritation, and bacterial culture for mastitis pathogens (Al-Abedi *et al.,* 2002).

Isolation of Candida tropicalis

Milk samples were added to Sabouraud's Dextrose agar treated with 0.05 mg/mL chloramphenicol and kept at 37 °C for 24 hours to a week. After incubation, morphological analyses at the macroscopic and microscopic levels were conducted to establish the genus level. The analytical profile index (API) technique was applied on the suspected isolates, then they were confirmed using amplification of the *18S rRNA* gene of *C. tropicalis* by c-PCR technique.

Conventional polymerase chain reaction technique

DNA extraction and oligonucleotide primers. The G-spin DNA extraction kit (Qiagen GmbH, Hilden, Germany) was used to extract yeast genomic DNA of *C. tropicalis* from milk samples in accordance with the directions of the manufacturer. Three oligonucleotide primers (forward and reverse) were used to amplify the *18S rRNA* gene (Al-Abedi *et al.,* 2023), and the most virulent genes of the *C. tropicalis*: agglutinin-like sequence (*ALST1*) gene and the secretory aspartyl proteinase like (*SAPT4*) gene (Zaugg *et al.,* 2001). All primers were provided from Macrogen company, South Korea (Table 1). The concentration and purity of the DNA extracted from the camel milk samples were determined using nanophotometer P-Class (IMPLEN, Germany). The concentration of DNA yield was determined by absorbance (A) at wavelength 260 nm. The purity was obtained by computing the ratio of A260 nm to A280 nm, as described by Liao *et al*. (2017).

DNA amplification. The PCR master mix reaction, with a total amount equal to

25 µL, was prepared using the Maxime PCR premix kit I-Taq method comprising a master mixture of 12.5 µL, each primer (forward and reverse) primers 1 µL (10 pmol), DNA template 3 µL, and 8.5 µL of nuclease-free water. The PCR machine was programmed for 30 cycles, according to Zaugg *et al.* (2001) and Al-Abedi *et al.* (2023). The thermocycler programme settings were as follows: predenaturation step 2 min at 95 °C (1 cycle), denaturation step 30 s at 95 °C, annealing step (58.3 °C for *18S rRNA*, 55.9 °C for *ALST1*, and 58.6 °C for *SAPT4*) 30 s, and extension step 50–60 s at 72 °C (29 cycles), with a final extension step of 5 min at 72 °C. The PCR results were moved to a 1.5% agarose gel dyed with Safe-Red™ dye, and run for 1 hour (100 V) on electrophoresis. The bands were examined through UV transillumination (BIO-RAD/USA).

RESULTS

From the total of 70 milk samples obtained from camels with mastitis, 15 camels (21.4%) were infected with *Candida tropicalis* based on cultural (Sabouraud dextrose agar) morphological features. API 20C AUX was detected in 10/15 (66.6%), as shown on Fig. 1.

Furthermore, the concentration of DNA ranged from 5.9 to 70.2 ng/mL, with a purity level from 1.8 to 2.08. In the present study *C. tropicalis* was identified

Table 1. Primers employed for the amplification of the *18S rRNA, ALST1*, and *SAPT4* genes in *Candida tropicalis*

Gene		Primer sequence (3′–5′)	Product size (bp)	Reference
18S rRNA gene	F	TCTGACGTGCTGGGGATAGA	542	Al-Abedi *et al.* (2023)
	R	TGGAATACCAAAGGGCGCAA		
ALST1 gene	F	AATGTATCACCGCAGCCAA	364	Zaugg *et al.* (2001)
	R	CGGCACCTTCGAAATTTGCT		
SAPT4 gene	F	CCGTACCAAGTTCTGAGTTTGC	452	
	R	TGTCGCTGCTGGAGGAAATT		

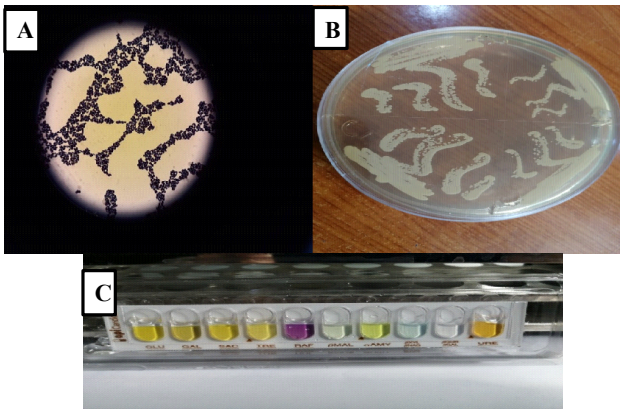

Fig. 1. A. Gram staining (1000× oil immersion); **B.** White creamy colonies of *Candida tropicalis* on SDA; **C.** API 20C AUX.

using conventional PCR technique, amplifying a particular partial gene of *18S rRNA*. This particular gene was present in all 15 analysed samples of *C. tropicalis*, accounting for 100% positive samples. The resulting PCR product exhibited a size of 542 base pairs (Fig. 2).

The outcomes of PCR amplification of the aforementioned virulence genes revealed that 12 out of 15 isolates (80%) affiliated with *C. tropicalis* exhibited a positive response for secreted aspartyl proteinase (*SAPT4*). The PCR product of this particular gene was determined to have a length of 452 base pairs (Fig. 3). Similarly, 10/15 isolates were positive outcome for agglutinin-like sequence (*ALST1*) (66.6%). The length of the PCR product for this gene was 364 base pairs (Fig. 4).

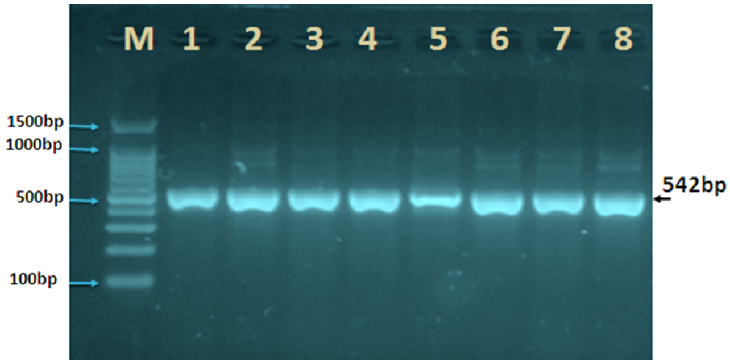

Fig. 2. Electrophoresis on 1.5% agarose gel displaying pathogenic *C. tropicalis* PCR product analysis. Lane M: Marker ladder (2000 bp); lanes 1–8: *C. tropicalis* isolates positive for the *18S rRNA* gene (542 bp).

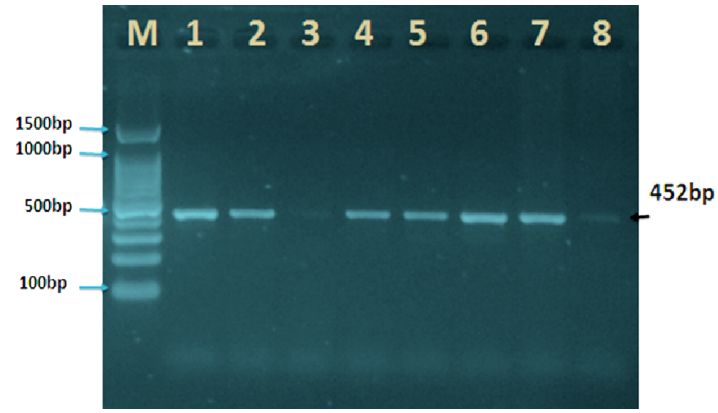

Fig. 3. Electrophoresis on 1.5% agarose gel displaying pathogenic *C. tropicalis* PCR product analysis. Lane M: Marker ladder (2000 bp); lanes 1,2, 4–8: *C. tropicalis* isolates positive for the *SAPT4* gene (452 bp).

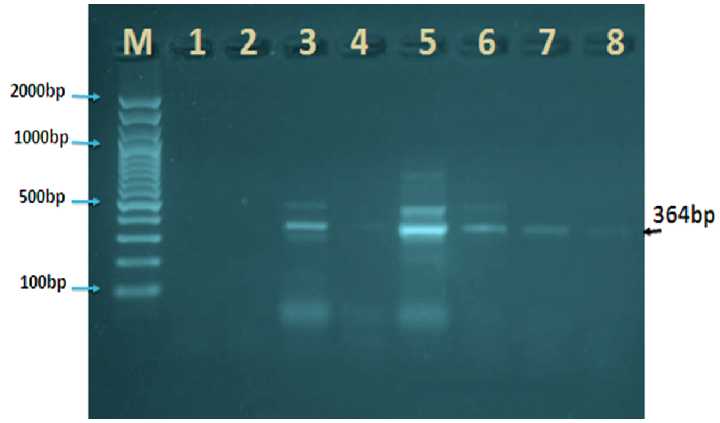

Fig. 4. Electrophoresis on 1.5% agarose gel displaying pathogenic *C. tropicals* PCR product analysis. Lane M: Marker ladder (2000 bp); lanes 3, 5–8: *C. tropicalis* isolates positive for the *ALST1* gene (364 bp).

DISCUSSION

The article describes the incidence of a serious camel disease (mycotic mastitis), and its potential effect on the camel production in Wasit province, South Iraq. In the current study, *C. tropicalis* were identified in 15/70 (21.4%) of camel mastitis samples based on milk cultured in Sabouraud's dextrose agar. The percentage of *C. tropicalis* isolated in this investigation was higher than that in a previous study conducted in Sudan, which identified 33.3% cases of *C. tropicalis* from mastitis camels (Mohammed *et al.,*1996). Furthermore, the API test allowing the identification of 10 (66.6%) isolates at the genus and species as *Candida tropicalis* demonstrated a higher percentage than those obtained in the Nairobi, Kenya study isolated from camel milk (Njage *et al.,* 2011). The discrepancy in the percentage of *C. tropicalis* distribution may be caused by geographic variation, the number of samples used, or variations in diagnostic techniques, season, age of camels (Mwangi *et al.,*2022).

In this investigation, a fragment of the mitochondrial gene encoding the main *18S rRNA* gene was amplified using appropriate primers to identify *Candida tropicalis.* The majority of primers were created in the most recent decades, more sequences have been added to public databases, which has resulted in the identification of new fungi and modifications to their taxonomy (Kanbe *et al.,* 2002; Banos *et al.*, 2018). All detected *C. tropicals* in camel milk (100%) were confirmed based on *18S rRNA* using conventional PCR technique. These findings differ those of Njage *et al.* (2011), who identified 73 isolates with restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD). The discrepancies in the proportions of each study may be due to variances in primers used in PCR procedures; the total number of isolates recruited in each investigation, and the competence of the laboratory examiners (Geresu *et al.,* 2021). The PCR approach is increasingly being used to detect *Candida* since it is quick, easy, specific, sensitive, and reliable (Galán *et al.,* 2006).

In the current study conventional PCR technique for screening of virulence factors showed that the secreted aspartyl proteinase (*SAPT4*) gene was detected in 12/15 (80%) while the agglutinin-like sequence (*ALST1*) gene: in 10/15 (66.6%) of isolates. No previous studies have published data from screening of *C. tropicalis* virulence factors from camel milk with mastitis. Nevertheless, the *ALS1-3* genes encode cell-surface adhesins that promote colonisation, as well as the *SAPT1-4* genes that encode secreted aspartyl proteinases of the causative agent, thus playing an important role in pathogenicity to produce the disease and identifying the severity of infection especially in immunocompromised subjects (Hajjeh *et al*., 2004; Valand *et al*., 2022).

CONCLUSION

According to the current study, *Candida tropicalis* is one of common fungi in milk from camels with mastitis in the Wasit area. The agglutinin-like sequence (*ALST1*) and secreted aspartyl proteinase (*SAPT4*) genes, which may be pathogen transmission vehicles, were detected in the majority of isolates from camel milk samples by conventional PCR.

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