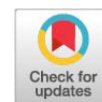
Research Article



Prevalence of Mycotic Mastitis and Evaluation of Some Virulence Potential of *Candida albicans* Isolated from Mastitic Goats

Saddam Hussein Mahmoud* and Shaimaa Nabhan Yassein

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Abstract | The aims of this study are to identify mycotic agents, assess the prevalence of mycotic mastitis, and evaluate the virulence of *C. albicans* that was isolated from Mastitic goats. To investigate the prevalence of mycotic mastitis, related to goats in Baghdad, 166 lactating goats were examined and 332 milk samples were collected from (January – July) 2022. 10 ml of milk were collected in sterile test tubes, an equal volume of milk and CMT reagent were gently shaken in a paddle and the reaction was noticed within (10-15) seconds. The clinical form of mastitis accounted 9.35%, while the subclinical form accounted 52.4%, with positive (+ve) California mastitis test (CMT) results which are high significant. All milk samples were cultured on Sabouraud dextrose agar (SDA) for (3-5) days at 37 °C. According to the result, 46.34% of the isolates had mycotic mastitis, with 33 isolates (34.74%) belonging to mold and 62 isolates (65.26%) returning to yeasts. *Candida albicans* had the highest percentage (high significant) of yeast isolation of (67.74%) with 42 isolates which were detected by using conventional methods such as the germ tube test, urease production, Chlamydospore formation, and chromogenic medium. Hemolysis, phospholipase activities, and biofilm formation were among the virulence tested factors. Hemolysis and phospholipase activities were detected in 76.2% and 83.33% of *C. albicans* isolates, respectively, while biofilm formation was detected in 100% of *C. albicans* isolates. The diagnosis was confirmed using 25 of the most virulent *C. albicans* isolates, by polymers chains reaction (PCR) to detect the KER1 gene revealed that all 25 samples were positive with the product size (658bp). Phospholipases B1 (PLB1) and the secretion of aspartyl proteinase 1 (SAP1) gene were used to detect *C. albicans* virulence with 100% and 80% accuracy, respectively, while Hyphal Wall Protein 1 (HWP1) gene was not detected. It is expected that these *C. albicans* isolates contained a significant proportion of virulence factors that were linked to pathogenicity and the severity of infection. There findings suggest that poor sanitation and a reduction in goat health and nutrition were to blame for the high morbidity incidence of mycotic mastitis in Iraqi goats.

Keywords | Goats, Dairy industry, Mycotic, Mastitis, *C. albicans*, virulence factors, Yeast's virulence and biochemical indices

Received | May 06, 2023; **Accepted** | June 22, 2023; **Published** | July 23, 2023
***Correspondence** | Saddam Hussein Mahmoud, Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; **Email:** saddam.hussein1101f@covm.uobaghdad.edu.iq
Citation | Mahmoud SH, Yassein SN (2023). Prevalence of mycotic mastitis and evaluation of some virulence potential of *Candida albicans* isolated from Mastitic goats. Adv. Anim. Vet. Sci., 11(9):1417-1427.
DOI | https://dx.doi.org/10.17582/journal.aavs/2023/11.9.1417.1427
ISSN (Online) | 2307-8316

Copyright: 2023 by the authors. Licensee ResearchersLinks Ltd, England, UK.
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

INTRODUCTION

Mastitis is a disease caused by a wide range of microorganisms that causes significant economic losses and damages to the dairy industry by reducing milk production, increasing antibiotic treatment, and culling costs (Yanuartono et al., 2019; Unny et al., 2020). It is a complicated condition that develops as a result of interactions between an agent, an animal, and the environment. It is a severe problem for both human and

animal health (Machado, 2018; Toledo and Dacey, 2021). When pathogens enter the mammary gland through the teat opening, they thrive in the udder, producing irritation (Pacha et al., 2020).

Bacteria, mycoplasmas, viruses, fungi, and algae are the most common causes of mastitis (Nitz et al., 2021). Yeasts are the primary pathogens that cause mycotic mastitis (Constable et al., 2017), and *Candida* species are the most common yeasts found in mycotic mastitis (Du et al., 2018).

Even though mycotic mastitis is less common than other agents (Sudhakara et al., 2018; Yanuartono et al. 2019; Mohammed and Yassein, 2020). It was rapidly increased over the past ten years and is usually cited as the significant cause of mycotic mastitis in ruminants (Mohammed and Yassein, 2020). There was high prevalence of several fungi in milk from goats in the Baghdad area with subclinical mastitis, which could be harmful to people's health (Hasan and Yassein, 2018).

Additionally, *C. albicans* is considered as normal flora in humans and animals (Abharian et al., 2018; Talapko et al., 2021). It is an opportunistic pathogen that causes infection in immunocompromised hosts. Several factors contribute to this yeast's virulence and pathogenicity, including the production of germ tubes, and extracellular hydrolytic enzymes, particularly phospholipase and proteinase, phenotyping switching, and biofilm formation. These virulence factors are linked to *C. albicans* pathogenicity (Udayalaxmi and D'Souza, 2014). Due to similarities in phenotypic traits between species, it is increasingly important to develop an accurate technique to accurately identify yeast species and prevent diagnostic errors when conducting morphological, biochemical, and physiological testing (Spanamberg et al., 2009). So, the aims of this study were to identify mycotic agents, assess the prevalence of mycotic mastitis, and evaluate the virulence of *C. albicans* that was isolated from mastitic goats.

MATERIALS AND METHODS

Collection of milk samples

This work was carried out in the period of (January – July) 2022. The number of milk samples were 332. These were collected from 166 goats in Abu-Ghraib region and tested clinically and to detect sub-clinical mastitis by using the California mastitis test (CMT). Ten milliliters of milk were collected in sterile test tubes under aseptic conditions. They were immediately transported to the laboratory in an ice box. Afterwards, all milk samples that collected from apparently healthy udder were subjected to the CMT test. By using a horizontal plane, an equal volume of milk and CMT reagent were gently shaken in a paddle. The reaction was scored as negative, trace, or positive within (10-15) seconds, according to the procedure of Coles' (1986) and Sadoon (2021).

Determination of mycotic isolates

Each milk sample was incubated at 37 °C for (3-5) days on SDA containing 0.5 mg/ml Chloramphenicol. Sub-culturing was used to obtain pure colonies, which were then examined macroscopically and microscopically according to Washinton et al. (2006) for Mold. Whereas for Yeast detection, particularly suspected *C. albicans*, conventional methods represented by germ tube production, Urease production, chlamydospore formation, and identification by CHROM agar were employed (Neppelenbroek et al., 2014).

Detection of virulence factors of *C. albicans*

Preparation of yeast suspension

C. albicans isolates were activated on SDA after an overnight incubation at 37 °C on Sabouraud dextrose broth. A small amount of activated pure colonies was transferred into 5 ml of phosphate buffered solution (PBS) at turbidity equal to 1.0 McFarland and contain 3×10^8 yeast cells/ml using a sterile loop (Deepa et al., 2015).

Detection of phospholipase production

Ten microliters of yeast suspension with a 1-McFarland turbidity level were inoculated onto the egg yolk agar surface and incubated there for 48 hours. Growing *C. albicans* on egg yolk agar and assessing the extent of the precipitation zone could determine the extracellular phospholipase activity of the yeast. According to the formula, the diameters of the colonies and the opacity zone were measured in millimeters (mm) (Mahmoudabadi et al., 2010).

Hemolytic activity

Ten microliters of the previously prepared yeast suspension were taken and spotted on SDA containing 7.0% human blood and 3.0% glucose. Each plate was incubated at 37°C for 48 hours. Any isolate that showed a clear zone of haemolysis around the colonies was considered positive for haemolytic activity (Favero et al., 2011). The enzymatic activity was measured manually for each isolate using the Hz value, which was calculated by the ratio of colony diameter to colony diameter plus zone of precipitation in (mm). The zone of enzymatic activity was calculated in accordance with Nazeer et al. (2020).

Determination of biofilm formation

C. albicans biofilm formation was assessed using the method described by Inci et al. (2012). A loop of yeast was cultured in a tube which contained 2.0 ml of Brain Heart Infusion Broth (BHIB) medium containing 0.25% glucose for 24 hours at 37°C. The tubes were then diluted at a 1:20 ratio with fresh prepared BHIB, and 200 µl was placed into

Links Researchers

sterile 96-well polystyrene microtiter plates, which were then covered with lids and incubated at 37°C for 24 hours. This plate was rinsed with PBS twice before being inverted to blot. Each well received 1.0 % crystal violet (200 µl) and was incubated for 15 minutes. After three rounds of PBS rinsing, 200 µl of an ethanol: Acetone combination (80:20v/v) were added to each well. The absorbance values were calculated at 590 nm using an ELISA reader. For each isolate, the biofilm formation experiment was done three times, average optical density (OD) values were determined, according to Rodrigues et al. (2010) and Ahmed (2015).

Molecular characterization

Extraction of *Candida albicans* DNA

Twenty-five of the most virulent *C. albicans* isolates were chosen to confirm the yeast. Some virulence genes were detected by molecular detection such as (PLB1, SAP1 and HWP1). The DNA was extracted from each isolate using a PrestoTM Mini gDNA Yeast Kit (Geneaid).

PCR amplification

The PCR reaction was carried out in a 25µl reaction 12.5 µl of Green Master Mix (Promega/USA), 1.0 µl of 10 picomol/µl primer (Table 1), 2.0 µl of DNA template, and the volume was brought up to 25 µl with nuclease-free water. Following PCR amplification, agarose gel electrophoresis was used to confirm gene amplification (Table 2).

Statistical analysis

The statistical analysis system- SAS (2018) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

RESULTS AND DISCUSSION

The number of collected samples were 332 from a total of 166 goats, 31 halves were detected as clinical mastitis (9.33%), while the remainder were subjected to the CMT test, which revealed that 174 samples gave positive results for CMT with 57.8% which are high significant but 127 samples (42.19%) gave negative results for this test (Table 3 and 4).

Table 1: The oligonucleotide primer pair.

Target genes		Primers sequences (5'- 3')	Amplicon size (base pair)	References
SC1	F	CGGAGATTTTCTCAATAAGGACCAC	658	Galan *et al.*, 2006
	R	AGTCAATCTCTGTCTCCCCTTGC		
PLB1	F	ATGATTTTGCATCATTTG	765	Mukherjee *et al.*, 2001 and Soliman *et al.*, 2020
	R	AGTATCTGGAGCTCTACC		
SAP1	F	GCTTTTGCTGGTTGATGCCA	479	Al-abidy *et al.*, 2019
	R	TGCTGATTGACCAGGACGAG		
HWP1	F	ATG ACT CCA GCT GGT TC	503	Inci *et al.*, 2013 Mohammadi *et al.*, 2021
	R	TAGATCAAG AAT GCA GC		

Table 2: The PCR thermocycler conditions.

Target genes	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	References
SC1	95°C, 2 min	94°C, 30 s	60°C, 30s	72°C, 10s	72°C, 10 min	Galan *et al.*, 2006; Abd El-Razik *et al.*, 2011
		40 cycles				
PLB1	95°C, 5min	94°C, 1 min	52°C, 1 min	72°C, 1 min	72°C, 5min	Mukherjee *et al.*, 2001
		35 cycles				
SAP1	95°C, 2min	95°C, 30s	58.3°C, 30s	72°C, 1 min	--	Al-abidy *et al.*, 2019
		30 cycles				
HWP1	95°C, 5 min	94°C, 30s	60°C, 40s	72°C, 50s	72°C, 10 min.	Mohammadi *et al.*, 2021
		34 Cycles				

Table 3: The Percentage of Mastitic and Non Mastitic Halves.

Mastitis Form	No. of infected and non infected halves	%
Clinical mastitis	31/ 332	9.33%
Subclinical mastitis	174/301	57.8%
Non infected with mastitis	127/301	42.19%

As shown in Table 5, clinical mastitis was found in 25 goats as 31 halves (9.35%), with 12 halves (38.70%) having subacute clinical mastitis, 14 halves (45.16%) having acute clinical mastitis which are high significant, and 5 halves (16.14%) having peracute clinical mastitis.

The results of the current investigation indicated that 174 halves (57.8) of the samples had subclinical mastitis, which

Links Researchers

Table 4: Forms of clinical mastitis in goats.

No. of Infected goats with clinical mastitis	No. of halves with clinical mastitis	No. of halves with subacute clinical mastitis	No. of halves with acute clinical mastitis	No. of halves with peracute clinical mastitis
25	31	12	14	5
%	100%	38.70%	45.16%	16.14%

was represented by 19 samples (10.92%) with a positive one (+1) CMT, 96 samples (55.18%) with a positive two (+2) CMT which are high significant, and 53 samples (33.9%) with a positive three (+3) CMT, as shown in Table 6.

Table 5: CMT score among milk samples of subclinical mastitis.

CMT score	No. of samples that give (+ve CMT)	%
Negative	0	0
Trace	0	0
+1	19	(10.92%)
+2	96	(55.18%)
+3	59	(33.90%)
Total	174	(100%)

Table 6: Percentage of mycotic mastitis.

No. of mycotic mastitis cases	No. of mold	No. of yeast
95	33	62
%	34.74%	65.56%

Percentage of mycotic mastitis

The current study found that mycotic mastitis affected 95/205 isolates (46.34%), with 33 isolates belonging to mold (34.74%), and 62 isolates returning to yeasts (65.26%) which are high significant (Table 7).

Table 7: Percentage of mold isolated from caprine subclinical mastitis.

Type of mold/ No. of isolate	No. of *Penicillium* spp.	No. of *A. fumigatus*	No. of *A. terreus*
33	11	15	7
100%	33.33%	45.45%	21.21%

Table 8: Percentage of yeast isolated from caprine subclinical mastitis.

Type of yeast/ No. of isolate	No. of *C. albicans*	No of *C. kruzi*	No. of *C. glabrata*	No. of *C. tropicalis*
62	42	7	11	2
100%	67.74%	11.29%	17.74%	3.22%

As shown in the Table 8, the mold types included 11 isolates *of Pencillinum* spp. (33.33%), 15 isolates of *A. fumigatus* (45.45%), and 7 isolates of *A. terreus* (21.21%).

Whereas 42 isolates of the different yeasts returned to the *C. albicans* (67.74%) which are high significant, 7 isolates of *C. kruzi* (11.29%), 11 isolates of *C. glabrata* (17.34%), and 2 isolates of *C. tropicalis* isolates (3.22%), (Table 9).

Table 9: Relation between CMT score and number of *C. albicans* isolated from subclinical mastitis.

CMT score	No. of *C. albicans* isolates	%
Negative	0	0
Trace	0	0
+1	1	(2.38%)
+2	22	(52.38%)
+3	19	(45.24%)
Total	42	(100%)

Percentage of caprine Candidal mastitis

The current work focused on the isolation of *C. albicans* from mastitic milk in Iraqi goats, with a 67.74% (high significant) overall isolation rate of yeasts (Table 9), and represented high ratio as curative agent of mycotic mastitis with 42/95 (44.21%).

Figure 1: macroscopical characteristic of *C. albicans* cultured on Sabouraud dextrose agar for 72 hours at 37 °C. The resulting colony is a white cream pasty colony.

Identification of *C. albicans* by conventional methods

The traditional diagnostic criteria were used to identify 42 clinical isolates of *C. albicans.* As shown in Figure 1, the macroscopical distinctive characteristics of *C. albicanes* were creamy, smooth, pasty, and convex colonies for 3–5 days after inoculation on SDA at 37°C that could become wrinkled with prolonged incubation. Lactophenol Cotton Blue stain was used for microscopic examination, which revealed the presence of pseudohyphae with clusters of

Links Researchers

budding cells. All of these isolates were then cultivated on specific medium, such as Corn Meal Agar, which is also known as the Dalmau plate method to promote the production of blastoconidia and chlamydospores, to confirm the diagnosis, as shown in Figure 2.

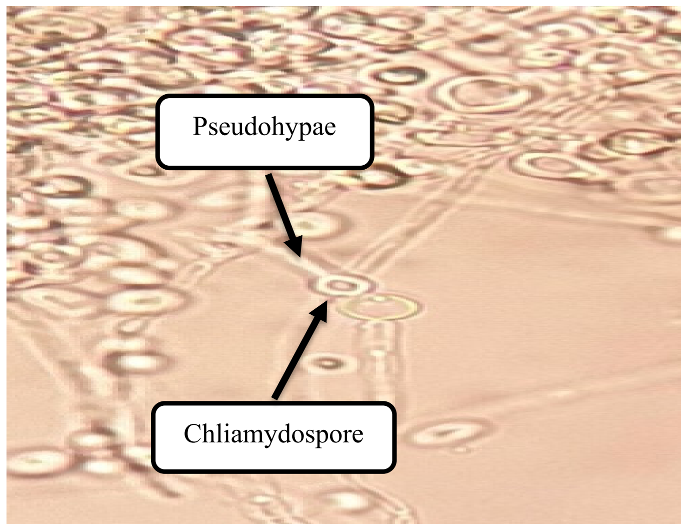


Figure 2: *C. albicans* pseudohyphae with chlamydospore/ after three days of growing on Corn Meal Agar containing 1% Tween 80 at 28°C. (40X).

The Germ tube test, also known as the Reynolds-Braude phenomenon, yielded positive results for these isolates and may be considered the primary test for *C. albicans* identification. As shown in Figure 3, Germ tube formation was observed in 100% of the *C. albicans* isolates in the current study.

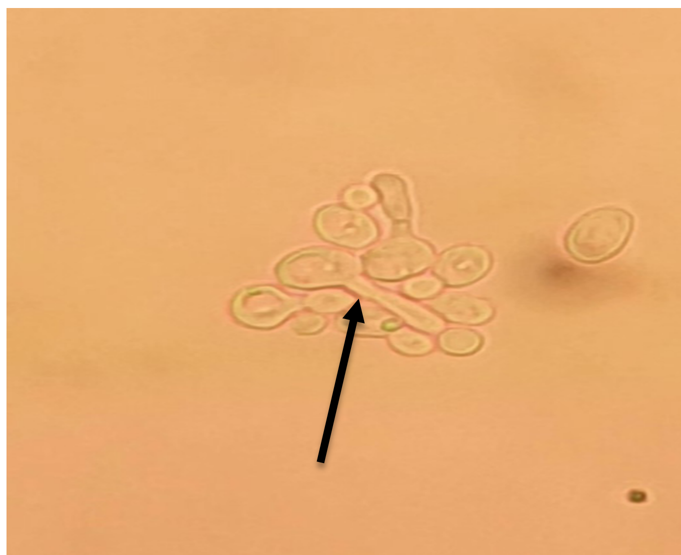


Figure 3: *C. albicans* producing germ tubes after one hour of development in fresh human serum at 37 ºC. (40X).

Another selective and deferential media employed for the detection of *Candida* spp. was *Candida* chromogenic agar, which was applied in accordance with the manufacturer's instructions. In this research, *C. albicans* generated green colonies, as depicted in Figure 4. Colonies of *C. glabrata* isolates on CHROM agar were pink with a mauve core. Colonies of *C. krusei* could be easily recognized from other yeasts smooth, brown to brown purple colonies on this agar after 48 hours of incubation. All *C. parapsilosis* isolates showed a distinctive dark blue gray core color after 48 hours of incubation.

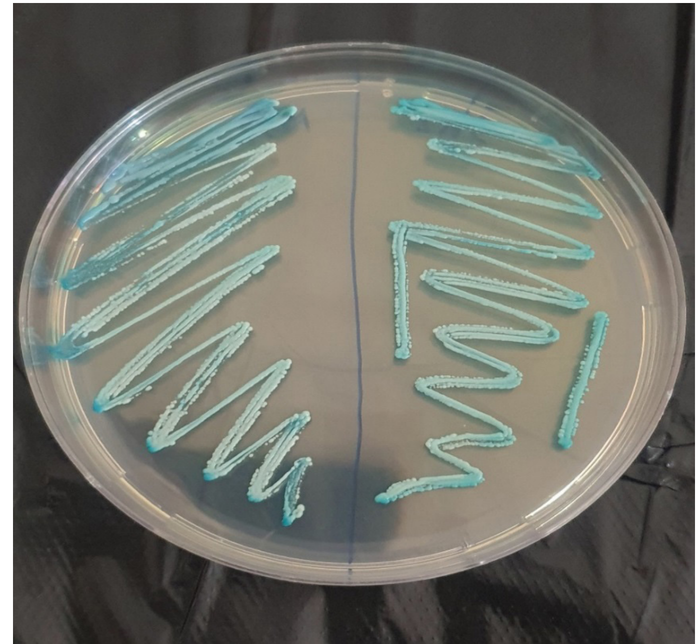


Figure 4: Morphology of *C. albicans* on CHROMagar reveals leaf-green colored colonies.

Figure 5: Urease test: negative Urease test for *C. albicans*.

Results of virulence factors

The current paper investigated some *C. albicans* virulence factors such as phospholipase and hemolysin enzymes, which were produced by 32/42 (76.2%) and 35/42 (83.33%), respectively, of the total *C. albicans* isolates (Tables 10, 11 and Figures 6, 7).

Links Researchers

Table 10: phospholipase activity of *C. albicans* isolates for subclinical caprine mastitis.

Pz value	No. of *C. albicans* isolates	Scored of isolates	% of isolates that produced phospholipase	
1	10	-ve	32/42	76.19%
0.99-0.9	3	(+)		
0.89-0.8	3	(++)		
0.79-0.7	7	(+++)		
<0.69	19	(++++)		

Enzymatic activity values were scored and categorized as follows: Pz = 1: no enzymatic activity (-ve); Pz = 0.9-0.99: weak activity (+); Pz = 0.8-0.89: mild activity (++); Pz = 0.7-0.79: strong activity (+++); Pz = < 0.69 : very strong activity (++++).

Table 11: Hemolysis activity of *C. albicans* isolates for subclinical caprine mastitis.

Ph value	No. of *C. albicans* isolates	Scored of isolates	% of isolates that produced Hemolysis activity	
1	7	-ve	35/42	83.33%
0.99-0.9	7	(+)		
0.89-0.8	11	(++)		
0.79-0.7	13	(+++)		
<0.69	4	(++++)		

Enzymatic activity values were scored and categorized as follows: Ph = 1: no enzymatic activity (-ve); Ph = 0.9-0.99 : weak activity (+); Ph= 0.8-0.89: mild activity (++); Ph = 0.7-0.79 : strong activity (+++); Ph = < 0.69 : very strong activity (++++).

Table 12: Biofilm formation of *C. albicans.*

No. of *C. albicans* isolates	Score of biofilm		
	Weak	Moderate	Strong
42 (100%)	19 (45.25%)	18 (42.85%)	5 (11.90%)

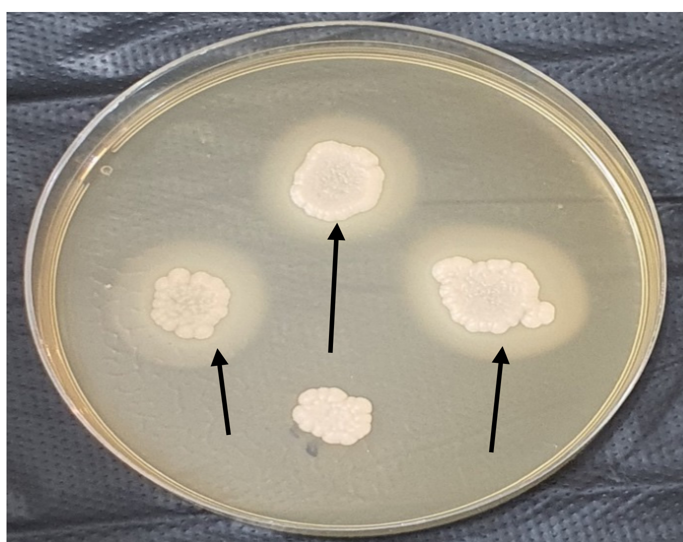


Figure 6: *C. albicans's* phospholipase activity on egg yolk agar medium at 37°C for (24–48) hours. Illustrate the zone of inhibition around the colony.

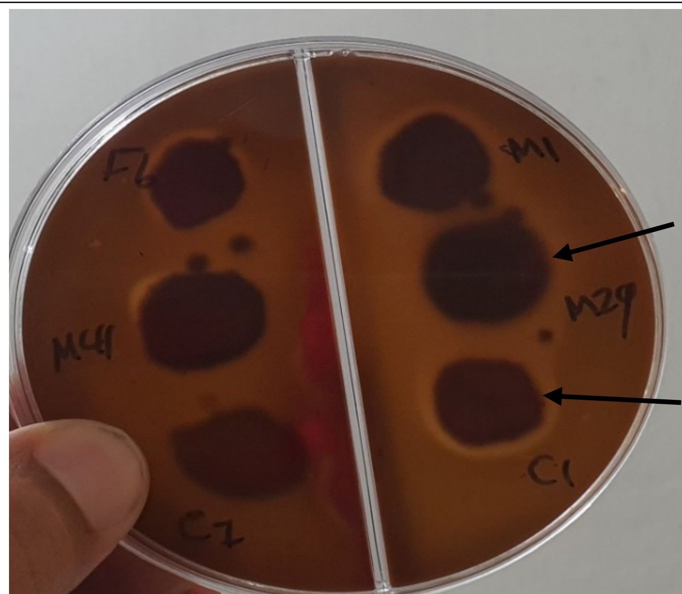


Figure 7: *C. albicans* haemolysin activity on Sabouraud dextrose blood agar with 3% glucose at 37 °C for 48 hours. Display a clear zone around the colony.

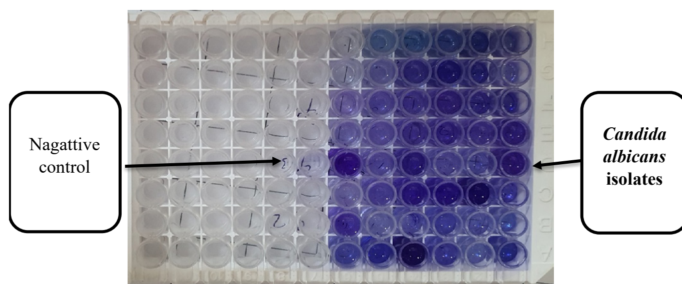


Figure 8: Microplate inoculated with *C. albicans* isolates with Negative control for detection of biofilm formation.

Another virulence factor is biofilm formation, which was found in 100% of the *C. albicans* isolates, with different degrees, indicating that these isolates exhibited a high ratio of virulence factors linked to pathogenicity and infection severity (Table 12, Figure 8).

Molecular identification of *C. albicans*

According to the findings of the virulence factor, 25 of the most virulent isolates of *C. albicans* were chosen for confirmation of the diagnosis using PCR. To extract DNA from 25 *C. albicams* isolates, a genomic DNA extraction kit was utilized. All *C. albicans* isolate DNA samples were successfully extracted, and the nano-drop device was used to check the purity and concentration of the DNA extracted then assessed using gel electrophoresis. varying from 75 to 100 ng/l, with a purity between 1.7 and 1.9. The extracted DNA sample was electrophoresed on an agarose gel at 75 volts for one hour before being seen under a UV light source (Figure 9).

KER1-specific gene was utilized to detect and identify *C. albicans* isolates by PCR, and SC1F and SC1R primers

Links Researchers

were employed to amplify the extracted DNA, which revealed a band of DNA fragmentation of 658 bp for detection *C. albicans* with 100% (Figure 10).

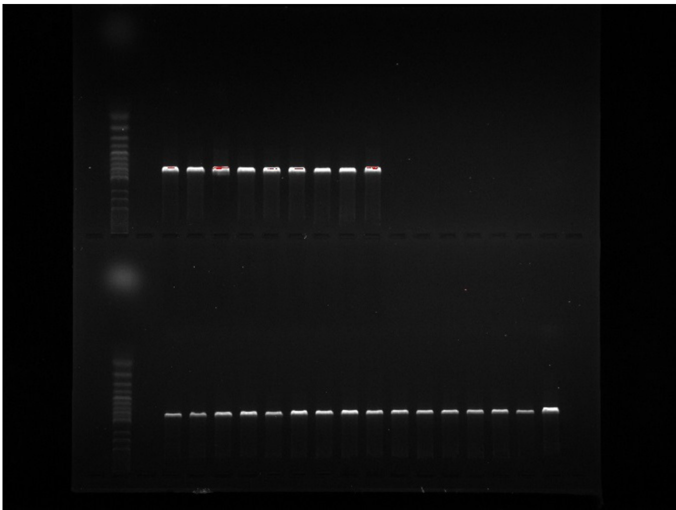


Figure 9: Gel electrophoresis for extracted DNA.

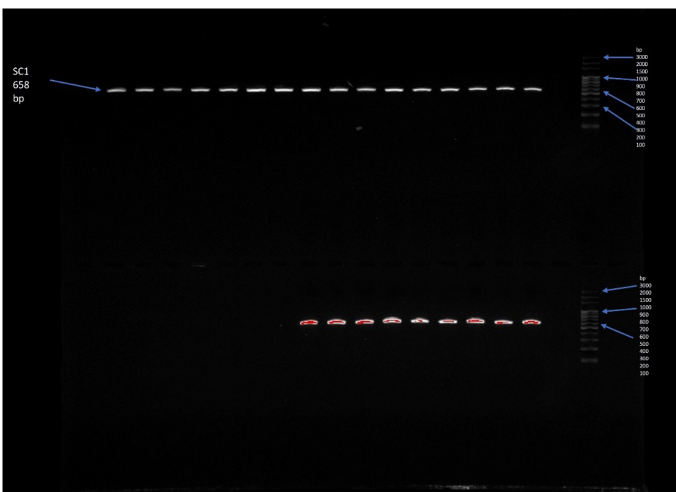


Figure 10: Agarose gel 1% electrophoresis picture with an electric current of 75 volts for 1 hour was performed, showing the PCR product analysis of the SC1 gene in *C. albicans* was a positive isolate. with a PCR product size of 658 bp, ladder (100-1500 bp).

The PCR data presented here indicate that SC1F and SC1R amplification is are species specific, and as a result, they may contribute in specifically identifying *C. albicans* strains. A single pair of primers (SC1F and SC1R) derived from the KER1 gene sequence specific to *C. albicans* is used in the described PCR-based approach. The KER1 gene is amplified by these primers in a 670 bp fragment. The anticipated 670 bp amplicon was produced by every single clinical *C. albicans* isolate.

While PLB1, SAP1 and HWP1 genes were employed for detecting *C. albicans* virulence factor genes isolates had positive results, While HWP1 was not found in any of the examined *C. albicans* isolates (0%), PCR analysis revealed that 25 strains of *C. albicans* produced unique bands with molecular sizes of around 765 bp and 479 bp that contained PLB1 and SAP1 genes, with 100% (25 strains) and 80% (20 strains), respectively (Figures 11, 12)

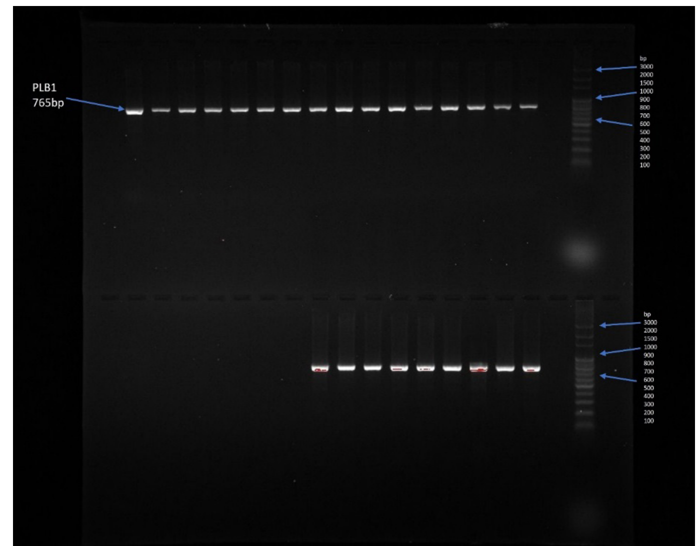


Figure 11: PCR products of the PLB1 gene of *C. albicans* were electrophoresed on a 1% agarose gel at 75 volts for an hour, with a product size of 765 bp.

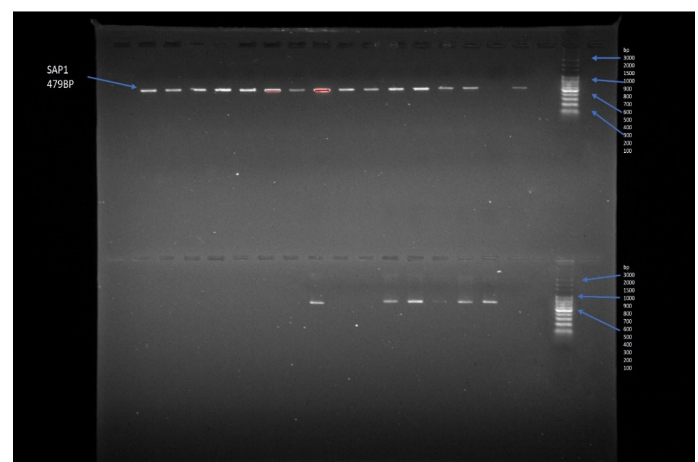


Figure 12: The *C. albicans* SAP1 gene gel electrophoresis on 1% agarose gel at 75 volts for an hour with a product size of 479 bp.

The present study recorded high percent of subclinical caprine mastitis, this finding was consistent with the findings of Stuhr and Aulrich (2010), who stated that a large proportion of intramammary infections in dairy goats can be classified as subclinical mastitis with no or rare visible clinical symptoms.

The result of the current study was consistent with the finding of Hasan and Yasein (2018) who reported (53%) of the tested samples gave positive for CMT. The current study agrees with Al-Dujaily and Mahmood (2021) who found that the CMT caused a high positive (3+ score) response in all tested animals.

Links Researchers

The present study demonstrated 42 isolates of *C. albicans* that were constituted high ratio of yeast infections when compared with other yeasts. This result is consistent with those of Hassan et al. (2012), who reported that the most commonly isolated species from goats with mastitis in Egypt were *C. albicans* (24%) and *C. parapsilosis* (14%).

In contrast to the current study, Ilhan et al. (2016) discovered that fungal agents were isolated from 19 of 170 goat milk samples (11.1%). In culture, 3 (1.7%) milk samples tested positive for *Candida albicans*, 2 (1.1%) for *C. lusitaniae*, 1 (0.5%) for *C. parapsilosis*, 1 (0.5%) for *C. glabrata*, and 2 (1.1%) for *Cryptococcus neoformans*. While Al-Abedi (2020), reported the presence of *C. albicans* in 4/11 (36.2%), *C. tropicals* in 3/11 (27.2%), *C. krusei* in 2/11 (18.1%), and *C. parapsilosis* and *C. glabrata* in 1/11 (9.09%) for each in his study.

All the macroscopic and microscopic characteristics of *C. albicans* and production of blastoconidia and chlamydospores, were similar to the findings of Atshan and Al-Haddad (2014) and Abdulla and Ismael (2023).

On the other hand, the current study was similar to the finding of Mattei et al. (2013) who confirmed that Germ tube formation was observed in 95% of the isolates and Phospholipase production was found to be high in 13 of the germ tube positive isolates.

This finding corroborated the findings of Manikandan and Amsath (2013). However, when the Urease test that was inoculated with *C. albicans* isolates, the test's color did not change and remained yellow as seen in Figure 5.

Numerous virulence factors characterize *C. albicans* pathogenicity, but the most important of which is the secretion of hydrolytic enzymes (e.g., phospholipases, proteases, and hemolysins) that promote colonization and tissue incursion (Menezes et al., 2016). The enzymatic activity of *Candida* spp. has been reported to vary depending on the species and source of isolates (Gultekin et al., 2011).

It is generally known that *C. albicans* is resistant to common antimicrobials, particularly azoles, especially when *Candida* cells are in the form of biofilms (Henriques and Silva, 2021). By creating a thick network of pseudohyphae in biofilms, *Candida* species can decrease medication penetration and boost antifungal resistance (Pereira et al., 2020).

The current investigation concurred with Mohammed et al. (2017) who discovered that all *C. albicans* isolates tested developed biofilm on polystyrene with varying strength.

On the other hand, Deepa et al. (2015), report that biofilm formation was present in 78.9% of *Candida* isolates, but Mohammadi et al. (2021) noted that biofilm formation was present in 20%, 11.4%, and 21.4% of isolated *candida* at mild (+), moderate (++), and strong (+++) levels in 54.3% of *Candida* species. Also, Hussain et al. (2020) found that 2 of isolates were strong and 6 of isolates were moderate.

The PCR assay is a potent approach for identifying various genes, it is quicker, easier, and more accurate (Mohsin and Ali, 2021; Sheet, 2022).

The present result contrasted with the result of Shrief et al. (2019) who examined the virulence genes of *C. albicans* and found that HWP1 gene represented (77%), SAP1 (65%), and PLB1 (52%). Also, these results are contrasted with Soliman et al. (2020) who discovered that 50% of *C. albicans* isolates had HWP1 and SAP4, while PLB1 was not detected in tested isolates (0%).

In contrast to this study, Vijayalakshmi et al. (2016) found that the HWP1, SAP1, and PLB1 virulence genes in 77%, 65%, and 52%, respectively, of multi-drug resistant *C. albicans*. Moreover, HWP1, SAP1, and PLB1 virulence genes were found in 90.9%, 59.09%, and 13.63% of the investigated *C. albicans* isolates, respectively, by Abdul-Lateef et al. (2015). The number of specimens under analysis and the numerous *C. albicans* isolation origins are factors that may contribute to the variability in the prevalence percentages of virulence genes as mentioned by (Vijayalakshmi et al., 2016). Abdulla and Ismael (2023) found that HWP1 genes were found in all *C. albicans* isolated.

CONCLUSIONS AND RECOMMENDATIONS

The current study discovered that mastitis continues to merit substantial attention for its control and preventions because it is thought to be the most financially burdensome disease. The most common fungi found in caprine mastitis are described in this study, and based on current observations of the higher percentage of fungi isolated from mastitic milk samples, may be due to unsanitary conditions and the development of antibiotic resistance in bacteria, which lengthens the course of treatment and increases the likelihood that fungi like *candida* will infect as a secondary invader.

This study concluded that poor sanitation and a reduction in goat health and nutrition were to blame for the high morbidity incidence of mycotic mastitis in Iraqi goats, and the *C. albicans* that isolated from subclinical caprine mastitis made up high ratio of all *Candida* spp. isolates

Links Researchers

were high ratio of virulence factors that associated with the pathogenicity and severity of infection.

Additional researches are needed to study other types of virulence factors for *C. albicans* and other types of *Candida* spp. that isolated from caprine mastitis.

ACKNOWLEDGEMENTS

The authors are thankful to all members in the laboratories of Ministry of Science and Technology for their assistance, help and generosity. And many thanks to staff of departments' Internal and Preventive Veterinary Medicine, college of veterinary medicine, University of Baghdad – Iraq for their support and providing the facilities during the samples processing.

NOVELTY STATEMENT

There is no data available in Iraq about molecular detection of *C. albicans* isolated from caprine mastitis and due to the high prevalence of this type of yeast in mastititic cases and its veterinary and economic importance in field animals, the study was conducted to explore the spread of candidal mastitis in goat and identified the species by novel gene accompanied with detection of some virulence genes that associated with pathogenesis of *C. albicans*

AUTHOR'S CONTRIBUTION

These authors each contributed equally.

Conflict of interest

There are no stated conflicts of interest by the authors.

REFERENCES

Abdulla NQF, Ismael HM (2023). The efficacy of antifungal medications and plant extracts against *Candida albicans* isolated from vulvovaginitis women. Iraqi J. Sci., 64(2): 560-572. https://doi.org/10.24996/ijs.2023.64.2.6

Abdul-Lateef LAR, Al-Kafhaji KA, Al-Saddam ASK (2015). Genetic identification of some virulence factors of *Candida albicans* in candidal intertrigo of diabetic patients in Hilla Province/ Iraq. Aust. J. Basic Appl. Sci., 9(36): 66-72.

Abharian PH, Dehghan P, Tollouei S (2018). Molecular characterization of *Candida dubliniensis* and *Candida albicans* in the oral cavity of drug abusers using duplex polymerase chain reaction. Curr. Med. Mycol., 4(1): 12-17. https://doi.org/10.18502/cmm.v4i1.29

Abd El-Razik, K.A.. Abdelrahman, K.A., Abd El-Moez, S.I. and Danial, E. N. (2011). New approach in diagnosis and treatment of Bovine Mycotic Mastitis in Egypt. African Journal of Microbiology Research Vol. 5(31). 5725-5732. ISSN 1996-0808. DOI: 10.5897/AJMR11.1200

Ahmed EF (2015). Antimicrobial and antibiofilm activity of mango seeds extract. Iraqi J. Sci. 56(4B): 3121-3129.

Al-Abidy HFHA, Khudaier BY, Al-Attraqchi AAF (2019). Conventional and molecular detection of *Candida Albicans* and *Candida Parapasilosis* isolated from bovine mastitis in Basrah-Iraq. Biochem. Cell. Arch., 19(2): 3285-3289.

Al-Abedi, S.F.H. (2020). Comparative Study for Candida Spp Isolated and Identification from Goats Milk with Mastitis Between Api Candida Kit Test, Candida Chromogenic Agar and Vitek 2 System. Biochem. Cell. Arch. 20 (2): 5329-5332.

Al-Dujaily AH, Mahmood AK (2021). The effectiveness of biogenic silver nanoparticles in the treatment of caprine mastitis induced by *Staphylococcus aureus.* Iraqi J. Vet. Sci., 35(III): 73-78. https://doi.org/10.33899/ijvs.2021.131415.1946

Atshan OFA, Al-Haddad ZAA (2014). Study the effectiveness of Artemisia herba-alba leaves extract on the experimental infection with Candida albicans isolated from urogenital tract in cows. Int. J. Adv. Res., 2(4): 998-1006.

Coles EH (1986). Veterinary clinical pathology, 4th Ed. P:345. W.B. Saunders Company, London. https://doi.org/10.1136/jcp.39.12.1365

Constable PD, Hinchcliff KW, Done SH, Grunberg W (2017). Veterinary medicine a textbook of the diseases of cattle, horses, sheep, pigs, and goats edition 11. pp. 1904-2004. Elsevier Ltd. All Rights Reserved.

Deepa K, Jeevitha T, Michae A (2015). *In vitro* evaluation of virulence factors of *Candidia* species isolated from oral cavity. J. Microbiol. Antimicrobe., 7(3): 28-32. https://doi.org/10.5897/JMA2015.0337

Du J, Wang X, Luo H, Wang Y, Liu X, Zhou X (2018). Epidemiological investigation of non-albicans Candida species recovered from mycotic mastitis of cows in Yinchuan, Ningxia of China. BMC Vet. Res., 14: 251-260. https://doi.org/10.1186/s12917-018-1564-3

Favero D, Franca EJG, Furlaneto-Maia L, Quesada RMB, Furlaneto MC (2011). Production of haemolytic factor by clinical isolates of Candida tropicalis. Mycoses, 54: e816-e820. https://doi.org/10.1111/j.1439-0507.2011.02035.x

Galan A, Veses V, Murgui A, Casanova M, Martinez JP (2006). Rapid PCR-based test for identifying *Candida albicans* by using primers derived from the pH-regulatedKER1 gene. FEMS Yeast Res., 6: 1094–1100. https://doi.org/10.1111/j.1567-1364.2006.00114.x

Gultekin B, Eyigor M, Tiryaki Y, Kırdar S, Aydın N (2011). Investigation of antifungal susceptibilities and some virulence factors of *Candida* strains isolated from blood cultures and genotyping by RAPD-PCR. Mikrobiyol. Bull., 45(2): 306–317.

Hasan KAM, Yassein SN (2018). Prevalence and type of fungi in milk from goats with sub clinical mastitis. Online J. Vet. Res., 22(8): 669-674.

Hassan AA, Hassan MA, El-Ahl RMS, Darwish AS (2012). Prevalence of yeast infections in small in ruminants with particular references to their treatment by some natural herbal extracts. Bull. Environ. Pharmacol. Life Sci., 1(3): 12-22.

Henriques M, Silva S (2021). Candida albicans virulence factors and its pathogenicity. Microorganisms, 9: 704. https://doi.org/10.3390/microorganisms9040704

Hussain NS, Latif II, Obaid HH (2020). Antimicrobial activity of non-bond colicin on *Candida albicans* Biofilm. Iraqi J. Sci., 61(11): 2831-2837. https://doi.org/10.24996/

ijs.2020.61.11.6

Ilhan Z, Ekin IH, Samet-Koltas S, Ozgul-Gulaydın O, Cihat-Ozturk C, Borum AE (2016). Occurrence of fungal agents in mastitis in dairy goats. J. Anim. Plant Sci., 29(3): 4691-4700.

Inci M, Atalay MA, Koç AN, Yula E, Evirgen Ö, Durmaz S, and Demir, G. (2012). Investigating virulence factors of clinical Candida isolates in relation to atmospheric conditions and genotype. Turk. J. Med. Sci., 42(2): 1476– 1483. https://doi.org/10.3906/sag-1204-119

Inci M, Atalay MA, Özer B, Evirgen Ö, Duran N, Motor VK, Koç, A.N.; Önlen, Y.; Kilinç, C. and Durmaz, S. (2013). Investigations of ALS1 and HWP1 genes in clinical isolates of *Candida albicans*. Turk. J. Med. Sci., 43: 125-130. https://doi.org/10.3906/sag-1205-90

Machado GP (2018). Mastitis in small ruminants. Anim. Husb. Dairy Vet. Sci., 2(4): 1-9. https://doi.org/10.15761/AHDVS.1000146

Mahmoudabadi AZ, Najafyan M, Alidadi M (2010). Clinical study of *Candida* vaginitis in Ahvaz, Iran and susceptibility of agents to topical antifungal. Pak. J. Med. Sci., 26(3): 607–610.

Manikandan C, Amsath A (2013). Isolation and rapid identification of *Candida* species from the oral cavity. J. Pure. App. Biosci., 1(2): 172-177.

Mattei AS, Alves SH, Severo CB, Guazzelli LS, Oliveira FM, Severo LC (2013). Determination of germ tube, phospholipase, and proteinase production by bloodstream isolates of *Candida albicans*. Rev. Soc. Brasil. Med. Trop., 46(3): 340-342. https://doi.org/10.1590/0037-8682-0045-2013

Menezes PR, Riceto MÉB, Borges AS, Röder BDV, Pedroso SR (2016). Evaluation of virulence factors of *Candida albicans* isolated from HIV-positive individuals using HAART. Arch. Oral. Biol., 66: 61-65. https://doi.org/10.1016/j.archoralbio.2016.02.004

Mohammadi F, Hemmat N, Bajalan Z, Javadi A (2021). Analysis of biofilm-related genes and antifungal susceptibility pattern of vaginal *Candida albicans* and *Non-Candida albicans* Species. Biomed. Res. Int., 2021: 5598907, 9 pages. https://doi.org/10.1155/2021/5598907

Mohammed NA, Ajah HA, Abdulbaqi NJ (2017). Detection the prevalence of adhesins and extracellular hydrolytic enzymes genes in *Candida albicans* biofilm formation. Iraqi J. Sci., 58(2): 988–1000. https://doi.org/10.24996/ijs.2017.58.2C.3

Mohammed SJ, Yassein SN (2020). Characterization of some virulence factors of *Candida albicans* isolated from subclinical bovine mastitis. Plant Arch., 20(1): 238-242.

Mohsin ZA, Ali WS (2021). Antagonistic activity of bacteriocin-producing *Lactobacillus* against *Candida* spp. Iraqi J. Sci., 62(7): 2153-2162. https://doi.org/10.24996/ijs.2021.62.7.4

Mukherjee PK, Seshan KR, Leidich SD, Chandra J, Cole GT, Ghannoum MA (2001). Reintroduction of the *PLB*1 gene into *Candida albicans* restores virulence *in vivo*. *Microbiology*, 147: 2585-2597. https://doi.org/10.1099/00221287-147-9-2585

Nazeer S, Naz SA, Zubair A, Shafique M, Sharafat S, Baig S, and Jabeen, G. (2020). Assessment of different enzymatic virulence traits contributing to pathogenicity of *Candida albicans*. Int. J. Endorsing Health Sci. Res., 8(3): 129-138. https://doi.org/10.29052/IJEHSR.v8.i3.2020.129-138

Neppelenbroek KH, Seó RS, Urban VM, Silva S, Dovigo LN, Jorge JH (2014). Identification of *Candida* species in the clinical laboratory: A review of conventional, commercial, and molecular techniques. Oral Dis., 20: 329–344. https://doi.org/10.1111/odi.12123

Nitz J, Wente N, Zhang Y, Klocke D, Seeth M, Krömker V (2021). Article, dry period or early lactation time of onset and associated risk factors for intramammary infections in dairy cows. Pathogens, 10: 224. https://doi.org/10.3390/pathogens10020224

Pacha PA, Munoz MA, Paredes-Osses E, Latorre AA (2020). Virulence profiles of *Staphylococcus aureus* isolated from bulk tank milk and adherences on milking equipment on Chilean dairy farms. J. Dairy Sci., 103(5): 4732-4737. https://doi.org/10.3168/jds.2019-17794

Pereira R, dos Santos FRO, de Brito EHS, Morais SM (2020). Biofilm of *Candida albicans*: Formation, regulation and resistance. J. Appl. Microbiol., 131: 11-22. https://doi.org/10.1111/jam.14949

Rodrigues LB, Santos LR, Tagliari VP, Rizzo NN, Trenhago G, OLiveira AP, Goetz, F. and Nascimento, V.P. (2010). Quantification of biofilm production on polystyrene by *Listeria, Escherichia coli* and *Staphylococcus aureus* isolated from a poultry slaughter house. Braz. J. Microbiol., 41(4): 1082-1085. https://doi.org/10.1590/S1517-83822010000400029

Sadoon AS (2021). Clinical and subclinical mastitis in buffalue in Mosul area, Iraq. Iraqi J. Vet. Sci., 36(1): 177-186. https://doi.org/10.33899/ijvs.2021.129644.1671

SAS (2018). Statistical analysis system, user's guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sheet OH (2022). Molecular detection of *mecA* gene in methicillin resistant *Staphylococcus aureus* isolated from dairy mastitis in Nineveh governorate, Iraq. Iraqi J. Vet. Sci., 36(4): 939-943. https://doi.org/10.33899/ijvs.2022.132643.2115

Shrief R, Zaki ME, El-Sehsah EM, Ghaleb S, Mofreh M (2019). Study of antifungal susceptibility, virulence genes and biofilm formation in *Candida albicans.* Open Microbiol. J., 13: 241-248. https://doi.org/10.2174/1874285801913010241

Soliman MMH, Kandil MM, Elnemr SA, Abuelnaga ASM (2020). Prevalence of virulence genes and antifungal resistance in *Candida albicans* isolated from raw goat milk. World Vet. J., 10(4): 670-677. https://doi.org/10.54203/scil.2020.wvj81

Spanamberg A, Ramos JP, Leoncini O, Alves SH, Valente P (2009). High frequency of potentially pathogenic yeast species in goats raw milk and creamed cheese in southern Brazil. Acta Sci. Vet., 37(2): 133-141. https://doi.org/10.22456/1679-9216.16239

Stuhr T, Aulrich K (2010). Intramammary infections in dairy goats: Recent knowledge and indicators for detection of subclinical mastitis. Landbauforschung - vTI Agric. For. Res., 4(60): 267-280.

Sudhakara RB, Sivajothi S, Deepika KG (2018). Successful management of fungal mastitis in goats. A report of three cases. Approaches Poult. Dairy Vet. Sci., 3(5): 288-289. https://doi.org/10.31031/APDV.2018.03.000575

Talapko J, Juzbašic M, Matijevic T, Pustijanac E, Bekic S, Kotris I, Škrlec I (2021). *Candida albicans* the virulence factors and clinical manifestations of infection. J. Fungi., 7: 79. https://doi.org/10.3390/jof7020079

Toledo I, Dacey J (2021). Mastitis in small ruminants. UF/IFAS Extension. Peer Reviewed. https://doi.org/10.32473/edis-an367-2021

Udayalaxmi SJ, D'Souza D (2014). Comparison between virulence factors of *Candida albicans* and *Non-Albicans*

species of *Candida* isolated from genitourinary tract. J. Clin. Diagn. Res., 8(11): DC15-DC17.

Unny NM, Pillai UN, Mani BK (2020). Biofilm associated staphylococcal mastitis in goats. Int. J. Livest. Res., 10(6): 1-2. https://doi.org/10.5455/ijlr.20200414025108

Vijayalakshmi P, Thenmozhi S, Rajeswari P (2016). The evaluation of the virulence factors of clinical *Candida* isolates and the anti-biofilm activity of *Elettaria cardamomum* against multi-drug resistant *Candida albicans*. Curr. Med. Mycol., 2(2): 8-15. https://doi.org/10.18869/acadpub.cmm.2.2.3

Washinton WJ, Stephan A, Willium J, Elmer K, Gail W (2006). Konemans color Atlas and Textbook of diagnostic Microbiology; 6th ed. pp. 1152-1232.

Yanuartono AN, Indarjulianto S, Raharjo S, Purnamaningsih H, Haribowo DN (2019). Review: Mycotic mastitis in ruminants. J. Ilmu-Ilmu Peternakan, 29(2): 109-130. https://doi.org/10.21776/ub.jiip.2019.029.02.03