2026, Scienceline Publication
World's Veterinary Journal
World Vet J, 16(2): 459-467.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2026.wvj43
PII: S232245682600043-16

The Emerging of Antifungal Resistance and Virulence in Non-Albicans *Candida* Derived from Bovine Mastitis

Mona Mohamed Hassan Soliman*, and Mai Mohamed Kandil

Department of Microbiology and Immunology, National Research Centre, Giza, Egypt

*Corresponding author's Email: mona_nrc.micro@yahoo.com

ORIGINAL ARTICLE
Received: March 11, 2026
Revised: April 10, 2026
Accepted: May 14, 2026
Published: June 30, 2026

ABSTRACT

Mastitis represents a considerable economic burden on the dairy industry, adversely affecting milk yield and quality. In mycotic mastitis, *Candida* (*C.*) species are frequently responsible for opportunistic fungal infections in cows. The present study aimed to isolate *C. tropicalis* from aseptically collected milk samples from 60 dairy cows (average age 4.2 years) that tested positive for the California mastitis test (CMT). Mycotic etiology was confirmed through direct microscopy (KOH wet mount), culture on Sabouraud dextrose agar, and biochemical identification (API 20C AUX system). Antifungal sensitivity testing was conducted employing the broth microdilution method in accordance with CLSI M27-A3 guidelines. Additionally, molecular detection of antifungal resistance genes (*ERG3* and *CDR1*) and virulence genes (*SAPT3*, *ALS2*, and *LIP1*) was carried out using polymerase chain reaction (PCR). A total of five *C. tropicalis* isolates were obtained from 60 mastitis milk samples, yielding a prevalence of 8.3%. Antifungal susceptibility profiles demonstrated that all tested isolates were sensitive to itraconazole, nystatin, and ketoconazole. However, two of five isolates (40%) were resistant to fluconazole, all five isolates (100%) were resistant to terbinafine, and three of five isolates (60%) were resistant to voriconazole. Virulence genes (*SAPT3*, *ALS2*, and *LIP1*) were identified in 100%, 40%, and 60% of the five examined isolates, respectively. Meanwhile, antifungal resistance genes (*CDR1* and *ERG3*) were found in 100% and 80% of the isolates, respectively. The present study underscored the prevalence of multidrug-resistant *C. tropicalis* in bovine mycotic mastitis, emphasizing the need for routine antifungal susceptibility testing and molecular screening in dairy farms.

Keywords: Antifungal resistance, *Candida tropicalis*, Mastitis, Resistance gene, Virulence gene

INTRODUCTION

Bovine mastitis represents the most significant economic challenge for the global dairy industry, resulting in substantial losses from decreased milk production, discarded milk, and the premature culling of high-value livestock (Devanathan et al., 2024). While bacterial pathogens have traditionally been the primary focus, mycotic mastitis is increasingly recognized as a significant concern within veterinary medicine. Among fungal agents, species of the genus *Candida* (*C.*), specifically *C. tropicalis*, act as opportunistic pathogens that exploit the mammary gland's environment, particularly in cows with compromised immune systems or those experiencing a disrupted microbial balance due to prolonged antibacterial therapy (Dawoud et al., 2024; Schikora-Tamarit and Gabaldón, 2024). The consequences of mycotic mastitis are often more severe than those of bacterial infections due to irreversible damage to the udder tissue. The pathogenicity of *C. tropicalis* is driven by a robust set of virulence factors, including secreted aspartyl proteinases (SAPs), phospholipases, and lipases. These enzymes facilitate the degradation of mammary epithelial tissue, leading to chronic inflammation, udder induration, and permanent loss of secretory function (Zuza-Alves et al., 2017). Additionally, *C. tropicalis* forms extensive three-dimensional biofilms, dense microbial communities covered by an extracellular matrix. This matrix shields the fungi from host immune responses and antifungal agents, making the infection highly resistant to treatment (Cangui-Panchi et al., 2023; Yogendraiah et al., 2025).

The zoonotic potential of *Candida* species in dairy environments highlighted the need for effective treatment. The potential transmission of resistant strains between animals and human handlers contributes to a cycle of antimicrobial resistance (AMR), posing a significant public health threat (Devanathan et al., 2024). Historically, *C. albicans* was considered the predominant pathogenic yeast; however, recent clinical and veterinary studies have indicated a significant increase in isolation rates of non-albicans *Candida* (NAC) species (Sadeghi et al., 2018). *Candida tropicalis* has become one of the most important NAC species in clinical settings, ranking second after *C. albicans* in causing invasive and persistent infections (Jiang et al., 2024).

Management of these infections is severely limited by the increasing prevalence of antifungal resistance. Azoles, particularly fluconazole, are the preferred treatment option due to their safety and low cost (Paul et al., 2022). *Candida tropicalis* has demonstrated a strong ability to quickly develop acquired resistance against antifungal medicines. Unlike

To cite this paper: Soliman MMH and Kandil MM (2026). The Emerging of Antifungal Resistance and Virulence in Non-Albicans *Candida* Derived from Bovine Mastitis. *World Vet. J.*, 16(2): 459-467. DOI: https://dx.doi.org/10.54203/scil.2026.wvj43

other species, *C. tropicalis* frequently exhibits cross-resistance, whereby exposure to a single azole agent confers resistance to subsequent azole derivatives, such as voriconazole (Wang et al., 2021). Recent studies have documented concerning resistance rates not only to azoles but also to amphotericin B and echinocandins, thereby limiting the therapeutic options available to veterinarians (Díaz-García et al., 2023; Dawoud et al., 2024; Schikora-Tamarit and Gabaldón, 2024). The necessity of the present study stemmed from the urgent need to understand the molecular factors driving resistance in the dairy industry. Identifying specific virulence and resistance genes in local bovine isolates improves the ability to predict clinical outcomes and facilitates the implementation of more targeted strategies for infection management. Therefore, the present study aimed to isolate *C. tropicalis* from bovine mastitis cases, characterize its antifungal susceptibility profiles, and utilize polymerase chain reaction (PCR) to detect key resistance genes (*ERG3* and *CDR1*) and virulence-associated genes (*SAP3*, *ALS2*, and *LIP1*).

MATERIALS AND METHODS

Ethical approval

The current investigation was conducted in accordance with the guidelines of the Department of Microbiology and Immunology, National Research Centre, Egypt. All procedures for milk sampling and animal handling were performed in accordance with institutional protocol.

Sampling and animals

A total of 60 milk samples were collected from 60 individual cows (one sample per animal) across three medium-scale dairy farms situated in Giza governorate, Egypt. The cows were primarily Holstein-Friesian crossbreeds, with an average age of 4.5 ± 1.2 years, with a mean body weight of 550±45 kg. Clinical mastitis was diagnosed through physical examination of the udder, noting symptoms such as swelling, heat, and pain, along with observable abnormalities in milk, including clots or discoloration. Subclinical mastitis was identified using the California mastitis test (CMT). Prior to sample collection, the teats were meticulously cleaned and disinfected with 70% ethyl alcohol, and the first three milk streams were discarded. Approximately 10 mL of mid-stream milk was collected aseptically into sterile screw-capped tubes, transported to the laboratory in an ice box at 4°C, and processed within 24 hours.

Fungal conventional isolation and identification

Each milk sample was streaked onto Sabouraud dextrose agar (SDA; Oxoid, UK) and incubated at 30°C for 48-72 hours. To differentiate *Candida* species, isolates were subcultured onto chromogenic *Candida* agar (CHROM agar; BD Diagnostics, USA). Pure colonies were identified based on morphology and conventional biochemical assays as described by Marinho et al. (2010).

Antifungal sensitivity testing

Antifungal susceptibility was determined according to the CLSI (2009) guidelines using the Kirby-Bauer disc diffusion method. Six antifungal agents were tested, including nystatin (100 IU), terbinafine (1 µg), itraconazole (8 µg), fluconazole (25 µg), ketoconazole (15 µg), and voriconazole (1 µg).

Molecular detection of antifungal resistance and virulence genes

Extracting DNA

Genomic DNA was extracted from five pure *C. tropicalis* isolates using the QIAamp DNA mini kit (Qiagen, Hilden, Germany). Briefly, 200 µL of the sample was incubated at 56°C for 10 minutes with 20 µL of Proteinase K and 200 µL of lysis buffer. After adding 200 µL of 100% ethanol, the lysate was washed and centrifuged. Nucleic acid was eluted in 100 µL of the provided elution buffer.

Primers

Primers from Bio Basic (Markham, ON, Canada) were used to detect resistance genes (*ERG3* and *CDR1*) and virulence genes (*SAP3*, *ALS2*, and *LIP1*). The primer sequences and detailed thermal cycling conditions, including denaturation, annealing, and extension, were adapted from Zhang et al. (2019), Paul et al. (2022), and Aboulela et al. (2023; Tables 1 and 2).

PCR amplification

Six µL of DNA template, one µL of each primer at 20 pmol, 4.5 µL of water, and 12.5 µL of EmeraldAmp Max PCR master mix (Takara, Japan) were combined to form a 25 µL reaction. The amplification reaction was performed using an Applied Biosystem 2720 thermal cycler.

Visualization of the PCR products

The PCR products were separated via electrophoresis on a 1.5% agarose gel (AppliChem GmbH, Darmstadt, Germany) in 1x Tris-Borate-Ethylenediaminetetraacetic acid (TBE) buffer. A consistent gradient of 5 Volts/cm was applied. Fragment sizes were determined using a GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Germany). Gels were photographed using the Alpha Innotech Gel Documentation System and subsequently analyzed with specialized software.

Table 1. Primers and PCR conditions of *ERG3* and *CDR1* resistance genes *in Candida tropicalis* isolated from mastitis cow's milk

Genes	Primers	Amplified fragment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
ERG3	ATTCCATTCACTATTGCCTGGTC AGTGCCTTATAACCACCAGTAGA	836 bp	94˚C 5 min	94˚C 30 sec	55˚C 40 sec	72˚C 50 sec	72˚C 10 min	Paul et al. (2022)
CDR1	TCGCCGTTTGCTGAAGAAGA GCAATCCCCAATTTCGATGGT	140 bp	94˚C 5 min	94˚C 30 sec	55˚C 30 sec	72˚C 30 sec	72˚C 7 min	Aboulela et al. (2023)

Min: Minute, Sec: Seconds

Table 2. Primers and PCR conditions of *SAPT3*, *ALS2*, and *L1P1* virulence genes *in Candida tropicalis* isolated from mastitis cow's milk

Genes	Primers	Amplified fragment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
SAPT3	AATTGGAATATAAATACGCT GGGGGTGAAACTACAATTTA	1325 bp	94˚C 5 min	94˚C 1 min	53˚C 1 min	72˚C 1.5 min	72˚C 12 min	Zhang et al. (2019)
ALS2	GGTAAAAGAAGTCGGATACGCAT CTACATCCGTTGCCATTACTATT	1214 bp	94˚C 5 min.	94˚C 1 min.	55˚C 1 min.	72˚C 1.5 min.	72˚C 12 min.	
L1P1	CCAAGGAGTCTATGGCTCAGTTA TAAGTGTAAAGTTGTCGGTGTTC	1945 bp	94˚C 5 min	94˚C 1 min	50˚C 1 min	72˚C 2 min	72˚C 12 min	

Min: Minute, Sec: Seconds

Statistical analysis

Data were analyzed using SPSS software (version 14.0). Descriptive statistics, including frequencies and percentages, were calculated to summarize the prevalence of *C. tropicalis* isolates, antifungal resistance patterns, and the distribution of virulence and resistance genes.

RESULTS AND DISCUSSION

In the present study, *C. tropicalis* was identified in 8.3% (5/60) of mastitis milk samples. These isolates formed creamy, shiny white colonies on SDA (Figure 1) and were verified as *C. tropicalis* by their distinct blue color on CHROM agar (Figure 2). The isolation rate observed in the present study aligns with the 10.1% reported by Seker (2010) in Turkey, but it markedly exceeds the 2% documented by Dworecka-Kaszak et al. (2012) in Poland. Conversely, higher prevalence rates have been reported by Eldesouky et al. (2016) at 19.5% in Egypt and 26.1% in Turkey (Erbas et al., 2017). These variations likely reflect differences in regional farming practices, humidity, and the hygiene protocols employed during milking. The emergence of *C. tropicalis* in dairy herds is often worsened by improper use of antibacterial agents. Although antibiotics effectively target bacteria, their indiscriminate use can disrupt the cow's natural mammary microbiota by eliminating beneficial bacterial communities. This microbial condition allows opportunistic, drug-resistant fungi such as *C. tropicalis* to proliferate and colonize the udder (Jiang et al., 2024). Furthermore, the present study suggested that prolonged antibiotic exposure may induce stress responses in fungal cells, potentially increasing their virulence and resilience against subsequent antifungal treatment (Devanathan et al., 2024). Antibiogram of *C. tropicalis* for six different antifungal agents (Figures 3-7; Table 3) demonstrated that *C. tropicalis* isolates were sensitive to itraconazole, nystatin, and ketoconazole. However, two of five isolates (40%) were resistant to fluconazole, all five isolates (100%) were resistant to terbinafine, and three of five isolates (60%) were resistant to voriconazole.

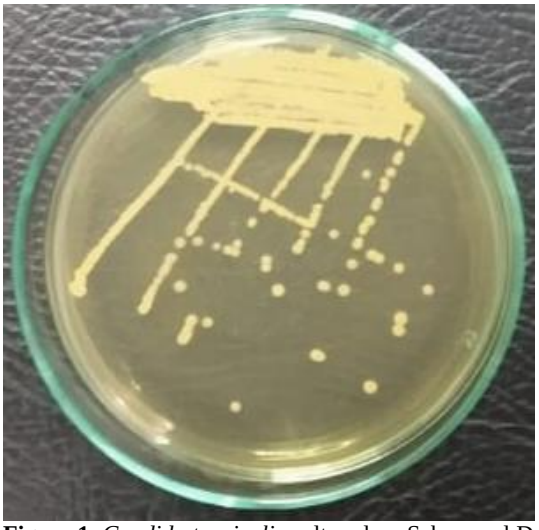

Figure 1. *Candida tropicalis* cultured on Sabouraud Dextrose Agar. The culture appears smooth, creamy-white, and convex colonies after incubation at 37°C for 48 hours.

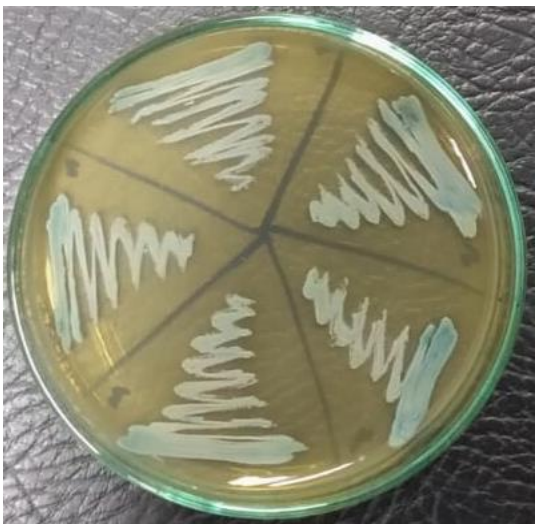

Figure 2. Differential identification of *Candida tropicalis* on chromogenic *Candida* agar (CHROM agar). *Candida* medium, and the colonies appeared blue

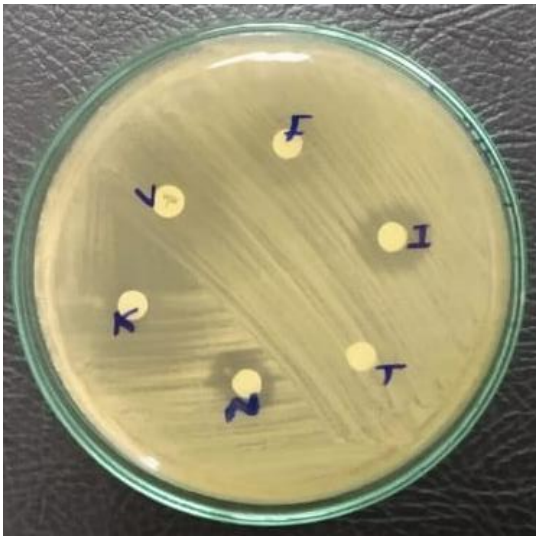

Figure 3. Antifungal susceptibility testing of *Candida tropicalis* using the disc diffusion method (Isolate 1). F: Fluconazole, I: Itraconazole, T: Terbinafine, N: Nystatin, K: Ketoconazole, and V: Voriconazole

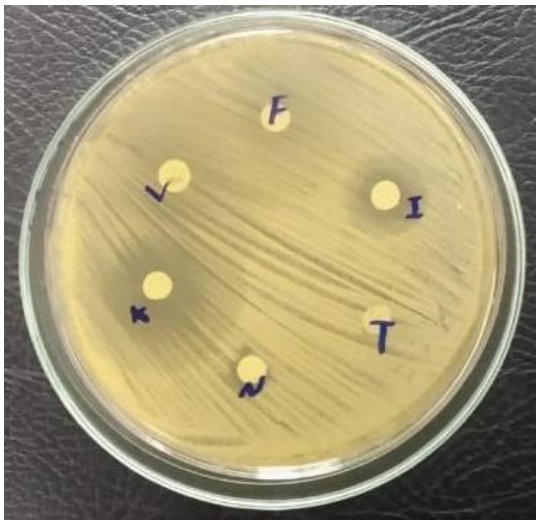

Figure 4. Antifungal susceptibility testing of *Candida tropicalis* using the disc diffusion method (Isolate 2). F: Fluconazole, I: Itraconazole, T: Terbinafine, N: Nystatin, K: Ketoconazole, and V: Voriconazole

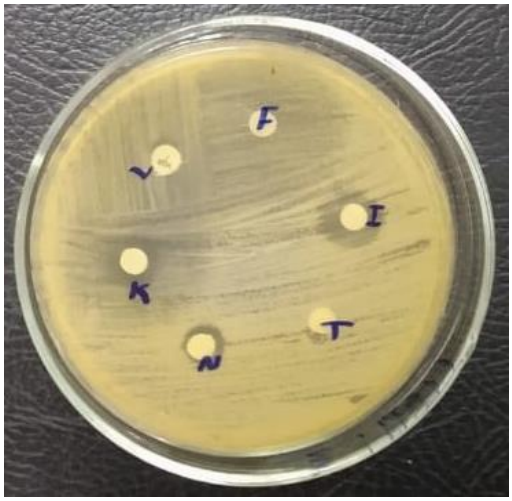

Figure 5. Antifungal susceptibility testing of *Candida tropicalis* using the disc diffusion method (Isolate 3). F: Fluconazole, I: Itraconazole, T: Terbinafine, N: Nystatin, K: Ketoconazole, and V: Voriconazole

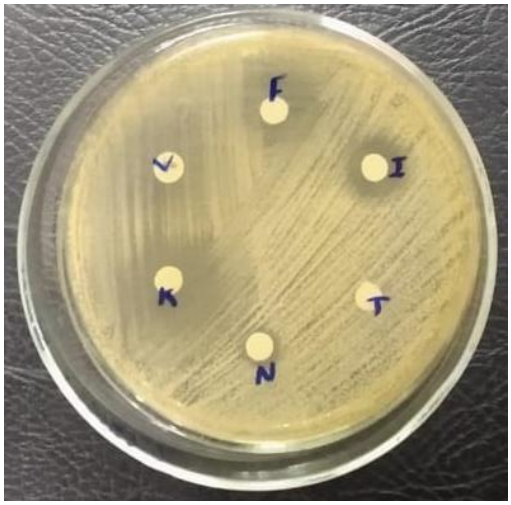

Figure 6. Antifungal susceptibility testing of *Candida tropicalis* using the disc diffusion method (Isolate 4). F: Fluconazole, I: Itraconazole, T: Terbinafine, N: Nystatin, K: Ketoconazole, and V: Voriconazole

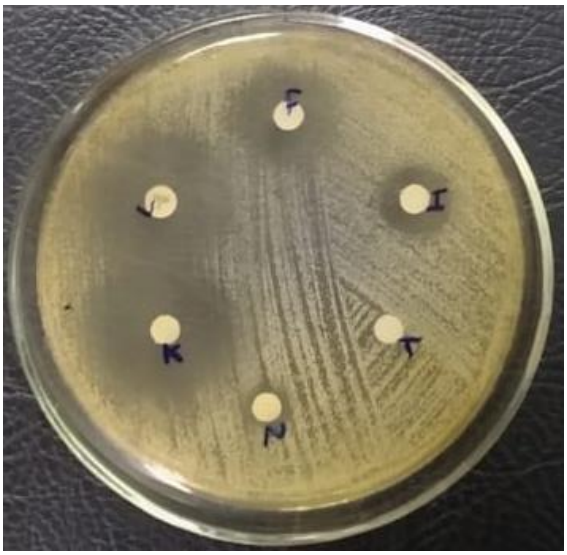

Figure 7. Antifungal susceptibility testing of *Candida tropicalis* using the disc diffusion method (Isolate 5). F: Fluconazole, I: Itraconazole, T: Terbinafine, N: Nystatin, K: Ketoconazole, and V: Voriconazole

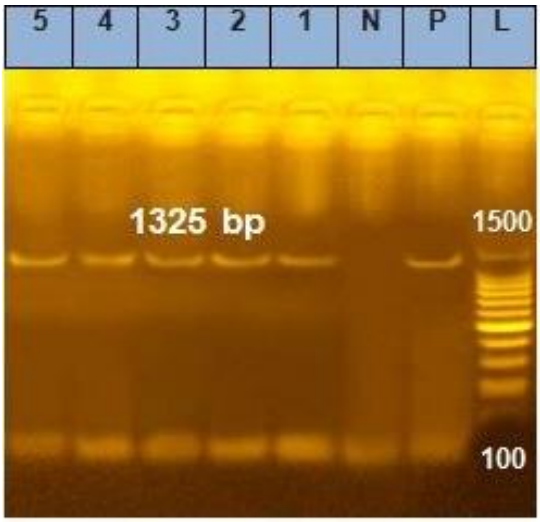

Figure 10. Agarose gel electrophoresis of the *SAP3* virulence gene (1325 bp) in *Candida tropicalis* isolates. Lane L: 100-1500 bp DNA ladder; Lane P: Positive control; Lane N: Negative control; Lanes 1, 2, 3, 4, 5: Positive isolates showing 100% prevalence of the *SAP3* gene.

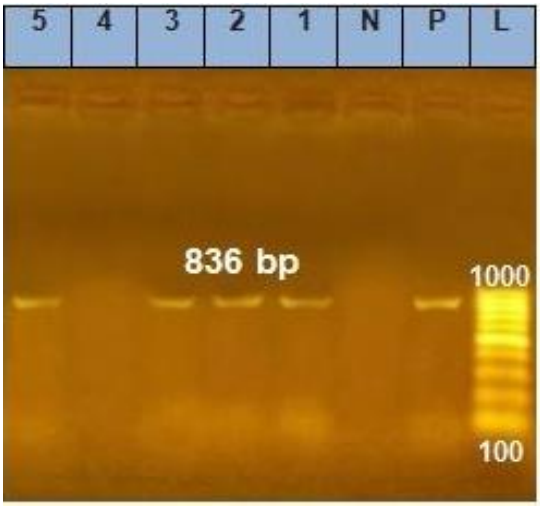

Figure 8. Agarose gel electrophoresis of the *ERG3* resistance gene (836 bp) in *Candida tropicalis* isolates. Lane L: 100-1000 bp DNA ladder, Lane P: Positive control, Lane N: Negative control, Lanes 1, 2, 3, 5: Positive isolates, Lane 4: Negative isolate.

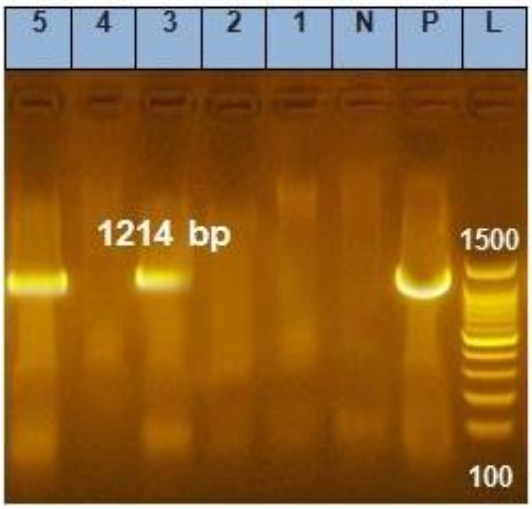

Figure 11. Agarose gel electrophoresis of the *ALS2* virulence gene (1214 bp) in *Candida tropicalis* isolates. Lane L: 100-1500 bp DNA ladder, Lane P: Positive control, Lane N: Negative control, Lanes 3, 5: Positive isolates, Lanes 1, 2, 4: Negative isolates.

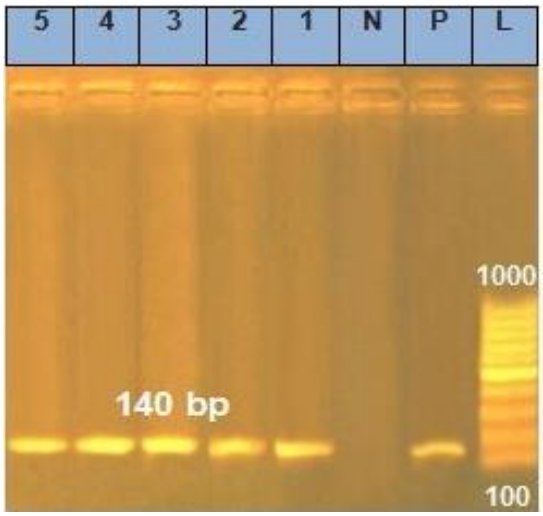

Figure 9. Agarose gel electrophoresis of the *CDR1* resistance gene (140 bp) in *Candida tropicalis* isolates. Lane L: 100-1000 bp DNA ladder; Lane P: Positive control, Lane N: Negative control, Lanes 1, 2, 3, 4, 5: All isolates positive for the LIP1 gene.

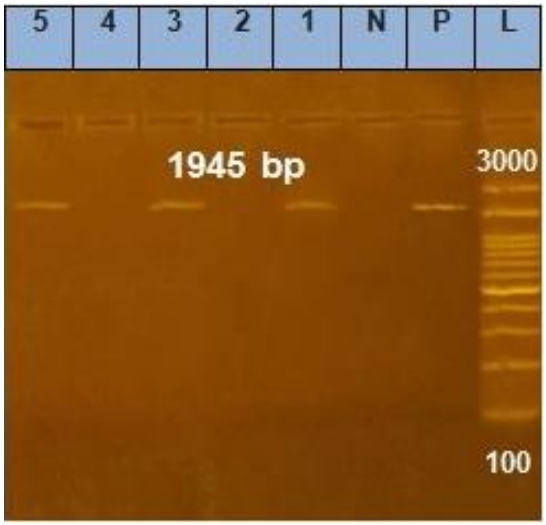

Figure 12. Agarose gel electrophoresis of the *L1P1* virulence gene (1945 bp) in *Candida tropicalis* isolates. Lane L: 100-3000 bp DNA ladder, Lane P: Positive control, Lane N: Negative control, Lanes 1, 3, 5: Positive isolates, Lanes 2, 4: Negative isolates.

Table 3. Antifungal sensitivity testing of *Candida tropicalis* isolated from mastitis cow's milk

C. troicalis isolates	Fluconazole	Itraconazole	Terbinafine	Nystatin	Ketoconazole	Voriconazole
Isolate (1)	12.3 ± 0.3	13.3 ± 0.3	R	9.3 ± 0.3	21.3 ± 0.3	16.3 ± 0.3
Isolate (2)	R	14.3 ± 0.3	R	7.7 ± 0.3	19 ± 0.6	R
Isolate (3)	R	14 ± 0.6	R	9.3 ± 0.3	12.3 ± 0.3	R
Isolate (4)	13.3 ± 0.3	15.3 ± 0.3	R	9.3 ± 0.3	19.3 ± 0.3	R
Isolate (5)	19.3 ± 0.3	15.3 ± 0.3	R	10 ± 0.0	26.3 ± 0.3	24 ± 0.6

The zones were measured in mm. R: Resistant, *C*: *Candida*

Table 4. Antifungal resistance genes and virulence genes of *Candida tropicalis* isolated from mastitis cow's milk

Sample	ERG3	CDR1	SAPT	ALS2	L1P1
1	+	+	+	-	+
2	+	+	+	-	-
3	+	+	+	+	+
4	-	+	+	-	-
5	+	+	+	+	+

+: Means the presence of this gene, -: Means the absence of this gene

The emergence of antifungal resistance in *C. tropicalis* results from multiple factors, including genetic changes and environmental influences (Paul et al., 2022). According to an investigation conducted in Turkey, Sonmez and Erbas (2017) reported that 25 Candida isolates demonstrated 100% resistance to miconazole, flucytosine, amphotericin B, and fluconazole. Additionally, Cândido et al. (2021) reported that *C. tropicalis* isolated from domestic and wild animals in Brazil demonstrated multidrug resistance, with 100% resistance to itraconazole but remaining susceptible to voriconazole. Antifungal resistance is mediated by point mutations in the *ERG11* gene and by increased expression of efflux pumps such as *CDR1* and *MDR1*, which help in avoiding common antifungal medicines. This resistance mainly stems from changes in the ergosterol biosynthesis pathway. Azole antifungal agents specifically target the enzyme lanosterol 14α-demethylase, which is encoded by the *ERG11* gene (Lee et al., 2021). In *C. tropicalis*, at least 31 distinct mutations in the *ERG11* gene have been identified; these structural alterations prevent antifungal drugs from binding to the target enzyme, making treatment ineffective (Lima et al., 2022).

Over the past decade, the resistance of *C. tropicalis* to fluconazole has been consistently observed across several nations and regions, with notably high resistance rates documented in Austria at 37.7% (Arendrup et al., 2023), Vietnam (18.8%; Ngo et al., 2023), India (21.1%; Lima et al., 2022), Singapore and Thailand (20%; Tan et al., 2016), and China (12.8%; Fan et al., 2019). In addition to fluconazole resistance, *C. tropicalis* has exhibited reduced susceptibility to other triazoles, such as itraconazole and voriconazole, across diverse geographic regions, including Brazil, Spain, and North America (Fernández-Ruiz et al., 2015; Chapman et al., 2017; Xisto et al., 2017). Notably, these resistance rates often align with those of fluconazole, reflecting the high prevalence rate of cross-resistance. This incident occurs because different azole medicines target the same enzyme (lanosterol 14α-demethylase enzyme); therefore, a single genetic mutation in the *ERG11* gene or the activation of multidrug efflux pumps (such as *CDR1*) can simultaneously make the fungus resistant to multiple medications within the same pharmacological class (Chong et al., 2012). In the current study, the molecular basis of this resistance was confirmed by the high prevalence of *CDR1* (100%) and *ERG3* (80%), as indicated in Figures 8 and 9 and Table 4. The *CDR1* gene encodes an Adenosine Triphosphate Binding Cassette (ABC) transporter that acts as an efflux pump, actively leading antifungal agents out of the fungal cell, lowering intracellular medicine levels to nonlethal levels (Prasad et al., 2019). Additionally, mutations or overexpression of *ERG3* (encoding sterol 5,6-desaturase) prevent the accumulation of toxic 14 alpha-methyl-3,6-diol, a byproduct of azole that typically results in fungal cell death. By altering this pathway, the fungus persists despite antifungal treatment (Paul et al., 2020).

Studies by Choi et al. (2016) and Rojas et al. (2023) have documented that *ERG11* and *ERG3* are the genes most frequently overexpressed in resistant isolates of *C. tropicalis*. This genetic adaptation typically occurs as a direct result of

prolonged and repeated exposure to azole antifungal agents, which apply selective pressure on the fungal population to overcome the drug's inhibitory effect on the ergosterol biosynthesis pathway. Although many studies have highlighted drug clearance, Jiang et al. (2013) found that efflux transporters may not be the main cause of azole resistance across all *C. tropicalis* populations. Jiang et al. (2013) suggested that alternative mechanisms play a more direct role in survival. These include modifications at the target site, such as mutations in the *ERG11* gene that decrease drug binding affinity, and the activation of bypass pathways in the sterol membrane, similar to those involving *ERG3* mutations. These mechanisms allow the fungus to preserve membrane integrity even when the main ergosterol pathway is inhibited.

In the present study, the virulence-associated genes *SAP3*, *ALS2*, and *LIP1* were detected in 100%, 40%, and 60% of the *C. tropicalis* isolates, respectively (Figures 10, 11, 12, and Table 4). These findings are consistent with the molecular pathogenesis models reported by Yu et al. (2016), who investigated the expression profiles of several virulence factors, including the secreted aspartyl proteinase family (*SAP1-4*), adhesins (*ALS1-2*), and lipases (*LIP1* and *LIP4*). Yu et al. (2016) demonstrated that the expression of the *ALS2* and *ALS3* genes, which encode critical cell-surface adhesins, was positively correlated with the extent of epithelial cell damage. This correlation highlighted an important stage in mycotic mastitis, indicating that *C. tropicalis* can adhere to the mammary epithelium, which is a crucial step in infection. Once adhered, the yeast can effectively utilize lytic enzymes, such as proteinases and lipases, to degrade host cell membranes. Thus, the high prevalence of *SAP3* (100%) and the presence of *ALS2* (40%) in the isolates during the present study indicated a strong genetic potential for both initial colonization and subsequent tissue destruction within the bovine udder.

CONCLUSION

The present study identified *C. tropicalis* as a significant opportunistic pathogen causing bovine mastitis, with an isolation rate of 8.3% in the examined dairy herds. Although isolates were sensitive to nystatin and ketoconazole, resistance rates to terbinafine (100%), voriconazole (60%), and fluconazole (40%) were high. The molecular analysis confirmed that this resistance was driven by the widespread presence of the *CDR1* efflux pump gene (100% of isolates) and the high prevalence of the *ERG3* gene (80%). Furthermore, the 100% detection rate for the *SAP3* virulence gene indicated that these isolates have a considerable capacity to degrade host tissue. The current results demonstrated that *C. tropicalis* was not merely a transient contaminant but a persistent pathogen with a strong genetic tendency for drug resistance and invading mammary tissue. Consequently, the current study suggested that routine veterinary diagnostics should shift from general fungal detection toward species-level identification and molecular resistance profiling. Future studies should focus on the transcriptomic profiles of *C. tropicalis* virulence genes during active infections and investigate the possibility of cross-transmission of these resistant strains between dairy handlers and livestock to enhance understanding of the zoonotic risks associated with mycotic mastitis.

DECLARATIONS

Availability of data and materials

The data supporting the study's conclusions would be provided by the corresponding author upon request.

Authors' contributions

Mona Mohamed Hassan Soliman designed the study, while Mona Mohamed Hassan Soliman and Mai Mohamed Kandil conducted data collection, practical work, analysis of results, and the preparation of the manuscript. All authors have read and approved the final edition of the manuscript before publication.

Competing interests

No conflicts of interest have been disclosed by the authors.

Ethical considerations

Each author has checked for ethical concerns, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, duplicate publication and/or submission, and redundancy. No AI tools were used to conduct or prepare the present study.

Funding

The current study did not receive any funding or financial support.

REFERENCES

Aboulela A, Idris S, Ragab D, Emad R, and El-Sawaf G (2023). Assessment of the accuracy of identification of clinical *Candida* species with reduced fluconazole susceptibility by rapid methods and their relative expression of major efflux pump genes. Microbes and Infectious Diseases, 4(3): 994-1007. DOI: https://www.doi.org/10.21608/MID.2023.220044.1553

Arendrup MC, Arikan-Akdagli S, Jørgensen KM, Barac A, Steinmann J, Toscano C, Arsenijevic AA, Sartor A, Lass-Flörl C, Hamprecht A et al. (2023). European candidaemia is characterised by notable differential epidemiology and susceptibility pattern: results from the ECMM Candida III study. Journal of Infection, 87(5): 428-437. DOI: https://www.doi.org/10.1016/j.jinf.2023.08.001

Cândido SL, Menezes IG, Nakazato L, Morgado TO, Ludwig A, Santurio JM, and Dutra V (2021). Isolation and resistance profile of *Candida* spp. of domestic and wild animals. Research, Society and Development, 10(10): e264101018541. DOI: https://www.doi.org/10.33448/rsd-v10i10.18541

Cangui-Panchi SP, Ñacato-Toapanta AL, Enríquez-Martínez LJ, Salinas-Delgado GA, Reyes J, Garzon-Chavez D, and Machado A (2023). Battle royale: Immune response on biofilms – host-pathogen interactions. Current Research in Immunology, 4: 100057. DOI: https://www.doi.org/10.1016/j.crimmu.2023.100057

Chapman B, Slavin M, Marriott D, Halliday C, Kidd S, Arthur I, Bak N, Heath CH, Kennedy K, Morrissey CO et al. (2017). Changing epidemiology of candidaemia in Australia. The Journal of Antimicrobial Chemotherapy, 72(4): 1103-1108. DOI: https://www.doi.org/10.1093/jac/dkw422.PMID:28364558

Choi MJ, Won EJ, Shin JH, Kim SH, Lee WG, Kim MN, Lee K, Shin MG, Suh SP, Ryang DW et al. (2016). Resistance mechanisms and clinical features of fluconazole-nonsusceptible *Candida tropicalis* isolates compared with fluconazole-less-susceptible isolates. Antimicrobial Agents and Chemotherapy, 60(6): 3653-3661. DOI: https://www.doi.org/10.1128/AAC.02652-15

Chong Y, Shimoda S, Yakushiji H, Ito Y, Miyamoto T, Shimono N, Kamimura T, and Akashi K (2012). Fatal candidemia caused by azole-resistant *Candida tropicalis* in patients with hematological malignancies. Official Journal of the Japan Society of Chemotherapy, 18(5): 741-746. DOI: https://www.doi.org/10.1007/s10156-012-0412-9

Clinical and laboratory standards institute (CLSI) (2009). Method for antifungal disk diffusion susceptibility testing of yeasts; Approved guideline, 2nd Edition. CLSI M44-A2. Available at: https://clsi.org/standards/products/microbiology/documents/m44/

Dawoud AM, Saied SA, Torayah MM, Ramadan AE, and Elaskary SA (2024) Antifungal susceptibility and virulence determinants profile of *candida* species isolated from patients with candidemia. Scientific Reports, 14(1): 11597. DOI: https://www.doi.org/10.1038/s41598-024-61813-w

Devanathan N, Vivek Srinivas M, Vasu J, Chaluva S, and Mukhopadhyay HK (2024). Molecular detection and genetic analysis of *Candida* species isolated from bovine clinical mastitis in India. Veterinary Research Forum: An International Quarterly Journal, 15(9): 509-514. DOI: https://www.doi.org/10.30466/vrf.2024.2008702.3968

Díaz-García J, Machado M, Alcalá L, Reigadas E, Sánchez-Carrillo C, Pérez-Ayala A, Gómez-García de la Pedrosa E, González-Romo F, Merino P, Cuétara MS et al. (2023). Antifungal resistance in *Candida* spp within the intra- abdominal cavity: Study of resistance acquisition in patients with serial isolates. Clinical Microbiology and Infection, 29(12): 1604.e1-1604.e6. DOI: https://www.doi.org//10.1016/j.cmi.2023.08.021

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, 2012: 196347. DOI: https://www.doi.org/10.1100/2012/196347

Eldesouky I, Mohamed N, Khalaf D, Salama A, Elsify A, Ombarak R, El-Ballal S, Effat M, and Alshabrawy M *(*2016). *Candida* mastitis in dairy cattle with molecular detection of *Candida albicans*. Kafkas Universitesi Veteriner Fakultesi Dergis, 22(3): 461-464. DOI: https://www.doi.org/10.9775/kvfd.2015.14843

Erbas G, Parin U, Kirkan S, Savasan S, Özavci V, and Yüksel HT (2017). Identification of *Candida* strains with nested PCR in bovine mastitis and determination of antifungal susceptibilities. Turkish Journal of Veterinary and Animal Sciences, 41(6): 757-763. DOI: https://www.doi.org/10.3906/vet-1704-39

Fan X, Xiao M, Zhang D, Huang JJ, Wang H, Hou X, Zhang L, Kong F, Chen SCA, Tong ZH et al. (2019). Molecular mechanisms of azole resistance in Candida tropicalis isolates causing invasive candidiasis in China. Clinical Microbiology and Infection, 25(7): 885-891. DOI: https://www.doi.org/10.1016/j.cmi.2018.11.007

Fernández-Ruiz M, Puig-Asensio M, Guinea J, Almirante B, Padilla B, Almela M, Díaz-Martín A, Rodríguez-Baño J, Cuenca-Estrella M, Aguado JM et al. (2015). *Candida tropicalis* bloodstream infection: Incidence, risk factors and outcome in a population-based surveillance. The Journal of Infection, 71(3): 385-394. DOI: https://www.doi.org/10.1016/j.jinf.2015.05.009

Jiang C, Dong D, Yu B, Jiang C, Dong D, Yu B, Cai G, Wang X, Ji Y, and Peng Y (2013). Mechanisms of azole resistance in 52 clinical isolates of *Candida tropicalis* in China. The Journal of Antimicrobial Chemotherapy, 68(4): 778-785. DOI: https://www.doi.org/10.1093/jac/dks481

Jiang C, Fang W, Chen S, Guo X, Gao X, Liu P, Hu G, Li G, Mai W, and Liu P (2024). Genetic framework sequencing analysis of *Candida tropicalis* in dairy cow mastitis and study of pathogenicity and drug resistance. BMC Microbiology, 24(1): 428. DOI: https://www.doi.org/10.1186/s12866-024-03522-y

Lee Y, Puumala E, Robbins N, and Cowen LE (2021). Antifungal drug resistance: molecular mechanisms in *Candida albicans* and beyond. Chemical Reviews, 121(6): 3390-3411. DOI: https://www.doi.org/10.1021/acs.chemrev.0c00199

Lima R, Ribeiro FC, Colombo AL, and de Almeida JN (2022). The emerging threat antifungal-resistant *Candida tropicalis* in humans, animals, and environment. Frontiers in Fungal Biology, 3: 957021. DOI: https://www.doi.org/10.3389/ffunb.2022.957021

Marinho SA, Teixeira AB, Santos OS, Cazanova RF, Ferreira CA, Cherubini K, and de Oliveira SD (2010). Identification of *Candida* spp. by phenotypic tests and PCR. Brazilian Journal of Microbiology, 41(2): 286-294. DOI: https://www.doi.org/10.1590/S1517-83822010000200004

Ngo TMC, Santona A, Fiamma M, Nu PAT, Do TBT, Cappuccinelli P, and Paglietti B (2023). Azole non-susceptible C. tropicalis and polyclonal spread of C. albicans in Central Vietnam hospitals. The Journal of Infection in Developing Countries, 17(4): 550-558. DOI: https://www.doi.org/10.3855/jidc.17599

Paul S, Singh S, Sharma D, Chakrabarti A, Rudramurthy SM, and Ghosh AK (2020). Dynamics of *in vitro* development of azole resistance in *Candida tropicalis*. Journal of Global Antimicrobial Resistance, 22: 553-561. DOI: https://www.doi.org/10.1016/j.jgar.2020.04.018

Paul S, Shaw D, Joshi H, Singh S, Chakrabarti A, Rudramurthy SM, and Ghosh AK (2022). Mechanisms of azole antifungal resistance in clinical isolates of *Candida tropicalis.* PLoS One, 17(7): e0269721. DOI: https://www.doi.org/10.1371/journal.pone.0269721

Prasad R, Nair R, and Banerjee A (2019). Multidrug transporters of *Candida* species in clinical azole resistance. Fungal Genetics and Biology, 132: 103252. DOI: https://www.doi.org/10.1016/j.fgb.2019.103252

Rojas AE, Cárdenas LY, García MC, and Pérez JE (2023). Expression of *ERG11*, *ERG3*, *MDR1* and *CDR1* genes in *Candida tropicalis*. Biomedica: revista del Instituto Nacional de Salud, 43(Sp. 1): 144-155. DOI: https://www.doi.org/10.7705/biomedica.6852

Sadeghi G, Ebrahimi-Rad M, Mousavi SF, Shams-Ghahfarokhi M, and Razzaghi-Abyaneh M (2018). Emergence of non-*Candida albicans* species: Epidemiology, phylogeny and fluconazole susceptibility profile. Journal de Mycologie Médicale, 28: 51-58. DOI: https://www.doi.org/10.1016/j.mycmed.2017.12.008

Schikora-Tamarit MÀ and Gabaldón T (2024). Recent gene selection and drug resistance underscore clinical adaptation across *Candida* species. Nature Microbiology, 9: 284-307. DOI: https://www.doi.org/10.1038/s41564-023-01547-z

Seker E (2010). Identification of *Candida* species isolated from bovine mastitic milk and their *in vitro* hemolytic activity in Western Turkey. Mycopathologia, 169(4): 303-308. DOI: https://www.doi.org/10.1007/s11046-009-9255-z

Sonmez M and Erbas G (2017). Isolation and identification of *Candida* spp. from mastitis cattle milk and determination of antifungal susceptibilities. International Journal of Veterinary Science, 6(2): 104-107. Available at: https://www.ijvets.com/pdf-files/Volume-6-no-2-2017/104-107.pdf

Tan TY, Hsu LY, Alejandria MM, Chaiwarith R, Chinniah T, Chayakulkeeree M, Shorman M, Krishnan PU, Myat TO, Jeon SY et al. (2016). Antifungal susceptibility of invasive Candida bloodstream isolates from the Asia-Pacific region. Medical Mycology, 54(5): 471-477. DOI: https://www.doi.org/10.1093/mmy/myv114

Wang D, An N, Yang Y, Yang X, Fan Y, and Feng J (2021). *Candida tropicalis* distribution and drug resistance is correlated with *ERG11* and *UPC2* expression. Antimicrobial Resistance and Infection Control, 10(1): 54. DOI: https://www.doi.org/10.1186/s13756-021-00890-2

Xisto MI, Caramalho RD, Rocha DA, Ferreira-Pereira A, Sartori B, Barreto-Bergter E, Junqueira ML, Lass-Flörl C, and Lackner M (2017). Pan-azole-resistant *Candida tropicalis* carrying homozygous erg11 mutations at position K143R: A new emerging superbug?. The Journal of Antimicrobial Chemotherapy, 72(4): 988-992. DOI: https://www.doi.org/10.1093/jac/dkw558

Yu S, Li W, Liu X, Che J, Wu Y, and Lu J (2016). Distinct expression levels of ALS, LIP, and SAP genes in *Candida tropicalis* with diverse virulent activities. Frontiers in Microbiology, 7: 1175. DOI: https://www.doi.org/10.3389/fmicb.2016.01175

Yogendraiah KM, Sadanandan B, Kyathsandra Natraj L, Vijayalakshmi V, and Shetty K (2025). Optimization of *Candida tropicalis* growth conditions on silicone elastomer material by response surface methodology. Frontiers in Bioengineering and Biotechnology, 13: 1572694. DOI: https://www.doi.org/10.3389/fbioe.2025.1572694

Zhang LJ, Yu SB, Li WG, Zhang WZ, Wu Y, and Lu JX (2019). Polymorphism analysis of virulence-related genes among *Candida tropicalis* isolates. Chinese Medical Journal, 132(4): 446-453. DOI: https://www.doi.org/10.1097/CM9.0000000000000069

Zuza-Alves DL, Silva-Rocha WP, and Chaves GM (2017). An update on *Candida tropicalis* based on basic and clinical approaches. Frontiers in Microbiology, 8: 1927. DOI: https://www.doi.org/10.3389/fmicb.2017.01927