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The Emergence of Pan- Antifungal Resistance in Candida spp Isolated from The Oral Mucosa of Cats and Owners

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Abstract:

Aims: the current study aimed to detect candida spp. from oral mucosa of domestic cats and their owners suffering from different clinical presentations.

Methods: A total of 119 oral swabs were collected equally from cats and their owners streaked on SDA and chrome agar. The isolates were tested via Vitek 2 compact system. Differentiation of candida spp. was done by cand primers. The Study of antifungal susceptibility patterns for Clotrimazole, Fluconazole, Nystatin and Amphotericin by Kirby-Bauer disc diffusion method.

Results: candida spp. was isolated from oral cavity of (16.67 %) cats and (8.40 %) cat owners. *Albicans* and *C. glabrata* were detected equally in (9.2%) cats. *C. albicans* was detected in **(**3.4**%)** and *C. glabrata in* (**5%)** of cat owners by cand primers. Significant inverse correlation was reported between stomatitis, glossitis, stomatitis and isolation of candida from oral mucosa of cats. Inverse significant correlation was reported between oral lesions and isolation of candida from cat owners. cat isolates of *C.albicans* were completely susceptible to clotrimazole and fluconazole. All cat isolates of *C. glabrata* have resistance to Clotrimazole and Fluconazole. All isolates of *C. albicans* and *C. glabrata* from cat have resistance to Nystatin and Amphotericin. *C.albicans* from cat owners were completely susceptible to clotrimazole and fluconazole. All isolates of C. glabrata from cat owners have resistance to Clotrimazole and Fluconazole. All isolates of C. albicans and C. glabrata from cat owners have resistance to Nystatin. A total of (20%) of *C.albicans* from cat owners have intermediate susceptibility and are resistant to Amphotericin.

Conclusions: A significant presence of *C. albicans* and *C. glabrata*, in the oral mucosa of domestic cats and their owners. The pan-resistant profile of all *C. glabrata* isolates common antifungal agents, azoles and polyenes. The complete resistance of all isolates to Nystatin and the emerging resistance

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to Amphotericin B highlights a serious therapeutic challenge. The inverse correlation between clinical lesions and yeast isolation suggests a complex host-pathogen interaction. These results underscore the potential for cross-species transmission of resistant strains and emphasize the need for antifungal stewardship in veterinary and human medicine.

Keyword: Antifungal Resistance, One Health, Feline Candidiasis, Zoonotic Transmission, Oral mycobiota

Introduction:

Fungi are among the most important pathogens in humans and animals, causing a wide range of diseases(Hassan; *et al.*, 2017; Al-Khalidi *et al.*, 2018; Al Khalidi *et al.*, 2020; AL-Ezzy & Abdulameer, 2021). However, some fungi can be beneficial in promoting human and animal health(Al-Khalidi *et al.*, 2020; Najem *et al.*, 2020).

The oral cavity is a primary reservoir for *Candida* species in both humans and cats. Colonization refers to the presence of the yeast without causing active disease, a state that is heavily influenced by the host's immune competence and the stability of the local microbiome(Patel, 2022).

In humans, *Candida albicans* is the most frequently isolated species from the oral cavity of healthy individuals, with carrier rates ranging from 30-60% in various populations. Other non-*albicans* species like *C. glabrata*, *C. krusei*, and *C. tropicalis* are also common commensals (Vila *et al.*, 2020). The balance between the host and the fungus is maintained by an intact immune system, competitive bacterial flora, and salivary flow. In healthy human owners, *Candida* typically exists as a harmless commensal (Refai & El-Yazid, 2017). This yeast is a natural component of the oral microbiome but can become pathogenic if the host's immune system is compromised, leading to opportunistic infections like oral thrush (Talapko *et al.*, 2021).

Candidiasis represents the most prevalent fungal infection of the oral cavity, caused by yeasts of the genus *Candida*(Peters et al., 2017). While historical estimates suggested oral *Candida* carriage in 35–80% of the population, contemporary molecular analyses indicate that these fungi are ubiquitous constituents of the normal human oral mycobiota(Lewis & Williams, 2017). *Candida albicans* remains the predominant species, isolated from over 80% of oral fungal samples in both healthy and diseased states. Other non-albicans species frequently identified include *C. glabrata*, *C. dubliniensis*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis* (Sav *et al.*, 2020)

The transition from commensalism to pathogenicity occurs when systemic, local, genetic, or environmental factors disrupt oral homeostasis. This dysbiosis can prompt uncontrolled fungal proliferation or altered expression of virulence determinants(Zdanavičienė *et al.*, 2017). Key local risk

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factors for candidiasis include poor oral hygiene, dental prostheses, xerostomia, tobacco use, inhaled corticosteroids, high-carbohydrate diets, and pre-existing mucosal pathology(Lewis & Williams, 2017).

The rise of antifungal resistance, particularly to azoles and echinocandins, complicates treatment outcomes in both human patients and companion animals, especially cats (Robbins et al., 2023; Ostrander et al., 2023).

In humans, non-albicans *Candida* species, notably *C. glabrata* and *C. auris*, demonstrate concerning resistance patterns. *C. auris* is particularly alarming due to its multidrug-resistant profile and nosocomial transmission potential(Rhodes & Fisher, 2019) . Azole resistance in *C. glabrata* often involves upregulation of efflux pumps (CDR1, CDR2) and mutations in the PDR1 gene (Whaley & Rogers, 2016). Echinocandin resistance, primarily due to FKS1 and FKS2 hotspot mutations, is increasingly reported in clinical isolates (Pappas *et al.*, 2016)

Recent studies indicate similar resistance patterns in feline isolates (Hizlisoy *et al.*, 2025). The parallel emergence of antifungal resistance in human and feline *Candida* isolates underscores the interconnected nature of fungal diseases. A coordinated One Health approach is essential for monitoring, preventing, and treating resistant candidiasis across species barriers.

Current study aimed to detect candida spp. from oral mucosa of domestic cats and their owners suffering from different clinical presentations.

Materials and Methods:

Ethical Consideration:

Current randomized experimental procedures were performed in accordance with the guides of institutional declaration and confirmed by the ethics committee at the pathology department, College of Veterinary Medicine, University of Diyala, Iraq. The approval No. PD, CVM-UOD-25/202

Area of the study:

Current study was achieved in veterinary clinics at Suleimani governorate.

Collection of Samples

This study was performed from 10th September 2024 until 25th June 2024. The study included 119 domestic cat breeders. Oral swabs were taken and transferred to mycology lab. at the college of veterinary medicine, University of Suleimani.

Culturing of oral swabs on Sabouraud's dextrose agar and CHROM agar candida

Direct Microscopical Examination:

A sample from the swab was transferred to a clean glass slide, covered with a coverslip, and briefly passed through a flame two to three times for heat fixation. The prepared slide was then ex-

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amined under light microscopy using 10X and 40X objectives to identify yeasts and pseudohyphae(Hall, 2013) . A second slide was heat-fixed and Gram stained to visualize yeast cells (Carroll *et al.*, 2024)

Indirect Examination:

Swabs were inoculated onto Sabouraud's Dextrose Agar (SDA) plates

(Himedia, Mumbai, India) ,containing chloramphenicol and incubated in petri plates at 37 °C for 24-48 hours (Musleh & Al-Saadi, 2022)

Isolation and Purification:

Individual colonies from all SDA-cultured samples were aseptically isolated and purified for diagnostic testing (Marsh & Martin, 2009)

Identification: Morphological Characteristics: Colonies growing on SDA were examined for their color, shape, texture, diameter, elevation, and odor (Ellis *et al.*, 2007).

Microscopic Characteristics:

A portion of the colony was aseptically transferred with an inoculation loop, emulsified in a drop of lactophenol cotton blue stain, and spread onto a sterile glass slide. A coverslip was applied, and the preparation was examined under light microscopy using 10X and 40X objectives to observe yeast cells, pseudohyphae, and blastoconidia (Ellis *et al.*, 2007; Al-Khalidi *et al.*, 2018).A second smear was prepared on a separate sterile slide, heat-fixed by passing through a flame, stained using the Gram stain technique, and examined microscopically for germ tube formation (Ellis *et al.*, 2007; Ali & Al-Ezzy, 2026).

Biochemical Tests

Chromogenic Agar Candida (CAC) Test:

Pure 24-hour yeast colonies grown on SDA were streaked onto Chromogenic Agar Candida (CAC) plates using a sterile inoculating loop. Plates were incubated at 37 °C for 24–48 hours. Strains were identified according to colony color and morphology per manufacturer guidelines

(AL-Ezzy & Abdulameer, 2021; Musleh & Al-Saadi, 2022; Ali & Al-Ezzy, 2026).

Candida albicans: Green colonies; *Candida glabrata*: Pale pink to cream colonies.

2. **Germ Tube Test** :according to (Ellis *et al.*, 2007)

Identification of candida spp. by Vitek 2 compact system

Yeast identification was performed using the automated VITEK 2 system (bioMérieux, France) according to Pure isolates presumptively identified as yeast were processed according to the manufacturer's specifications (Ismail *et al.*, 2024).

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PCR Based Molecular Methods:

DNA Extraction

DNA was extracted from candida by using Favor Prep™ total DNA mini Kit (FAVORGEN, Taiwan) according to the protocol stated by the Mini Kit manufacturer

dsDNA Quantitation by Qubit 4.0

The assay is highly selective for double-stranded DNA (dsDNA) over RNA and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay. The standard and short procedure.

Materials used for thermal Cycling

Primers Selection and identification of candida was illustrated in table (1)

PCR working solution

Molecular Detection of candida

Molecular detection of cand oligonucleotides primers

This step was Carried by adding 12.5µL from oneTaq(NEB ®) master mix,3 µL of DNAsample,1µL from one each primer,7.5 µL from Nuclease-Free water(New England Biolabs, 2024), the reaction done under the optimum PCR conditions shows in Table(1)

Protocol of Gel Electrophoresis

Preparation of 1X TAE Buffer

Two hundred milliliter of (TAE) buffer 50x (0.08 M Tris, 0.08 M Acetic acid and 0.02 M EDTA) was diluted to 10X by taking 200 ml of 50X TAE and added to 800 ml of deionized distilled water (ddH_2O). This 10X buffer re-diluted to 1X (working concentration) by taking 100 ml and added to 900 ml of deionized distilled water(ddH_2O)(Alkhuwailidy & Alrufae, 2022; Hadi *et al.*, 2025a).

Preparation of Agarose Gel 2% and Loading of Samples into Gel

Ten microliters of PCR product and DNA ladder have been loaded into the wells of gel. The voltage of power supply was fixed at 80V for 80 minutes. At the end of run, gel documentation with high resolution camera have been used to capture image and analyze the bands(Alkhuwailidy & Alrufae, 2022; Hadi *et al.*, 2025b).

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Table (1): Specific Primers Used for Detection Of candida spp

GENES	PRIMER SEQUENCE (5′-3′)	SIZE OF PROD-UCT BP	REFERENCE
CAND F	Forward primer (5`AGCTTGCGTTGATTAC-GTCCCTGCCC3`)	C. albicans 850bp C. glabrata 1000bp	**(GARCÍA-SALAZAR *ET AL.*, 2022; DÍAZ-HUERTA *ET AL.*, 2025)**
CAND R	**REVERSE PRIMER (5`TTCACTCGCCGC-TACTAAGGCAATCCC3`)**	***C. ALBICANS* 850BP** ***C. GLABRATA* 1000BP**	

Antifungal susceptibility test:

The disk diffusion susceptibility testing method was employed for Candida spp. isolates against the following antifungal agents: fluconazole (25 µg), clotrimazole (25 µg), nystatin (25 µg), and amphotericin B (25 µg) (Himedia, Mumbai, India). Briefly, a standardized suspension of each isolate, adjusted to a turbidity equivalent to a 0.5 McFarland standard in sterile 0.85% saline, was used to inoculate Mueller-Hinton Agar (MHA) (Himedia, Mumbai, India). The MHA was supplemented with 2% (w/v) glucose to support optimal fungal growth and 0.5 /ml methylene blue dye to enhance the definition of zone edges. Inoculated plates were incubated at 35°C for 24 hours. Following incubation, zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) interpretive breakpoints (Ismail *et al.*, 2024; Hadi *et al.*, 2025b)

Statistical Analysis:

Results were analyzed using Statistical Package for Social Scientist (SPSS version 18.0) Significant difference among means of the groups was determined by chi test. Values were considered significant when $p<0.05$(Al-Ezzy, 2016, 2017; Al-khalidi *et al.*, 2020), correlations were determined by correlation coefficient (Hameed *et al.*, 2024; Hameed & Al-Ezzy, 2024).

Results:

Table (2) and figures (1&2) show the frequency of candida species isolated from cat owners according to morphological features on chrome agar, SDA, germ tube formation; Viteck 2 compact system. The total number of candida *spp.* isolated from oral cavity of cat owners was 10/119, (8.40 %). The total number of candida *spp.* isolated from oral cavity of cat was 22/119, (16.67 %).

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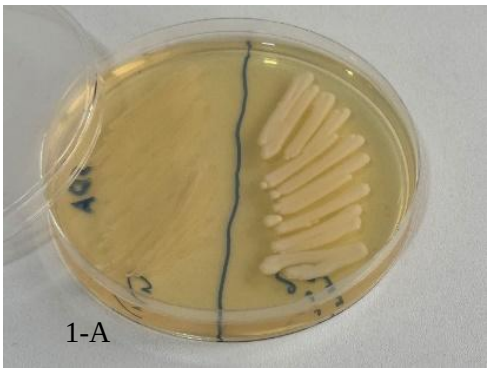
Table (2): Morphological Identification of candida Species Isolated from oral cavity of owners and **cats**

Source of sample	Isolation status on SDA and chrome agar	Total No. of swabs
Oral cavities of cat owners	No growth	109(91.6%)
	Candida albicans	4 (3.4%)
	C. glabrata	6(5%)
	Total	119(100%)
Oral cavities of cats	No growth	97(81.5%)
	Candida albicans	11(9.2%)
	C. glabrata	11(9.2%)
	Total	119(100%)



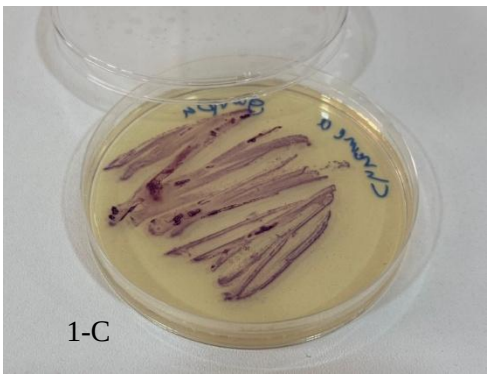


Figure (1): **A)** Macroscopic appearance of candida spp. swabbed from oral cavity of cat at 37c on Sabouraud s dextrose agar for 48 hours
B) Macroscopic appearance of candida swabbed from oral cavity of cat owner at 37c on Sabouraud's dextrose agar for 48 hours
C): Macroscopic appearance of candida swabbed from oral cavity of cat owner at 37c on chrome agar for 48 hours *Candida glabrata* usually appears as mauve (pinkish purple) colonies

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Molecular Identification Of *candida spp.* Isolated from Cats and owners by Conventional PCR

All Candida *positive* samples were subjected to confirmatory step by conventional PCR using specific primer pairs for Candida. Cand primers were used to confirm the detection of candida albicans (850 bp) and *C.glabrata* 1000bp, as shown in figure (3).

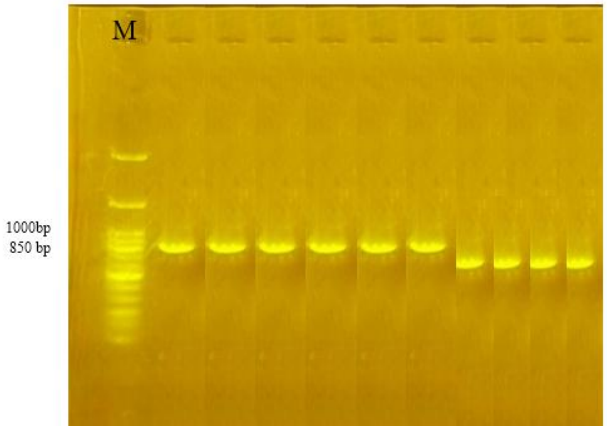

Figure (3): DNA products of *candida* generated through Cand primers

DNA products of *candida albicans* generated through Cand primers, stained with Ethidium bromide. M: Molecular marker (100bp); lane 2 (850bp), *candida albicans;* lane 1 (1000bp), *candida glabrata*

Oral Diseases and Isolation of *Candida* from Oral cavity of cat owners

As presented in Table 3, most cat owners (104/119; 87.39%) were free of oral lessons. Among this asymptomatic cohort, *Candida* species were isolated from a small subset, with *C. albicans* identified in 3 individuals (2.52%) and *C. glabrata* in 2 individuals (1.68%). In contrast, among the cat owners presenting with stomatitis (3/119; 2.52%), *Candida* species were also isolated. Specifically, *C. albicans* recovered from 1 individual (0.84%) and *C. glabrata* from 2 individuals (1.68%) within this group.

A statistically significant difference was observed in the prevalence of *Candida* isolation between individuals with and without oral lesions ($p = 0.000$). Furthermore, a significant inverse correlation was identified between the presence of oral lesions and the isolation of *Candida* species from the oral cavity of domestic cat owners ($p = 0.000$).

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. **Table (3):** Oral Diseases Associated with *candida* Infection among Domestic cat owners

DISEASES	CANDIDA SPECIES ISOLATED FROM ORAL CAVITY OF CAT OWNERS				
	No growth	***C. albicans***	***C. glabrata***	**Total positive candida spp. isolates**	**Total**
NO ORAL LESION	99(83.19%)	3(2.52%)	2(1.68%)	5(4.20%)	104(87.39%)
GLOSSITIS	2(1.68%)	0(0%)	0(0%)	0(0%)	2(1.68%)
SOFT TISSUE TRAUMA	1(0.84%)	0(0%)	0(0%)	0(0%)	1(0.84%)
CHEEK BITING	3(2.52%)	0(0%)	0(0%)	0(0%)	3(2.52%)
STOMATITIS	4(3.36%)	1(0.84%)	2(1.68%)	3(2.52%)	7(5.88%)
ORAL THRUSH AND STOMATITIS	0(0%)	0(0%)	2(1.68%)	2(1.68%)	2(1.68%)
TOTAL	109(91.59%)	4(3.36 %)	6(5.04%)	10(8.40%)	119(100%)
X2			51.520		
P VALUE			0.000		
R			-0.495		
P VALUE			0.000		

Clinical Signs Associated with *candida* Infection among Domestic cats

As shown in table (4), candida spp. was isolated frequently from cats presented with stomatitis 12/119, (10.08%) in which *C. glabrata* was isolated from 7/119, (5.88%) versus 5/119, **(7.56%)** for *C. albicans.* candida spp. was isolated at the second level from cats presented with glossitis 4/119, (3.36%) in which *C. glabrata* was isolated from 1/119, (0.84%) versus 3/119, (2.52%) for *C. albicans.* candida spp. was isolated at the third level from cats presented with stomatitis and thrush 3/119, (2.52%) in which only *C. glabrata* was isolated from 3/119, 3(2.52%).

Significant difference was reported among cats according to clinical presentation in isolation rate of candida (p value=0.000). Significant correlation was reported between clinical presentation and isolation of candida from oral cavity of cats (p value=0.000).

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Table (4): Correlation Between Clinical Signs and Isolation Of *candida* from oral cavity of domestic cats

Disease	**Candida species Isolated from oral cavity of cat**				Total
	C. glabrata	*C. albicans*	negative	**Total positive candida spp. isolates**	
no	0(0%)	0(0%)	93(78.15%)	0(0%)	93(78.15%)
glossitis	1(0.84%)	3(2.52%)	0(0%)	4(3.36%)	4(3.36%)
Soft Tissue Trauma	0(0%)	0(0%)	3(2.52%)	0(0%)	3(2.52%)
Cheek Biting	0(0%)	0(0%)	1(0.84%)	0(0%)	1(0.84%)
stomatitis	5(4.20%)	7(5.88%)	0(0%)	12(10.08%)	12(10.08%)
thrush	2(1.68%)	0(0%)	0(0%)	2(1.68%)	2(1.68%)
glossitis and thrush	0(0%)	1(0.84%)	0(0%)	1(0.84%)	1(0.84%)
stomatitis and thrush	3(2.52%)	0(0%)	0(0%)	3(2.52%)	3(2.52%)
Total	11(9.24%)	11(9.24%)	97(81.51%)	22(18.48%)	119(100%)
χ^2	158.667				
P value	0.000				
R	-0.875				
P value	0.000				

Antifungal activity against Candida Isolated from Oral cavities of Domestic cat owners

As shown in table (5), ten candida isolates were tested for susceptibility to commonly used anti-fungal agents. *C.albicans were* completely susceptible to clotrimazole and fluconazole (4/10,40%) with mean ± SE zone of inhibition (14.20± 5.79mm) for clotrimazole and (16.20 ± 6.61mm) for fluconazole. All isolates of *C. glabrata* were resistant to Clotrimazole and Fluconazole. All isolates of *C.albicans* and *C. glabrata* were resistant to Nystatin. A total of (20%) of *C.albicans* have intermediate susceptibility to Amphotericin with zone of inhibition range from (12-16mm). On the other hand, (20%) of *C.albicans* have resistant to Amphotericin with zone of inhibition (<12 mm) .

Antifungal Activity Against Candida Isolated from Oral Cavity of Cats

As shown in table (6), and figure (5), 22 candida isolates were tested for susceptibility to commonly used anti-fungal agents. *C.albicans* was completely susceptible to clotrimazole and fluconazole (11/11,100%) with mean ± SE zone of inhibition (17.09± 3.73 mm) for clotrimazole and (19.22 ± 4.19mm) for fluconazole. All isolates of *C. glabrata* were resistant to Clotrimazole and Fluconazole. All isolates of *C.albicans* and *C. glabrata* were resistant to Nystatin. All isolates of *C.albicans* and *C. glabrata* were resistant to Amphotericin with zone of inhibition (<12 mm).

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Table (5): Sensitivity of A. niger Isolated from Oral cavity Of Cat Owners to Antifungal agents

Antifungal agents	Minimum zone of inhibition (mm)	Maximum zone of inhibition (mm)	Mean ± SE zone of inhibition (mm)	Sensitivity of *C. albicans* isolates			Sensitivity of *C. glabrata* isolates		
				Susceptible ≥18mm	Intermediate 14-18mm	Resistant <14 mm	Susceptible ≥18mm	Intermediate 14-18mm	Resistant <14 mm
Clotrimazole(10mcg)	0	36	14.20± 5.79	4/10, (40%)	0/10, (0%)	0/10,(0%)	0/10,(0%)	0/10, (0%)	6/10, (60%)
Fluconazole(10mcg)	0	41	16.20 ± 6.61	4/10, (40%)	0/10, (0%)	0/10,(0%)	0/10,(0%)	0/10, (0%)	6/10, (60%)
Nystatin(100IU)	0	0	0.00 ± 0.00	0/10,(0%)	0/10,(0%)	4/10, (40%)	0/10,(0%)	0/10,(0%)	6/10, (60%)
Antifungal agents	**Minimum zone of inhibition (mm)**	**Maximum zone of inhibition (mm)**	**Mean ± SE zone of inhibition (mm)**	**Sensitivity of C. albicans isolates**			**Sensitivity of *C. glabrata* isolates**		
				Susceptible ≥16mm	**Intermediate 12-16mm**	**Resistant <12 mm**	**Susceptible ≥16mm**	**Intermediate 12-16mm**	**Resistant <12 mm**
Amphotericin(100µg)	0	12	4.40 ± 1.80	0/10,(0%)	2/10, (20%)	2/10, (20%)	0/10,(0%)	0/10,(0%)	6/10, (60%)

Table (6): Sensitivity of candida Isolated from Oral cavity Of Cats to Antifungal agents

Antifungal agents	Minimum zone of inhibition (mm)	Maximum zone of inhibition (mm)	Mean ± SE zone of inhibition (mm)	Sensitivity of C. albicans isolates			Sensitivity of *C. glabrata* isolates		
				Susceptible ≥18mm	Intermediate 14-18mm	Resistant <14 mm	Susceptible ≥18mm	Intermediate 14-18mm	Resistant <14 mm
Clotrimazole	0	35	17.09± 3.73	11/11, (100%)	0/11, (0%)	0/11,(0%)	0/11, (0%)	0/11, (0%)	11/11, (100%)
Fluconazole	0	39	19.22 ± 4.19	11/11, (100%)	0/11, (0%)	0/11,(0%)	0/11, (0%)	0/11, (0%)	11/11, (100%)
Nystatin	0	0	0.00 ± 0.00	0/11,(0%)	0/11,(0%)	11/11, (100%)	0/11, (0%)	0/11, (0%)	11/11, (100%)
Antifungal agents	**Minimum zone of inhibition (mm)**	**Maximum zone of inhibition (mm)**	**Mean ± SE zone of inhibition (mm)**	**Sensitivity of C. albicans isolates**			**Sensitivity of *C. glabrata* isolates**		
				Susceptible ≥16mm	**Intermediate 12-16mm**	**Resistant <12 mm**	**Susceptible ≥16mm**	**Intermediate 12-16mm**	**Resistant <12 mm**
Amphotericin	0	11	5.13 ± 1.12	0/11,(0%)	0/11,(0%)	11/11, (100%)	0/11, (0%)	0/11, (0%)	11/11, (100%)

Discussion:

In the current study, the total number of candida spp. isolated from oral swabs of cat owners was 10/119, (8.40 %)which considered relatively low compared with (Logien, 2022), stated that *C.albicans* represent (68%) and *C.glabrata* (2%) of total fungal isolates from oral cavity of patients in Benghazi, Libya .On the other hand , (Khadim & Alfayyadh, 2025) reported that *C.albicans* was isolated from 30% of oral swabs from human cases with periodontal issues. On the other hand (Alizadeh *et al.*, 2021) stated that candida spp. was isolated from oral cavity of (24.2%) of adults in which *C.albicans* represent 58.2% of total isolates where was *C. glabrata* represent (19.8%), while (Al-Qaysi & AL-Rubaie, 2024) reported that candida was isolated from (56.86%) of oral swabs. Previous reports and studies reported that nearly 10% of the common species in the oral

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cavity behave as opportunistic yeast pathogens, and cause infections and diseases like oral candidiasis (Sardi *et al.*, 2013; Pinto-Almazán *et al.*, 2022).

Variation of candida recovery from oral cavity is a consistent and controversial results were obtained as reported in a study conducted by (Mun *et al.*, 2016),the oral yeast carriage rate among clinically healthy adults with no mucosal lesions was found to be 48.3%. This prevalence did not differ significantly from that observed in a demographically matched group of individuals wearing removable dental prostheses.

In current study, the frequency of *Candida* species isolated from oral swabs of cats, as identified by colonial and microscopic morphological characteristics on SDA and CHROM agar™ Candida, was 16.67% (22 out of 119 samples),which is lower than that reported by (Nikaein *et al.*, 2023),stated that yeast was isolated from (9/60),15% oral swabs and candida spp. was isolated from (5/9,55.1%) oral swabs of cats. Potential explanations for this observation may include the superior innate antifungal activity of feline saliva, variations in environmental exposure to fungal sources, and/or distinctions in the composition and inhibitory activity of the concomitant bacterial microbiome. Alternatively, methodological discrepancies in sampling techniques or culture conditions may account for the reported differences(Nikaein *et al.*, 2023) .In contrary to the current result,(Krumbeck *et al.*, 2021),stated that candida was not isolated from oral cavity of cats with gingivostomatitis. On the other hand, the study conducted in Turkey by (Hizlisoy *et al.*, 2025) came close to the results of the current study, which reported that candida albicans was isolated from oral cavity of cats and dogs (9.6%) attended to outpatient clinic of Erciyes University, Turky . The observed discrepancies in results may be attributed to variations in isolation and identification methodologies, as well as host-related factors including dietary habits, environmental conditions, and animal husbandry practices.

The comparatively lower prevalence of fungal species within the oral cavity of cats may be attributed to both the qualitative and quantitative properties of their salivary secretions. Specific salivary components, such as lysozyme and histidine, have demonstrated inhibitory effects against various microorganisms (Altin *et al.*, 2021). Furthermore, increased salivary flow may reduce microbial adhesion and colonization through mechanical clearance (Zdanavičienė *et al.*, 2017; Nikaein *et al.*, 2023). Nevertheless, current understanding of the feline oral microbiome remains limited, underscoring the need for further molecular investigations to better elucidate these mechanisms(Nikaein *et al.*, 2023).

In the present study, most cat owners (87.39%) were asymptomatic and presented with no clinical oral lesions. Among this group, *Candida* species were isolated in a subset of individuals, with *C. albicans* detected in 2.52% and *C. glabrata* in 1.68% of asymptomatic participants. Among cat owners presenting with stomatitis (2.52%), *Candida* species were also identified. Within this symptomatic group, *C. albicans* was isolated from 0.84% and *C. glabrata* from 1.68% of individuals. This result come in agreement with (Tata *et al.*, 2019) ,stated that *C.albicans* was isolated from

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gingival and dental surfaces from Thai patients presented with denture associated stomatitis(10.52%) and gingivitis (3.28% On the other hand *C.glabrata* was isolated from patents presented with denture associated stomatitis(2.63%) and gingivitis(0.65%).

In current study, A statistically significant difference was demonstrated between the presence of oral lesions and the isolation of *Candida* spp. (p = 0.000). Furthermore, a significant inverse correlation was observed between oral lesions and the recovery of *Candida* species from the oral cavity of cat owners (p = 0.000).

These results come in accordance with that reported by (Akram *et al.*, 2018; Alrabiah *et al.*, 2019; Mokeem *et al.*, 2019) stated that several *Candida spp.* form part of the commensal oral microbiota and are frequently isolated from the oral cavities of healthy individuals *Candida albicans* is the most prevalent species colonizing this environment(Akram *et al.*, 2018). Although these yeasts typically exist in a dormant state under normal physiological conditions, they can become opportunistic pathogens under conducive circumstances, leading to infections such as oral candidiasis (thrush) (Javed *et al.*, 2009; Fukatsu *et al.*, 2022). A clinical study indicated that the subgingival biofilm serves as a significant reservoir for enhanced *Candida* colonization (Matic Petrovic *et al.*, 2019). In vulnerable populations—particularly those with inadequate oral hygiene—proliferation of oral *Candida* may contribute to the advancement of periodontal conditions, including chronic periodontitis (Canabarro *et al.*, 2013).

Clinical Signs Associated with candida Infection among domestic cats

In current study, *Candida* species were most frequently isolated from cats presenting with stomatitis, with a prevalence of 10.08%. Among these, *C. glabrata* was identified in 5.88% of cases, while *C. albicans* was isolated in 7.56%. At the second level of frequency, *Candida* species were isolated from cats with glossitis at a rate of 3.36%, with *C. glabrata* and *C. albicans* detected in 0.84% and 2.52% of cases, respectively. At the third level, *Candida* species were isolated from cats presenting with both stomatitis and thrush at a rate of 2.52%, with all isolates identified as *C. glabrata*.

A statistically significant difference was observed in *Candida* isolation rates among cats with different clinical presentations (stomatitis, glossitis, stomatitis and thrush). Additionally, a significant inverse correlation was found between the type of clinical presentation and the recovery of *Candida* species from the feline oral cavity.

The **feline oral microbiome** comprises a diverse community of bacteria, fungi, and viruses that exist in a delicate equilibrium. Recent studies have demonstrated that approximately 15% of healthy cats harbor cultivable yeast in their oral cavities, with Candida species representing the most prevalent genus (55.5% of isolates) (Nikaein *et al.*, 2023). This baseline colonization occurs without clinical signs in immunocompetent hosts, suggesting that **commensal colonization** is typical rather than exceptional. However, under conditions of immune dysregulation or mucosal compromise,

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these typically commensal organisms can proliferate and contribute to pathological processes (Hizlisoy *et al.*, 2025; Shaw *et al.*, 2025) .

Clinical presentations of oral disease in cats vary considerably in their manifestation and severity. Stomatitis (generalized oral inflammation), glossitis (tongue inflammation), and oral thrush (pseudomembranous candidiasis) represent distinct clinical entities that may reflect different underlying pathophysiological processes. The observed inverse correlation between clinical severity and Candida isolation rates challenges conventional assumptions about the relationship between fungal burden and disease manifestation, suggesting more complex host-pathogen interactions than previously recognized (Hizlisoy *et al.*, 2025; Shaw *et al.*, 2025) .

The differential isolation rates of Candida species across various clinical presentations reveal intriguing patterns that may reflect distinct pathogenic **mechanisms. In** current study, cats with stomatitis demonstrated the highest prevalence of Candida isolation (10.08%), with *C. glabrata* being more frequently isolated (5.88%) than *C. albicans* (7.56%). This predominance of non-albicans Candida species in stomatitis cases is particularly noteworthy, as it diverges from patterns observed in human oral candidiasis where *C. albicans* typically predominates (Dolgun *et al.*, 2025; Hizlisoy *et al.*, 2025).

Cats presenting with glossitis showed intermediate Candida isolation rates (3.36%), but with a reversal of the species distribution observed in stomatitis cases – *C. albicans* (2.52%) was more prevalent than *C. glabrata* (0.84%). This differential species distribution suggests that specific Candida species may exhibit tissue tropisms or varying pathogenic mechanisms in different oral locations(Shaw *et al.*, 2025). The anatomical and physiological differences between the oral mucosa lining the palatoglossal folds (typically affected in stomatitis) and the specialized epithelium of the tongue may create distinct microenvironments that favor colonization by different Candida species (Dosenberry *et al.*, 2025).

Most surprisingly, cats with the most severe clinical presentation – concurrent stomatitis and thrush – demonstrated the lowest Candida isolation rates (2.52%), with all isolates identified as *C. glabrata*. This inverse correlation between clinical severity and fungal recovery challenges conventional clinical intuition and suggests that the most severe inflammatory presentations may create conditions less favorable for Candida colonization or detection(Hizlisoy *et al.*, 2025; Shaw *et al.*, 2025).

The statistically significant difference ($p = 0.000$) in isolation rates across these clinical presentations indicates that the relationship between Candida colonization and oral inflammation is not straightforward. The inverse correlation between clinical severity and recovery of Candida species further suggests that factors beyond simple fungal proliferation are involved in disease pathogenesis (Hizlisoy *et al.*, 2025; Shaw *et al.*, 2025).

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Potential Mechanisms for Inverse Correlation

Inflammation-Mediated Suppression

The **local inflammatory response** associated with severe oral lesions may create an environment hostile to Candida colonization and survival. Activated mucosal immunity in conditions like stomatitis involves increased production of antimicrobial peptides (e.g., defensins, histatins), enhanced phagocytic activity, and elevated inflammatory cytokines – all of which can suppress fungal growth (Dosenberry *et al.*, 2025). This phenomenon represents a paradox where the inflammatory process intended to clear pathogens may simultaneously reduce detectable colonization while contributing to tissue damage(Shaw *et al.*, 2025) .

The composition of inflammatory infiltrates may further influence Candida detection. Conditions characterized by neutrophilic infiltration more effectively clear fungal elements through phagocytosis, resulting in lower culture positivity despite significant inflammation. In contrast, conditions with predominantly lymphocytic or plasmacytic infiltrates (as often seen in chronic stomatitis) may be less efficient at fungal clearance but still create an inflammatory environment that limits fungal proliferation(Dosenberry *et al.*, 2025; Shaw *et al.*, 2025).

The **oral microbiome** represents a complex ecological network where different microorganisms compete for resources and space. In inflammatory conditions like stomatitis, shifts in bacterial communities may create competitive pressures that affect Candida survival. Studies of feline chronic gingivostomatitis (FCGS) have demonstrated significant alterations in both bacterial and fungal components of the oral microbiome during inflammatory states(Shaw *et al.*, 2025) .

Recent metatranscriptomic analyses have revealed that inflammatory environments favor the growth of certain bacterial species that may competitively inhibit Candida through various mechanisms including nutrient competition, production of inhibitory compounds, or modulation of the local environment(Shaw *et al.*, 2025). The observed inverse correlation might thus reflect successful bacterial dominance in severely inflamed oral cavities, with Candida being outcompeted despite the presence of clinical signs suggesting fungal infection.

Medication history represents a crucial confounding factor when interpreting Candida isolation patterns. Cats with more severe clinical presentations often receive more aggressive therapeutic interventions, including antibiotics and corticosteroids, which can significantly alter oral microbiota composition and fungal detection rates(Nikaein *et al.*, 2023; Shaw *et al.*, 2025) .

Antibiotic therapy reduces bacterial competition, potentially facilitating Candida overgrowth. However, prolonged antibiotic exposure might also select for antibiotic-resistant bacterial strains that competitively inhibit Candida through mechanisms unrelated to antibiotic susceptibility. **Corticosteroid administration** suppresses inflammatory responses, which might reduce inflammation-mediated suppression of Candida while simultaneously masking clinical signs, creating complex interactions that complicate interpretation of culture results (Dosenberry *et al.*, 2025; Shaw *et al.*, 2025).

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Comorbidities such as feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infections significantly influence oral immunity and Candida dynamics. These viral infections alter lymphocyte function and mucosal immunity, potentially creating environments conducive to Candida proliferation despite minimal inflammation, or alternatively, permitting uncontrolled fungal growth with severe inflammation(Dosenberry *et al.*, 2025; Hizlisoy *et al.*, 2025) .

Diabetes mellitus and other metabolic disorders that impair immune function may similarly affect the relationship between clinical presentations and Candida detection. The complex interplay between systemic immunity, local inflammation, and fungal proliferation creates multivariate relationships that cannot be captured by simple correlation analyses (Dosenberry *et al.*, 2025).

Antifungal activity against Candida Isolated from Oral cavities of Domestic cat owners

In the present study, ten *Candida* isolates were evaluated for susceptibility to commonly used antifungal agents. All isolates of *C. albicans* (4/10, 40%) demonstrated complete susceptibility to clotrimazole and fluconazole. These results come in contrary with that reported by (Esmael & Shekhany, 2023),stated that *C. albicans* was sensitive to clotrimazole and fluconazole ,58.6%, and 24.0% respectively ,while resistant were reported in 42.6%, and 10.6% to clotrimazole and fluconazole respectively .

In contrast, in current study all isolates of *C. glabrata* exhibited resistance to both clotrimazole and fluconazole. Universal resistance to nystatin was observed across all isolates of both *C. albicans* and *C. glabrata* which come in contrary with that reported by (Esmael & Shekhany, 2023) stated that 23.9% of *C.albicans* was sensitive to nystatin .On the other hand ,(Salman & Ahmed, 2022)recorded that Nystatin demonstrated efficacy in antifungal susceptibility testing, with 56.7% of candida isolates classified as sensitive . In contrast, 41.7% of candida isolates exhibited resistance, while 1.7% showed intermediate susceptibility.

Nystatin exerts its antifungal effect primarily through disruption of the fungal cytoplasmic membrane via binding to ergosterol, a key sterol component of fungal cells. This interaction leads to the formation of transmembrane channels, resulting in the leakage of intracellular ions, particularly potassium and magnesium, and other essential cellular constituents. The consequent disruption of electrochemical gradients across the membrane impairs cellular homeostasis and ultimately leads to fungal cell death.

Nystatin demonstrates high affinity for ergosterol and comparatively low binding to human cholesterol derivatives, such as 3-hydroxy or oxysterols, contributing to its selective toxicity against fungal cells. However, this mechanism also limits its clinical indications compared to broader-spectrum azole antifungals. Although the emergence of nystatin-resistant strains remains relatively uncommon and most fungal species are considered susceptible, its use is often reserved for topical applications due to systemic toxicity concerns. Nevertheless, nystatin remains a viable treatment option for Candida infections, particularly in cases of localized mucosal candidiasis(Sariguzel *et al.*, 2015; Murtiastutik & Maharani, 2019).

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In current study, amphotericin B, 20% of *C. albicans* isolates showed intermediate susceptibility, while an additional 20% were resistant which indicates a significant proportion of isolates are not fully susceptible to this antifungal, a phenomenon with increasing reports. This suggests a potential for antifungal treatment failure and highlights the importance of antifungal susceptibility testing to guide therapy, especially since resistance can develop during treatment.

The antifungal susceptibility profiles observed in this study align with findings reported by (Mohammed *et al.*, 2017; Salman & Ahmed, 2022) ,wherein Candida albicans isolates exhibited the highest susceptibility to amphotericin B, followed by ketoconazole, fluconazole, and clotrimazole, respectively. Correspondingly, (Salman & Ahmed, 2022) reported an elevated resistance against ketoconazole, with comparatively lower resistance to fluconazole and clotrimazole, whereas minimal resistance was observed against amphotericin B.

In a study conducted in Diyala province(Salman & Ahmed, 2022) recorded that C. albicans demonstrated moderate to high susceptibility to clotrimazole, fluconazole, and amphotericin B, but reduced susceptibility to ketoconazole.

Antifungal Activity Against Candida Isolated from Oral cavity Of Cats

In current study, all C. albicans isolates from cats, demonstrated complete susceptibility to both clotrimazole and fluconazole. In contrast, all *C. glabrata* isolates exhibited resistance to both clotrimazole and fluconazole This difference is explained by variations in the organisms' mechanisms for developing antifungal resistance.

Azole drugs (e.g., fluconazole) are popular due to their oral bioavailability and safety profile. However, their efficacy against *C. glabrata* is notoriously unreliable.

Resistance is predominantly caused by the overexpression of drug efflux pumps, specifically the ABC transporters(Lee et al., 2023). Upregulation of efflux pump genes encoding ATP-Binding Cassette transporters (e.g., CDR1) and major facilitator superfamily transporters (e.g., MDR1) are overexpressed, actively pumping azoles out of the fungal cell. On the other hand ,Overexpression of efflux pumps is primarily regulated by gain-of-function (GoF) mutations in the transcription factor CgPDR1(Castanheira et al., 2022). The genomic character of *C.glabrata* plays a role in development of resistance, in which, *C.glabrata* is a haploid organism, so resistance can be conferred by a mutation in a single allele. This allows for faster development of antifungal drug resistance. Last proposed mechanism ,*C.glabrata* appears to use a "stealth" strategy to evade the host immune response and cause less tissue damage, which may be linked to its drug resistance and persistence .(Gull, 2025)

The result of current study come in accordance with that reported by (Rodrigues et al., 2014) ,Stated that *C.glabrata* has emerged as the second most prevalent causative agent of both mucosal and systemic candidiasis, following only *C. albicans* in clinical significance . *C. glabrata* has its intrinsic low susceptibility to azoles, especially fluconazole (Hassan *et al.*, 2021).In general, this is because azoles are the first prophylactic choice against fungal infections due to their low cost, and

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the second choice for invasive infections produced by different *Candida* species, generating cross-resistance to the other azoles (Allen *et al.*, 2015; Frías-De-León *et al.*, 2021) .

Currently accounting for 15–20% of all documented Candida infections, the relative incidence of *C. glabrata* continues to rise annually (Costa et al., 2016). This trend is largely attributable to the widespread and often prolonged use of antifungal agents for both therapeutic and prophylactic purposes, which has driven the selection of intrinsically resistant fungal pathogens. The clinical challenge posed by these infections is compounded by their substantial mortality rates, which are primarily linked to the remarkable capacity of these yeasts to rapidly develop multidrug resistance mechanisms (Rezaei et al., 2009; Costa et al., 2016)

As stated by (Fothergill *et al.*, 2014),Azoles inhibit 14-α-sterol demethylase, encoded by the *ERG11* gene, which is an enzyme involved in the biosynthesis of the fungal-specific membrane sterol ergosterol. As some non *C albicans* species exhibit intrinsic resistance to azoles, their use is likely a contributing factor to the more frequent incidence of infections caused by these NAC species (Lortholary *et al.*, 2011) Moreover, many studies have documented the ability of *Candida* to develop high-level resistance to azole antifungals.

In current study, Universal resistance to Nystatin and amphotericin B were observed across all isolates of both C. albicans and C. glabrata.

According to the current results, the observation of universal resistance to Nystatin and amphotericin B in *C. albicans* and *C. glabrata* from cat oral cavities is a highly unusual finding, as other studies report susceptibility to these drugs, particularly amphotericin B, in both *C. albicans* and *C. glabrata* isolates from various sources, including oral cavities and other clinical samples(Cai *et al.*, 2020; Frías-De-León *et al.*, 2021). While resistance to Nystatin and amphotericin B is possible in some Candida species or under specific conditions like systemic comorbidities , it is not a universal trait for these species in general, according to the provided context(Savastano *et al.*, 2016; Ghojoghi *et al.*, 2024).

The most important polyenes commonly used in the treatment of candidiasis are amphotericin B and nystatin. The most important agent, as far as development of resistance is concerned, is amphotericin B. Polyenes act by causing disruption of fungal cytoplasmic membrane, i.e. by interacting with ergosterol – an important component of fungal cell membrane, essential for maintaining fluidity and integrity of the membrane as well as for proper functioning of the membrane – bound enzymes. Amphotericin B intercalates into the membrane and generates channels and pores, through which many cellular components, particularly potassium and magnesium ions, come out and destroy the proton gradient within the membrane and cause death of the fungal cell (Morace *et al.*, 2014).

Several quantitative changes in ergosterol content of Candida spp. that contribute to development of resistance to polyenes including amphotericin B and nystatin include: Decrease in the

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content of ergosterol because of inhibition of its synthesis ;alteration of sterol content, i.e. replacement of ergosterol with sterols with reduced affinity and alterations in the ratio of sterol to phospholipids (Arikan & Rex, 2010).

Several qualitative changes in ergosterol of candida spp. that lead to development of resistance to polyenes including amphotericin B and nystatin include reorientation or masking of ergosterol in the cell membrane because of which there is no binding with polyenes (Hoenigl *et al.*, 2024); Changes in cell wall permeability to polyenes ; increased catalase activity, which diminishes oxidative damage caused by amphotericin B and nystatin (Farajzadeh *et al.*, 2011).

Conclusions:

In conclusion, this study reveals a significant presence of *Candida* spp., specifically *C. albicans* and *C. glabrata*, in the oral mucosa of domestic cats and their owners. A critical finding is the pan-resistant profile of all *C. glabrata* isolates to common antifungal agents, including azoles and polyenes. Furthermore, the complete resistance of all isolates to Nystatin and the emerging resistance to Amphotericin B highlight a serious therapeutic challenge. The inverse correlation between clinical lesions and yeast isolation suggests a complex host-pathogen interaction. These results underscore the potential for cross-species transmission of resistant strains and emphasize the need for antifungal stewardship in veterinary and human medicine.

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