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Experimental murine model of intra-abdominal infections caused by some non-albicans *Candida* species

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Abstract:

Background: Even though intra-abdominal candidiasis (IAC) has been increasingly recognized, with associated high morbidity and mortality rates, its pathogenesis remains poorly understood. This model aims to study the pathogenicity and *invivo* susceptibility of non-*albicans Candida* species associated with IAC in human in order to predict the frequency of infections, outcome of clinical disease and response to antifungal therapy.
Methodology: Both immunosuppressed and immunocompetent female CD-1 mice were challenged intraperitoneally with 5 x 10^8 CFU/ml inoculum of five non-*albicans Candida* strains; *Candida glabrata*, *Candida parapsilosis*, *Candida lipolytica*, *Candida tropicalis* and *Candida guilliermondii*. Mice were closely observed for symptoms. Treated groups received voriconazole (40 mg/kg/day) or micafungin (10 mg/kg/day) 24 hours after infection depending on *invitro* susceptibility results. Survival rate, mean survival time and fungal tissue burdens were recorded for all groups.
Results: All infected groups developed hepatosplenomegaly, peritonitis and multiple abscesses on intra-abdominal organs and mesenteries. *C. glabrata* and *C. lipolytica* represented the most and the least virulent strains respectively in terms of survival rate, mean survival time and fungal burden in both immunosuppressed and immunocompetent models. Following treatment, all immunocompetent animals survived the entire duration of experiments (0% mortality rate), while mortality rate was relatively high (20-60%) in immunosuppressed mice. Treatment failed to eradicate the infection in immunosuppressed mice despite significant decrease of the fungal burden and increase mean survival time.
Conclusion: This study reports an increasing pathogenicity of non-*albicans Candida* species, with persistent infection among immunosuppressed animals.

Keywords: Intra-abdominal, Candidiasis, non-*albicans*, *invivo*, mice.

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Modèle murin expérimental d'infections intra-abdominales causées par certaines espèces de *Candida* non albicans

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Abstrait:

Contexte: Bien que la candidose intra-abdominale (CAI) soit de plus en plus reconnue, avec des taux de morbidité et de mortalité élevés associés, sa pathogenèse reste mal comprise. Ce modèle vise à étudier le pouvoir pathogène et la susceptibilité in vivo d'espèces de *Candida* non *albicans* associées à l'IAC chez l'homme afin de prédire la

fréquence des infections, l'évolution de la maladie clinique et la réponse au traitement antifongique.
Méthodologie: Des souris femelles CD-1 immunodéprimées et immunocompétentes ont été stimulées par voie intrapéritonéale avec un inoculum de 5 x 10^8 UFC / ml de cinq souches *Candida* non *albicans*; *Candida glabrata, Candida parapsilosis, Candida lipolytica, Candida tropicalis* et *Candida guilliermondii*. Les symptômes ont été observés de près chez les souris. Les groupes traités ont reçu du voriconazole (40 mg/kg/jour) ou de la micafungine (10 mg/kg /jour) 24 heures après l'infection, en fonction des résultats de sensibilité *invitro*. Le taux de survie, la durée de survie moyenne et la charge en tissu fongique ont été enregistrés pour tous les groupes.
Résultats: Tous les groupes infectés ont développé une hépatosplénomégalie, une péritonite et de multiples abcès aux organes intra-abdominaux et au mésentère. *C. glabrata* et *C. lipolytica* représentaient respectivement les souches les plus et les moins virulentes en termes de taux de survie, de durée de survie moyenne et de charge fongique dans les modèles immunodéprimés et immunocompétents. Après le traitement, tous les animaux immunocompétents ont survécu à toute la durée des expériences (taux de mortalité de 0%), tandis que le taux de mortalité était relativement élevé (20 à 60%) chez les souris immunodéprimées. Le traitement n'a pas réussi à éradiquer l'infection chez les souris immunodéprimées malgré une réduction significative de la charge fongique et une augmentation du temps de survie moyen.
Conclusion: cette étude rapporte une pathogénicité croissante des espèces de *Candida* non *albicans*, avec une infection persistante chez les animaux immunodéprimés.

Mots-clés: intra-abdominal, candidose, non *albicans*, *invivo*, souris.

Introduction:

Candida species are important fungal pathogens that can survive in different anatomical sites (1, 2). Invasive candidiasis is the most frequently encountered fungal disease among hospitalized patients. This includes candidaemia and intra-abdominal candidiasis (IAC), which manifests as peritonitis and intra-abdominal abscesses (3, 4). Despite the high frequency of IAC among critically ill patients, it remains understudied compared to candidemia (5-7). Over the last decades a progressive increase in the epidemiology of non-*albicans Candida* infections has been recorded (8-11). The expanding population of patients with severe illnesses or immunosuppression, abdominal surgeries, use of broad-spectrum antibiotics, intravenous catheters and parentheral nutrition are factors contributing to the changing epidemiology of these infections (11, 12).

The shift toward non-*albicans Candida* pathogens is accompanied with the emergence of resistance against commonly used antifungal agents rendering the treatment challenging especially in critically ill individuals (9-11, 13). IAC has been associated with poor outcomes in some patients (11, 14). Lack of adequate therapy and source control and severity of illness are the most important determinants of poor outcome (12). Research on IAC has several limitations including diagnosis difficulties due to the clinical heterogeneity of the disease (7, 14, 15). Moreover, treatment of this type of infection is challenging because it is usually based on previous case reports and experience gained from other forms of fungal disease (5). In addition, the emergence of strains resistant to the available antifungals has further complicated the treatment of IAC (16).

Animal models simulating IAC will provide a useful tool for understanding of disease pathogenicity, host response and evaluation of the therapeutic strategies in the absence of clinical trials (14,17). Since most animal models of candidiasis have been used for disseminated infections through intravenous route (3), the present study aims to provide a model simulating IAC caused by non-*albicans Candida* species in both immunocompetent and immunosuppressed mice via intraperitoneal introduction of pathogen and for *invivo* study of the pathogenicity and evaluation of treatment outcomes.

Material and Methods:

Test strains

Five non-*albicans Candida* strains were selected among isolates obtained from patients previously diagnosed with IAC at Ain Shams University Specialized Hospital (ASUSH), Cairo, Egypt. Isolates were identified using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry analysis (MALDI-TOF MS) (VITEK® MS, bioMérieux Inc., Marcy l' Etoile, France). The *invitro* susceptibility of the strains to fluconazole, voriconazole, caspofungin, micafungin, amphotericin B and flucytosine was determined using the Vitek2 system (bioMérieux Inc., Marcy l' Etoile, France). (This was part of a surveillance

Table 1: non-*albicans Candida* species used in this study

Candida species	Clinical origin	Type of IAI	Patient type	AUMC no.
C. glabrata	Peritoneal fluid	Secondary peritonitis	Liver transplantation	13949
C. parapsilosis	Peritoneal fluid	Secondary peritonitis	Appendectomy	13952
C. lipolytica	Peritoneal fluid	Secondary peritonitis	Liver transplantation	13950
C. tropicalis	Peritoneal fluid	Secondary peritonitis	CAPD	13948
C. guilliermondii	Bile	Cholecystitis/cholangitis	Liver transplantation	13951

IAI = Invasive Abdominal Infection; AUMC = Assiut University Mycological Centre; CAPD = Continous Ambulatory Peritoneal Dialysis

study on IAC previously presented as an e-poster in the 20th Congress of the International Society of Human and Animal Mycology, ISHAM, Amsterdam, 2018).

MALDI-TOF MS and Vitek2 assays were performed at The Children's Cancer Hospital Egypt 57357 (CCHE), Cairo, Egypt. All the isolates were deposited in the culture collection of Assiut University Mycological Centre (AUMC), Assiut University, Assiut, Egypt, and their numbers and identification are listed in Table 1.

Test animals

Pathogen-free female outbred CD-1 mice aged 4-5 weeks old (22-30 gm) purchased from The Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt; were used for the *invivo* studies. The test animals were maintained in the animal facility of The Research and Training Centre on Vectors of Diseases, Faculty of Science, Ain Shams University, Cairo, Egypt. They were housed in a non-stressful environment in cages of 5 mice each representing a group and left to acclimatize for one week before infection, with access to food and water *ad libitum*.

Inoculum preparation

The yeast strains were cultured on CandiSelect™ 4 chromogenic media (Bio-Rad, Marnes la Coquette, France) to ensure purity, and then cultured overnight in yeast extract-peptone dextrose broth (YPD) (1% yeast extract, 2% peptone, 2% D-glucose) with shaking at 30°C. Yeast cells were harvested by centrifugation, washed twice with phosphate buffered saline (PBS) and adjusted to the desired concentration of 5×10^8 CFU/ml (3) on a haemocytometer using PBS. Dilutions of the inocula were plated on SDA plates to ensure the correct inoculum was injected.

Infection assay

Each yeast strain was tested on both immunosuppressed and immunocompetent mice. Mice were immunosuppressed by administering cyclophosphamide intraperitoneally at a dose of 200 mg/kg for 3 consecutive days prior to infection. Both immunosuppressed and immunocompetent mice were then infected intraperitoneally. A control group was injected with PBS only. Challenged mice were closely observed daily for signs of the disease. Infected mice were sacrificed following administration of a dose of 200 mg/kg sodium pentobarbitone intraperitoneally when infected mice show severely reduced mobility, inability to reach food or water, weight reduction, hunched posture and fur ruffling.

Antifungal therapy

For all treatments, therapy began 24 hours after infection. According to Vitek2 results for each test strain, treatment was initiated with intraperitoneal dosage of 40 mg/kg of voriconazole (Vfend; VRC, Pfizer Inc., Egypt) once daily for 7 consecutive days. Micafungin (Mycamine; Astellas Toyama Co., Ltd. Japan) was administered intraperitoneally with a dose of 10 mg/kg/day for 6 consecutive days in a 0.2 ml volume. The doses were determined according to previous studies (18, 19). To prevent bacterial infections, mice received 5mg/kg/day ceftazidime subcutaneously.

Fungal burden and histopathology

For tissue burden, mice were sacrificed on day 3 post-infection and one day after treatment was completed in treated groups. The kidney, liver, spleen and pancreas were aseptically excised and washed with sterile PBS and homogenized in 5 ml sterile PBS on ice. The serially diluted homogenates were plated

onto SDA and incubated at 37°C. Colonies were counted to determine the colony-forming units (CFU) per gram tissue. Counts were expressed as log10 CFU per gram tissue. To assess the presence of peritonitis, the peritoneal cavity was washed with PBS, the effluent was collected, centrifuged and the pellet was re-suspended in 600 μl sterile water containing 0.01% bovine serum albumin. The undiluted suspension was plated on SDA plates and the CFU was counted (20).

For histopathological analysis, the liver, kidneys, spleen and pancreas were excised and immediately fixed in 10% neutral buffered formalin and embedded in paraffin. 5 μm sections were cut and stained with haematoxylin and eosin (H&E) for tissue morphology and Periodic Acid-Schiff (PAS) reagent to visualize fungal elements in tissue. Tissue sections were examined using light microscope (B-500T, Optika, Italy) and images were captured using a 3.1-megapixel eyepiece USB camera (C-B3 Optika, Italy) with Scope Image 9.0 software.

Statistical analysis

Data were analyzed using IBM® SPSS® Statistics for Windows, Version 25.0 (2017, Armonk, NY: IBM Corp.) A one-way analysis of variance (ANOVA test) was used when comparing more than two means. For multiple comparisons between different variables, Post Hoc tests: Tukey HSD was done and $p<0.05$ was considered significant.

Results:

Five non-*albicans Candida* species used in this study were *C. glabrata*, *C. parapsilosis*, *C. lipolytica*, *C. tropicalis* and *C. guilliermondii*. Survival studies revealed that *C. glabrata* and *C. tropicalis* caused the highest mortality in both immunosuppressed and immuno-competent models (Table 2).

Table 2: Mean survival time (days), survival range (days) and mortality rates (%) among different study groups

	C. glabrata	*C. parapsilosis*	*C. lipolytica*	*C. tropicalis*	*C. guilliermondii*
Immunosuppressed group					
Mean ± SD	7.20±2.17	7.60±1.34	32.6±16.7[a]	7.80±1.48	9.80±1.92
Range	(5-10)	(6-9)	(14-45)	(6-10)	(8-13)
Mortality %	100	100	40	100	100
Immunosuppressed-treated group					
Mean ± SD	29.00±14.68	32.60±16.99	40.00±11.18	39.60±12.07	33.00±16.45
Range	(16-45)	(13-45)	(20-45)	(18-45)	(14-45)
Mortality %	60	40	20	20	40
Immunocompetent group					
Mean ± SD	11.80±1.64[b]	25.8±17.54	45.00±0.00[c]	11.40±1.14[d]	32.00±17.80[e]
Range	(10-14)	(12-45)	45	(10-13)	(12-45)
Mortality %	100	60	0	100	40
Immunocompetent-treated group					
Mean ± SD	45.00±0.00[f]	45.00±0.00	45.00±0.00	45.00±0.00[g]	45.00±0.00
Range	45	45	45	45	45
Mortality %	0	0	0	0	0

a vs other immunosuppressed *Candida* groups, $p\leq0.001$.
b, d vs *C. lipolytica* and *C. guilliermondii* immunocompetent infected groups, $p<0.05$.
c, e vs *C. glabrata* and *C. guilliermondii* immunocompetent infected groups, $p<0.001$ and $p<0.05$ respectively.
f vs *C. glabrata* immunocompetent infected group, $p<0.001$.
g vs *C. tropicalis* immunocompetent infected group, $p<0.001$.

Candida parapsilosis and *Candida guilliermondii* caused 100% mortality rates among immunosuppressed mice but a relatively lower mortality rates among immunocompetent ones (60% and 40% respectively). *Candida lipolytica* caused the lowest mortality rates in both immuno-suppressed and immunocompetent groups. From the study of mean survival time, *C. lipolytica* infected immunosuppressed mice had significantly longer survival time (p<0.001) while there was no significant difference in the mean survival time among other *Candida* species. Among the immunocompetent groups, mice infected with *C. lipolytica* and *C. guilliermondii* had the highest mean survival time while those infected with *C. glabrata* and *C. tropicalis* had the least mean survival time (Fig. 1).

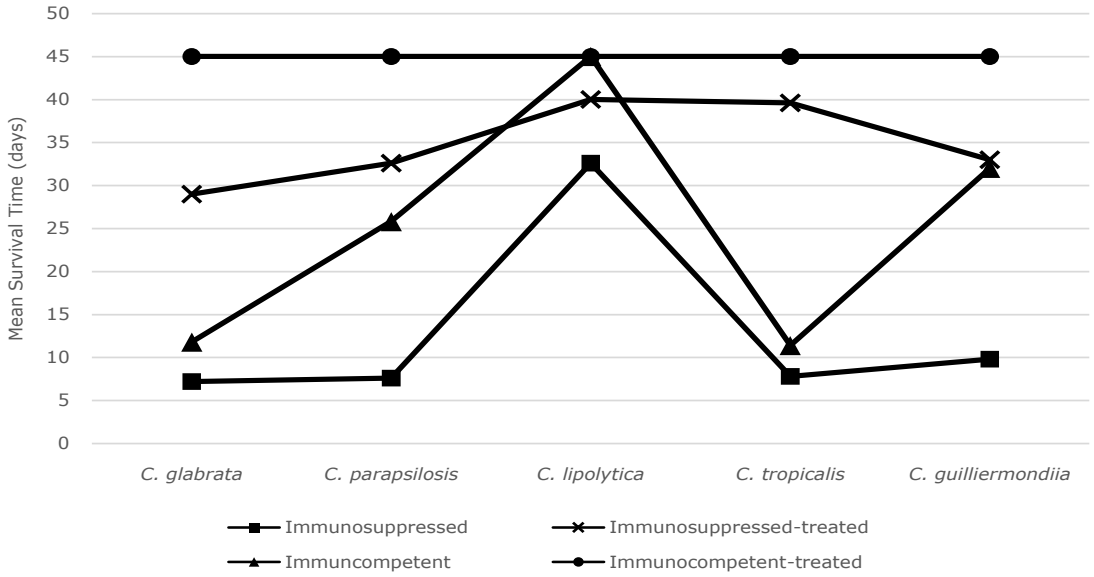

Fig. 1: Mean Survival Time of infected groups before and after treatment

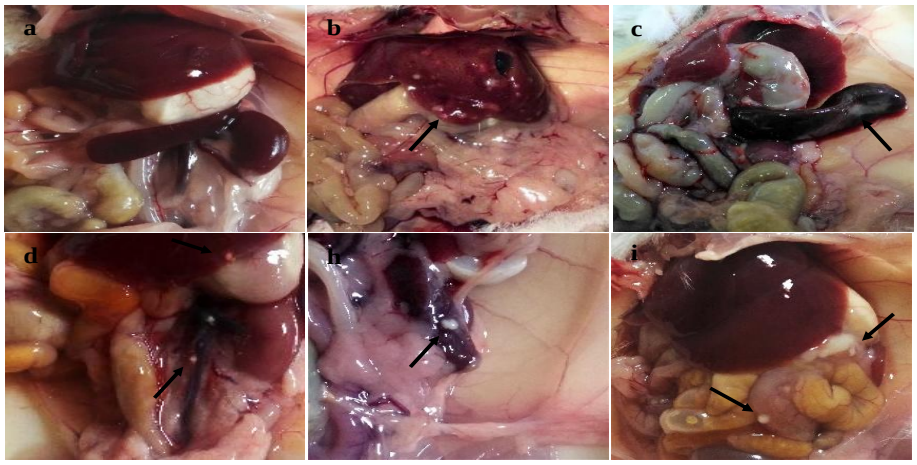

Fig. 2: Gross morphology of intra-abdominal organs of mice infected with *Candida* species. (a) normal control (b) multiple liver abscess (arrows) caused by *C. glabrata*, (c) severe spleen enlargement (arrows) due to *C. tropicalis* infection, (d) liver and mesenteric abscesses (arrows) caused by *C. parapsilosis* and (h) & (i) abscess formation (arrows) by *C. guilliermondii* and *C. lipolytica* respectively

On gross morphology of organs (Fig. 2), hepatosplenomegaly was the first notable sign in nearly all the infected immuno-suppressed mice in all *Candida* groups. Enlarged, mottled, and markedly congested liver with multiple abscesses of varying sizes were observed especially in *C. glabrata*. However, some immunocompetent mice showed macroscopically normal livers even though microscopic abnormalities were seen on histological examination. Significant splenic enlargement was observed in all groups, with some mice showing severe congestion of the spleen accompanied with abscesses and white patches. Kidneys were slightly enlarged with white patches in some infected animals and color change in others. Few pancreatic abscesses were observed only in immuno-suppressed animals infected with *C. tropicalis*. Abscesses were also observed on the peritoneum and mesenteric membranes.

Haematoxylin and Eosin (H&E) tissue preparations (Fig.3) revealed that kidneys from all infected groups exhibited variable pathological changes represented in glomerular and tubular damage with marked infiltration of lymphocytes and interstitial haemorrhage. Liver sections also showed disorganized hepatic parenchyma with congestion and dilation of blood sinusoids and inflammatory cells infiltration, and diffuse Kupffer cells was observed between sinusoids and around congested central veins. Spleen of infected mice showed extravasation of RBC's with multifocal granulomatous inflammation and abundant neutrophilic infiltration. Pancreatic tissue preparations from *C. tropicalis* infected immunosuppressed mice revealed scanty epithelial cells with mixed acute and chronic inflammatory infiltrates (Fig. 4).

PAS-stained tissue sections confirmed the presence of extensive fungal elements in all organs of both immunocompetent and immunosuppressed infected groups (Fig. 5).

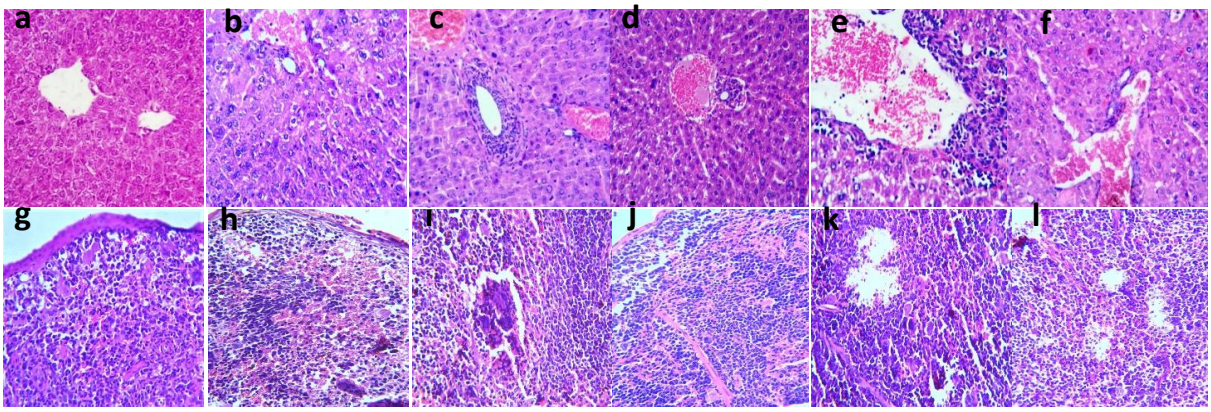

Fig. 3: Photomicrograph of the liver and spleen sections from different *Candida* groups. (a) Control mouse liver section, (g) Control spleen section, (b, h) liver and spleen sections from *C. glabrata* group, (c, i) liver and spleen sections from *C. parapsilosis* group, (d, j) liver and spleen sections from *C. lipolytica* group, (e, k) liver and spleen sections from *C. tropicalis* group, (f, l) liver and spleen sections from *C. guilliermondii* group (H&E 400x)

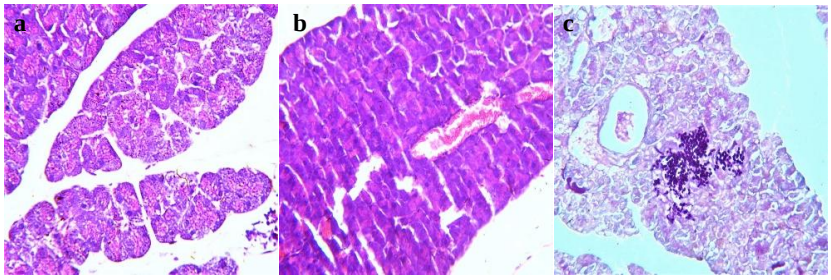

Fig. 4: photomicrograph of pancreatic tissue preparations, (a) control pancreas mouse section (H&E 400x), (b) pathological changes caused by *C. tropicalis* (H&E 400x), (c) fungal element s of *C. tropicalis* (PAS 400x)

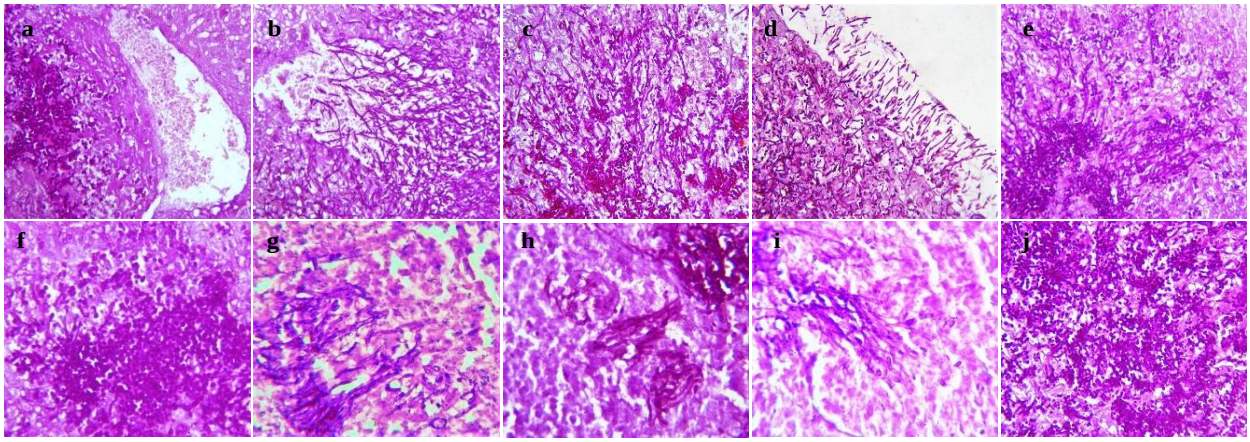

Fig. 5: Photomicrograph showing fungal elements in liver (a-e) and spleen (f-j) tissue preparations of infected mice. (a,f) *C. glabrata*, (b,g) *C. parapsilosis*, (c,h) *C. lipolytica*, (d,i) *C. tropicalis*, (e,j) *C. guilliermondii*. (PAS 400x)

Table 3: Fungal tissue burden of infected mice in all groups

Organ	Fungal Tissue Burden (Log_{10}CFU/gm)				
	C. glabrata	*C. parapsilosis*	*C. lipolytica*	*C. tropicalis*	*C. guilliermondii*
Immunosuppressed					
Kidney	6.60 ± 0.78^{a}	5.45 ± 0.17	3.86 ± 0.29^{d}	5.21 ± 0.45	5.19 ± 0.30
Liver	5.80 ± 0.67^{b}	4.94 ± 0.33	3.70 ± 0.47^{e}	4.48 ± 0.19	4.52 ± 0.37
Spleen	6.48 ± 0.69^{c}	5.22 ± 0.32	3.54 ± 0.27^{f}	5.14 ± 0.30	5.02 ± 0.36
Pancreas	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.09 ± 0.50^{h}	0.00 ± 0.00
Peritoneal wash	3.77 ± 0.35	3.60 ± 0 .40	2.53 ± 0.3^{g}	3.83 ± 0.12	3.53 ± 0.05
Immunosuppressed-treated ^{i}					
Kidney	2.19 ± 0.99	1.64 ± 0.32	1.43 ± 0.21	1.69 ± 0.49	1.74 ± 0.37
Liver	1.92 ± 0.83	1.33 ± 0.29	1.52 ± 0.10	1.43 ± 0.33	1.37 ± 0.31
Spleen	2.15 ± 1.15	1.38 ± 0.34	1.57 ± 0.36	1.55 ± 0.52	1.88 ± 0.47
Pancreas	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Peritoneal wash	1.27 ± 0.06	1.22 ± 0.24	1.14 ± 0. 06	1.07 ± 0.05	1.08 ± 0.63
Immunocompetent					
Kidney	4.97 ± 0.56^{j}	3.69 ± 0.19	2.26 ± 0.10^{n}	3.54 ± 0.35	3.72 ± 0.85
Liver	4.82 ± 0.15^{k}	3.53 ± 0.17	2.28 ± 0.12^{o}	3.20 ± 0.19	3.57 ± 0.26
Spleen	4.67 ± 0.08^{l}	3.63 ± 0.27	2.33 ± 0.07^{p}	3.65 ± 0.33	3.66 ± 0.10
Pancreas	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Peritoneal wash	3.86 ± 0.08^{m}	3.37 ± 0.06	2.16 ± 0.09^{q}	3.12 ± 0.14	3.41 ± 0.21
Immunocompetent-treated ^{r}					
Kidney	0.09 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.03 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pancreas	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Peritoneal wash	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

(a-c) vs other immunosuppressed groups; $p \leq 0.005$, $p < 0.05$, $p \leq 0.001$ respectively
(d-g) vs other immunosuppressed groups; $p \leq 0.001$, $p < 0.05$, $p < 0.001$ respectively
(h) vs other immunosuppressed groups; $p < 0.001$
(i) vs immunosuppressed groups; $p < 0.001$
(j-m) vs other immunocompetent groups; $p < 0.001$
(n-q) vs other immunocompetent groups; $p < 0.001$
(r) vs immunocompetent groups; $p < 0.001$

Fungal burden results among immunosuppressed mice (Table 3) showed that *C. glabrata* had significantly higher kidney, liver and spleen burdens than other *Candida* species (Fig. 6). *Candida parapsilosis*, *C. tropicalis and C. guilliermondii* had statistically similar fungal burden in the kidneys but higher *C. guilliermondii* spleen burdens.

All tested *Candida* species were capable of causing peritonitis with no significant difference between their peritoneal wash burdens. *C. lipolytica* showed the least fungal burden of all organs among immuno-suppressed mice. Of note, only *C. tropicalis* displayed pancreatic fungal burden.

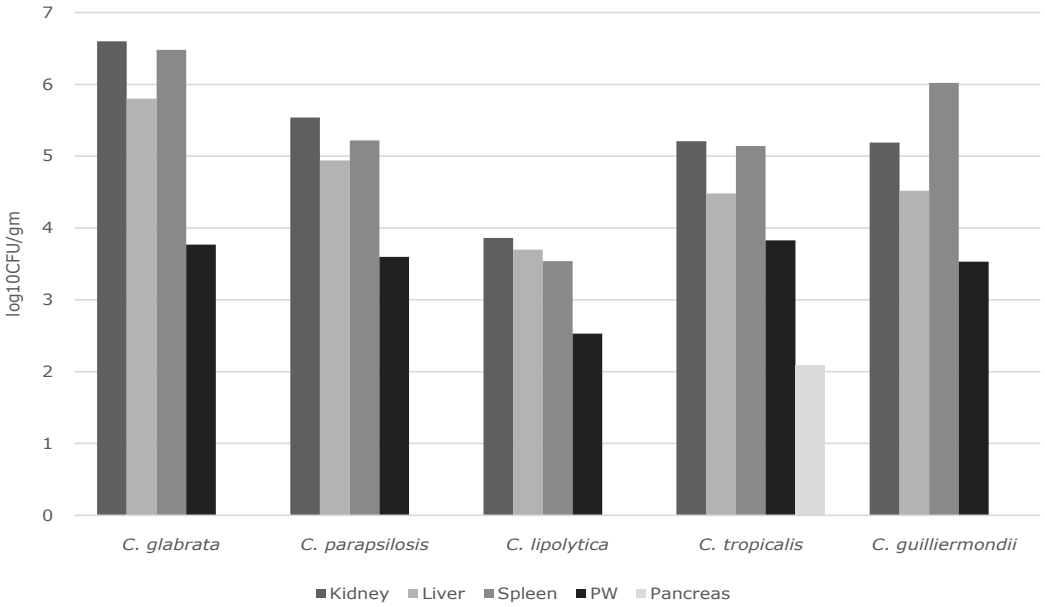

Fig. 6: Fungal tissue burden among immunosuppressed groups

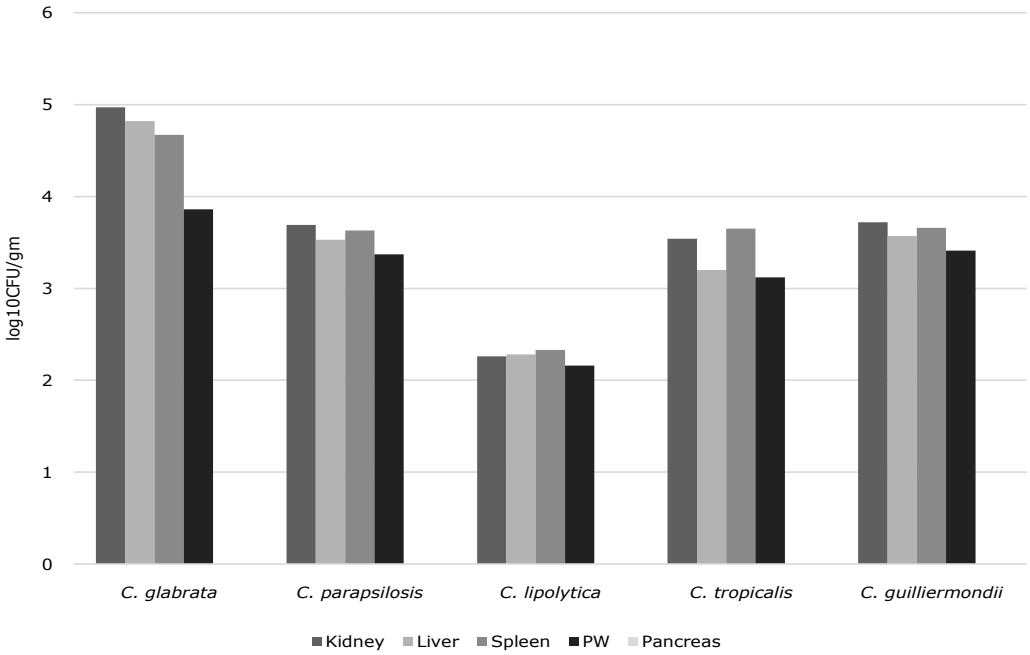

Fig. 7: Fungal Burden among immunocompetent groups

Comparatively, kidneys, liver and spleen, of *C. glabrata* and *C. lipolytica* infected animals had the highest and lowest fungal tissue burden respectively among immunocompetent mice (Fig. 7). The peritoneal effluent burdens of *C. glabrata*, *C. parapsilosis* and *C. guilliermondii* were similar statistically but much higher than those of *C. tropicalis* and *C. lipolytica*. None of the tested organisms was able to initiate infection in the pancreas in immunocompetent mice. Generally, immunosuppressed mice had significantly higher tissue burdens than immunocompetent ones ($p<0.001$) except for liver burdens in *C. glabrata* infected groups and peritoneal effluent counts in *C. glabrata*, *C. parapsilosis* and *C. guilliermondii* where no significant difference was recorded.

Liver and spleen represented the most affected intra-abdominal site of infection with no significant difference between their tissue burdens in immunosuppressed mice infected with *C. glabrata*, *C. parapsilosis* and *C. lipolytica*. Similarly, in immunocompetent models, *C. glabrata*, *C. parapsilosis* and *C. lipolytica* had statistically similar tissue burdens in the liver, spleen and peritoneal effluent. In case of *C. tropicalis*, spleen had the highest fungal burden followed by liver and peritoneal fluid respectively in both immunosuppressed and immunocompetent models. Similarly, in *C. guilliermondii* infected neutropenic mice, spleen was the most affected organ, while there was no significant difference between all organs among immunocompetent mice. Of note, the least fungal burdens were observed peritoneal effluent in almost all tested *Candida* species. The fungal disease progressed to the kidneys in both immunosuppressed and immunocompetent models of all *Candida* species. The Kidneys shared statistically similar fungal burdens with infected intra-abdominal organs in all *Candida* species and higher burdens than the liver in *C. tropicalis* and *C. guilliermondii* infected immunosuppressed models and the spleen in *C. glabrata* immunocompetent models.

Treatment was started for both immunosuppressed and immunocompetent models, using voriconazole for groups infected with *C. parapsilosis*, *C. lipolytica* and *C. guilliermondii* and micafungin for *C. tropicalis* and *C. glabrata* groups from the Vitek 2 system results (Table 4). All *Candida* species caused relatively high mortality (20 - 60%) in spite of the significant increase in the mean survival time after receiving treatment in all immunosuppressed mice. No significant difference in the mean survival time between immunosuppressed mice infected with different *Candida* strains after treatment.

In the treated immunocompetent model, all the tested animals survived the entire duration of the experiment, however, the increase in survival time compared to untreated groups was significant only for *C. glabrata* and *C. tropicalis*. Also, the difference in the mean survival time between treated immunosuppressed and treated immunocompetent mice was significant in case of *C. glabrata* only.

Table 4: Antifungal susceptibility of the tested strains using Vitek2 System

	FLU	VOR	CAS	MIC	AB	AFC
C. glabrata	≥64	≥8	≤0.25	≤0.06	0.5	≤1
C. parapsilosis	2	≤0.12	1	1	≤0.25	≤1
C. lipolytica	2	≤0.12	0.5	0.5	≤0.25	≤1
C. tropicalis	≤1	≤0.12	≤0.25	≤0.06	≤0.25	≤1
C. guilliermondii	16	0.25	1	2	8	≤1

FLU: Fluconazole; VOR: Voriconazole; CAS: Caspofungin; MIC: Micafungin; AB: Amphotericin B; AFC: flucytosine

Interestingly, despite the significant reduction of fungal burden among immuno-suppressed animals to voriconazole and micafungin, their fungicidal effect (i.e., organ sterilization) was never observed. Also, groups of immunosuppressed mice infected with different *Candida* species had statistically similar fungal burdens in all organs after receiving treatment. For each *Candida* strain, there was no significant difference between tissue burdens of different organs, except for *C. glabrata* where kidneys had the highest burden of all organs. In contrast, organ homogenates from immunocompetent mice showed no detectable growth after treatment except the kidneys, liver and spleen of *C. glabrata* infected mice.

Discussion:

This model aims to study the pathogenicity and *in vivo* susceptibility of non-*albicans Candida* species associated with IAC in human to predict the frequency of infections, the outcome of clinical disease and response to antifungal therapy. By mimicking clinical situations in humans as closely as possible, experimental non-*albicans Candida* infections were induced in both immunocompetent and immunosuppressed mice intraperitoneally with *Candida* strains associated with human disease isolated from cases of abdominal surgeries, liver transplantation and continuous ambulatory peritoneal dialysis (CAPD).

Our results showed persistent infection in the peritoneal cavity with peritonitis, hepatosplenomegaly and abscess formation upon infection with non-*albicans Candida* species, especially in immunosuppressed animals. Liver and spleen were the main intra-abdominal sites of infection and pancreas was affected only in case of *C. tropicalis*-infected immunosuppressed mice. This result is at variance with a previous intra-abdominal model of infection (3), where abscess formation on intra-abdominal organs was the main manifestation and pancreas being the most predominant site of involvement in the case of intraperitoneal injection of *C. glabrata* along with sterile feces. Cheng et al., (3) also reported that neutropenic mice injected with *C. glabrata* alone did not develop abscess which was not the case in our model, where abscesses developed upon challenging mice with different *Candida* species using the same inoculum ($5x10^8$ CFU/ml) without feces. Inspite of its retroperitoneal position and the inconsistency reported in previous studies (3), the kidney was included in our study as an indicator to assess progression of infection following intraperitoneal introduction of the fungal pathogen. The disease progressed to the kidneys with similar or higher burdens of fungal pathogens detected in the kidneys than intra-abdominal organs.

When comparing the virulence of the non-*albicans Candida* species tested in the present study in terms of mortality rates, survival time and tissue burden at predetermined points after infection, it was shown that among both naïve and immunosuppressed mice*, C. glabrata* was the most virulent species followed by *C. tropicalis* which had similar survival rates and mean survival time but less tissue burden than *C. glabrata. C. parapsilosis* and *C. guilliermondii* shared almost similar virulence, while *C. lipolytica* was the least virulent of all. Our results are at variance with the study conducted by Arendrup et al., (21), where *C. tropicalis* was reported to be more virulent than *C. glabrata* in an experimental model that compared different medically important *Candida* species

Previous animal model studies reported low virulence of *C. glabrata* with inability to cause severe illness or mortalities even with high intravenous inoculum (21-23). Our study reports the ability of *C. glabrata* to cause acute infection with high mortality in both immunosuppressed and immunocompetent animals. *C. tropicalis* was previously reported to be highly virulent even in immunocompetent mice (21, 24). These findings agree with our results where 100% mortality rate in both immunocompetent and immunosuppressed mice models and high tissue burdens were recorded. Many studies have reported the low virulence of *C. parapsilosis* with the fungi failing to initiate infection even in immunosuppressed mice (21,24,25). However, de Bernardis et al., (26) reported that *C. parapsilosis* could initiate infection when high inoculum is administered. Similarly, *C. guilliermondii* and *C. lipolytica* was reported to be weak, low-virulent fungi pathogens causing no mortalities among infected animals (21, 27-29). Interestingly, our study showed high virulence of *C. parapsilosis* and *C. guilliermondii* with mortalities in immuno-suppressed and immunocompetent mice which explained their high incidence in our last survey on IAC, where they represented the most prevalent non-*albicans Candida* species (30). Although *C. lipolytica* was the weakest pathogen in terms of mortality rates, survival time and tissue burdens in both

immunocompetent and immunosuppressed mice in our study, it caused mortality in immunosuppressed mice only, and lower than those caused by other tested species.

An increasing concern is being expressed about the emergence of antifungal resistance with accompanying increase in the prevalence of non-*albicans Candida* species among critically ill patients (9). Amphotericin B, fluconazole and echinocandins are the most commonly used antifungals in treating IAC (14). In our study, amphotericin B and fluconazole were excluded from treatment options according to the *invitro* susceptibility results. Micafungin was used *invivo* to treat mice infected with *C. tropicalis*, which reduced the fungal burden in all organs, and prolonged the survival time to some degree, however, it was not able to significantly reduce mortality rates or sterilize all organs in our immunosuppressed model. This finding agrees with the results of other studies, where micafungin did not completely resolve infections with *C. glabrata* (19, 31) and *C. tropicalis* (32).

Voriconazole was used in our study to treat *C. parapsilosis*, *C. lipolytica* and *C. guilliermondii*, which lowered the mortality rate and tissue burden in the mice organs. Zhao et al., (33) reported that fluconazole, itraconazole and posaconazole should not be used to treat *C. lipolytica*, but voriconazole may be useful for treatment. Our results agree with a previous experimental murine infection by *C. guilliermondii* where voriconazole was reported to be experimentally effective (34). Previous studies also reported that *C. parapsilosis* exhibited good susceptibility to voriconazole *in vitro* (35,36). However, voriconazole failed to completely eradicate the infection in immunosuppressed mice in our study.

In conclusion, IAC in Egypt is witnessing predominance of non-*albicans Candida* with species distribution and pathogenicity different from those reported worldwide. Further studies involving larger number of strains, tested with different inocula and several antifungal agents against each strain should be conducted to better understand the pathogenesis of the disease and predict treatment outcomes in clinical practice.

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Conflict of interest:

Authors declare no conflict of interest.

Compliance with ethical standards:

All applicable international, national, and/or institutional guidelines and ethical standards for the care and use of animals were followed.

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