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Clinical and pathological manifestations of *Candida auris* infections in mouse eyes

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Abstract

Background: The eye yeast infection is one of the leading causes of vision loss, especially in developing areas. Such infections may involve various body parts, either placed around the eye or inside the eye. Although ocular yeast infections are not as prevalent as bacterial infections, they are associated with more severe complications, particularly in immunocompromised individuals and those who overuse contact lenses. Fungal species, such as *Candida auris*, can infect the eyes using a variety of virulence factors that depend on environmental and anatomical factors. The study was designed to test the virulence and pathogenicity of a *C. auris* isolate through experimental induction of ocular infections in mice. **Methodology:** Mice were experimentally infected with a *C. auris* strain (PV715838.1). The concentration of 1×10^8 live yeast cells/ml were used to infect 30 BALB/c mice, which were divided into three groups: a negative control group, a positive control group (received eye drops containing *C. auris*), and the dexamethasone-treated group before being infected. Three weeks later, the mice were euthanized, and their eye tissues were harvested and subjected to histopathology examination. **Results:** The mice in the negative control group were healthy and did not present any signs of an ocular infection. Contrastingly, the infected group produced mild to moderate ocular lesions soon after inoculation, and signs included conjunctival redness and mild corneal opacities. The immunosuppressed infected group demonstrated worse ocular lesions, such as significant conjunctival hyperemia and dense corneal opacities, which developed to ulceration. These symptoms emerged faster and were more intense than those in the non-immunosuppressed infected group. The immunosuppression favored the growth of the yeast and tissue invasion, which resulted in intensive necrosis and tissue disorganization. **Conclusions:** This research proves that *C. auris* is able to induce ocular infections in mice, and the severity of the lesions is strongly associated with the immune status of the host.

Keywords: *Candida auris*, histopathology, mice, ocular infection

Introduction

Eye fungus continues to cause high levels of ocular morbidity and vision impairment, particularly in the developing world (Kalkanci & Ozdek, 2011). Infections caused by fungi of the eye are categorized with respect to their anatomical location. These infections may happen in the ocular adnexa or in the anterior and posterior segments of the eye (Sodhi *et al.*, 2016). Yeast infections of the eye are less frequent than bacterial infections, but they may cause severe complications when not treated (Naji, 2010). *Candida*-induced fungal keratitis is a frequent infection of immunocompromised people or those who use contact lenses frequently (Thomas & Kaliamurthy, 2013). The ability to cause eye infection and colonization of the yeast may depend on several virulence factors, including adhesion and colonization capabilities, enzyme production, immune evasion, stress resistance, and toxin production. Moreover, both human and animal eyes have anatomical structures that can be the source of the risk of these infections (Salman & Faraj, 2015; Ranjith *et al.*, 2017).

Environmental factors also contribute to the incidence of fungal infections of the eye. Significant factors include host immunity, climatic conditions, and high prevalence of fungi in the tropical areas. Specifically, the cornea is highly prone to fungal infections compared to other tissues of the eye, particularly in the regions where the infections are widespread (Slowik *et al.*, 2015; Jafer & Kassab, 2025). The research aimed to measure the virulence and pathogenicity of *C. auris* by experimentally inducing ocular infections in mice.

Materials and Methods

Ethical approval

This research was approved by the Local Animal Research Ethics Committee of the College of Veterinary Medicine, University of Baghdad (Approval No.: P-G1450, dated 17/6/2025).

Yeast isolation

Candida auris was isolated from swabs collected in a prior epidemiological survey from pigeons suffering from eye infections. The infected bird is characterized by swelling, cloudy and discolored eyes, and eye discharge, as shown in Figure 1. The strain registered in NCBI/ GenBank with the accession number PV715838.1 was used to induce experimental infection in the eyes of mice.



Figure 1: A pigeon's eye infected with *C. auris* shows swelling, a discolored eye, and discharge of pus

Preparation of the experimental infected dose

The infection dose applied in the experiment was prepared from a previous isolation (as mentioned above). Briefly, 2-3 colonies of *C. auris* were cultured on blood agar (HiMedia, India) and incubated at 37°C for 18 h. They were subsequently inoculated on brain heart infusion (BHI) agar, where they were incubated to proliferate in bulk at 37°C for 18-24 h. Then, the growth was harvested with sterile normal saline and centrifuged for 10 min at 2,000 rpm. The resulting sediment was washed three times after mixing with sterile 0.9% sodium chloride saline at pH

7.2, followed by suspending in the same buffer to reach an infective dose of 1×10^8 live yeast cells/ml (Jarullah, 2022). The solution was put into a sterile tube, and the concentration of the cells was checked through the use of a hemocytometer chamber (Al-Delemy, 2003). The calculations were done based on the following equation: $Z \times 4 \times 10^6 / N$, where: **Z**: The counts of blastospores of the yeast isolate, **N**: The number of squares counted (totally 80 squares) (Davie *et al.*, 1995). Lastly, 0.9% sodium chloride saline was added to the harvest to make sure that 1 ml of the solution had 1×10^8 live yeast cells/ml.

Experimental design

The experiment involved the use of 30 BALB/c mice with an age of 6-8 weeks (30-35 g). The mice were separated into three groups of 10 mice each: First group (negative control): This group had ten untreated mice, second group (positive control): This group consisted of ten mice that were administered a dose of 1×10^8 live yeast cells/ml. One drop only of 20 μ l was applied by using a micropipette. The animals were gently restrained by hand, and the drop of the infectious dose was instilled into the left eye only of mice, third group: Ten mice in this group were injected with dexamethasone sodium intramuscularly in the thigh at a dose of 0.5 mg/kg daily for one week. Following this treatment, they were subjected to the same infection procedure as in the second group (Saud & Ghani, 2006). During the three-week experimental period, food and water were provided freely. On day 21, all animals were examined clinically before performing necropsy, and tissue specimens from their eyes were collected, minced, and preserved in 10% formalin for histopathological examination.

Histopathological examination

Tissue preparation

Histological sectioning was performed in the histology laboratory of the Department of Pathology and Poultry Diseases at the College of Veterinary Medicine, University of Baghdad, using the method described by Luna (1968).

Staining

Histological sections were stained using hematoxylin and eosin, following the method outlined by Maniatopoulos *et al.* (1986).

Results

Mice in the negative group were normal, and their eyes appeared healthy without any symptoms, as shown in Figure 2. Symptoms of *C. auris* infection in the second group (positive control) were observed specifically in the left eye. The symptoms began to appear nine days post-infection. They included a loss of the natural sparkle of the eye, eyelid swelling, eye redness, white spots, and alopecia, as illustrated in Figure 3.

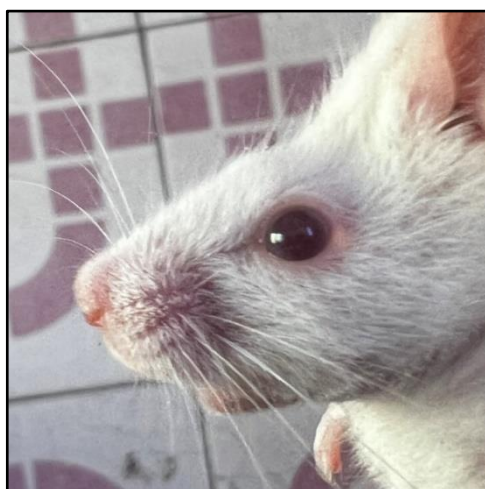


Figure 2: The eye of a mouse in the first negative control group appears healthy

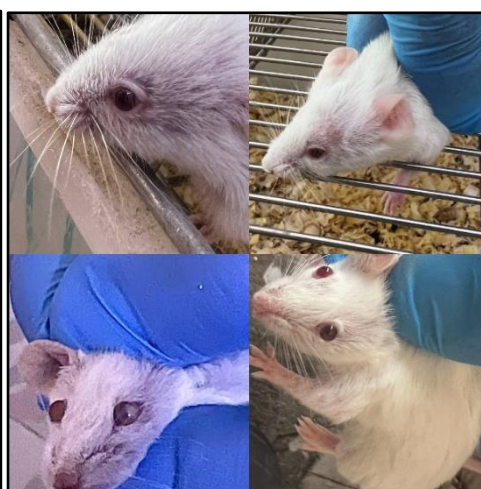


Figure 3: Clinical signs caused by *C. auris* infection in the eyes of mice in the second positive control group

Symptoms of *C. auris* infection were observed in the third group of mice that received dexamethasone sodium for one week, followed by yeast infection. This led to several complications, including swelling around the eyes, conjunctival hyperemia, purulent discharge, corneal opacity, alopecia, and proptosis (Figure 4). In some mice, the entire left side of the face was affected, resulting in subcutaneous edema, abscesses, and swollen skin (Figure 5).

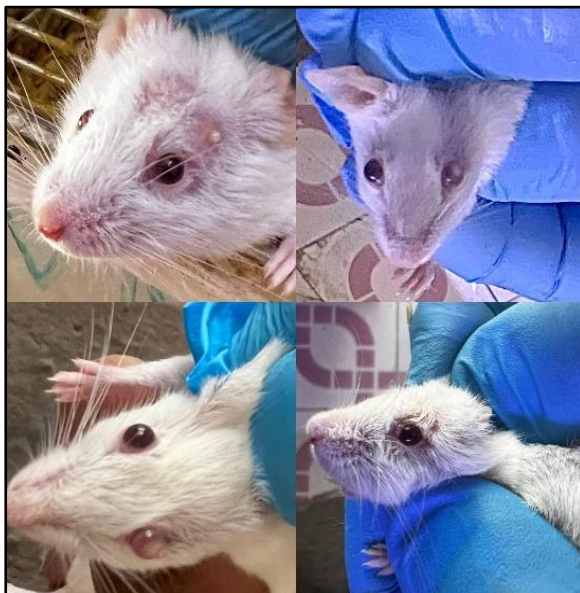


Figure 4: Clinical signs in the eyes of mice infected with *C. auris* in the third group

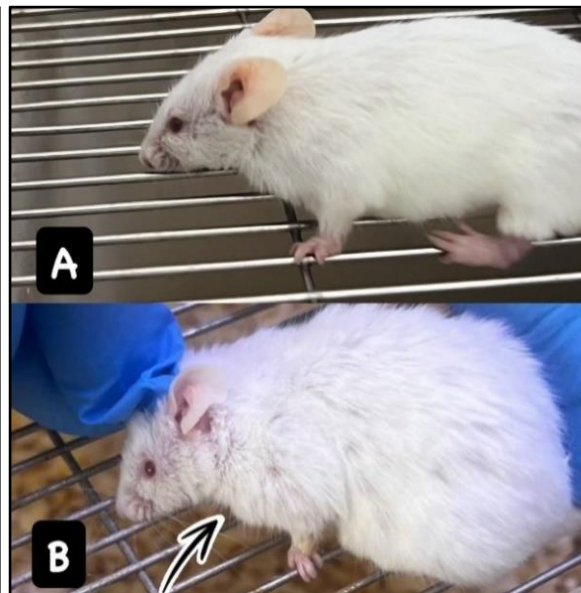


Figure 5: Swelling and subcutaneous edema of a mouse whose eyes are infected with *C. auris* in the third group: A: normal mouse; B: infected mouse

Histopathological changes

In the first negative control group, the histopathological examination of the eyeball revealed normal appearances of the lens, cornea, eyelids, ciliary processes, retina, and lacrimal sacs, as illustrated in Figures 6 and 7.



Figure 6: A histopathological section of the eyeball (in a negative control mouse) shows the normal appearance of the lens (L), cornea (C), eyelid (black arrow), ciliary process (red arrow), retina (R), and lacrimal sac (S). H&E, 40x



Figure 7: A histopathological section of the eyeball (in a negative control mouse) shows a normal appearance of the corneal stroma with anterior and posterior epithelium (C), conjunctival lining cells with substantial propria containing lacrimal and sebaceous glands (black arrow), and a normal lacrimal sac (S). H&E, 100x

In the second group, the histopathological examination of the right eyeball, which represents the non-infected side, showed that the lens, cornea, ciliary process, retina, and lacrimal sac appeared normal. However, the images of the conjunctival membrane revealed slight infiltration of mononuclear leukocytes (Figures 8 and 9).

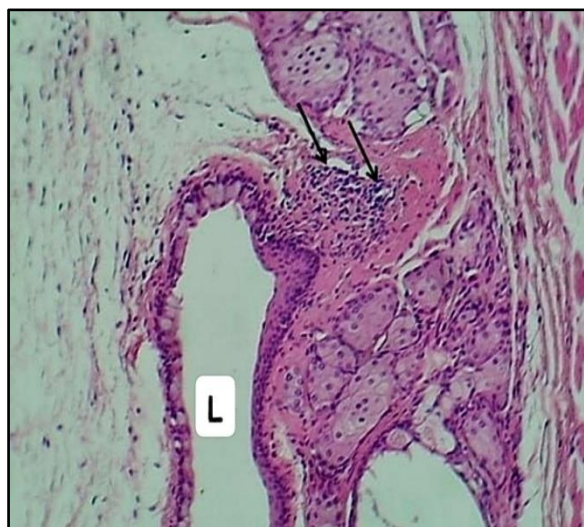


Figure 8: A histopathological section of conjunctiva (in the second group, right eye) shows a normal lacrimal sac (L) with mild infiltration of inflammatory cells in propria tissue (arrows). H&E stain, 100x

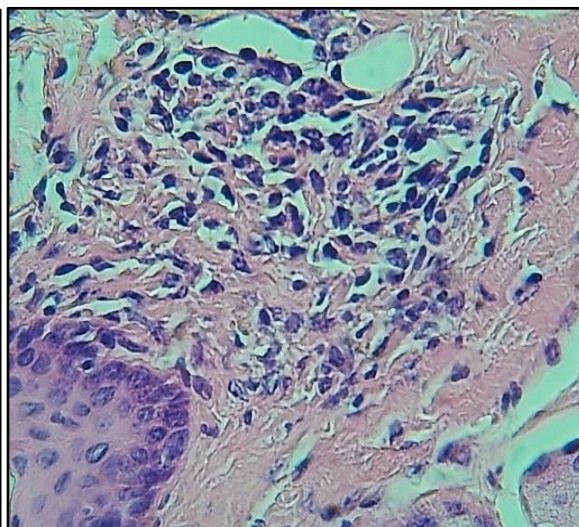


Figure 9: A histopathological section of the conjunctiva (in the second group, right eye) shows minor infiltration of mononuclear cells. H&E stain, 400x

The histopathological examination of the left eyeball revealed hyperplasia accompanied by mucoid degeneration of goblet cells, hyperplasia of conjunctival epithelial lining, in addition to the appearance of inflammatory cells and severe aggregation of leukocytes around glands, and the presence of edema (Figures 10 and 11). During histopathological examination of the right eye (non-infected side) in the third group, which included infections associated with immunosuppression, mild eyelid thickening was observed. This condition was accompanied by vascular congestion, edema, and minimal leukocyte infiltration (Figure 12). The histopathological examination of the left eye of the eyeball in the third group of animals revealed severe thickening of the eyelid tissue, which was associated with significant vascular congestion, edema, hemorrhage, and marked infiltration of mononuclear leukocytes (Figure 13).

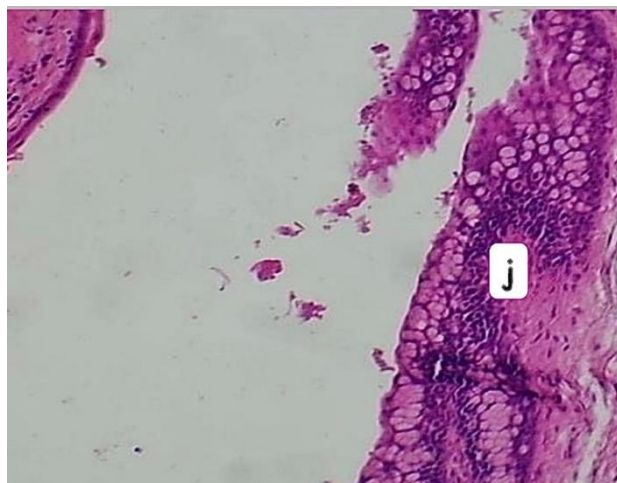


Figure 10: A histopathological section in the left eye of the second group shows mucoid degeneration, goblet cells hyperplasia of conjunctival epithelial lining (j). H&E stain, 100x

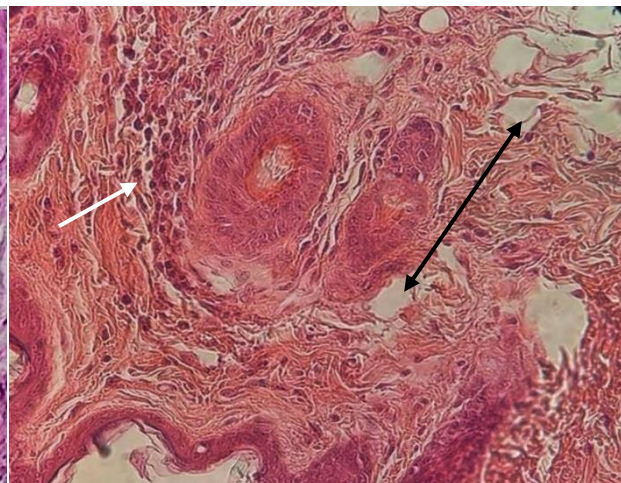


Figure 11: A histopathological section in the eyelid of the second group, left eye, shows severe aggregation of leukocytes around glands (white arrow) and the presence of edema (black arrow). H&E stain, 400x

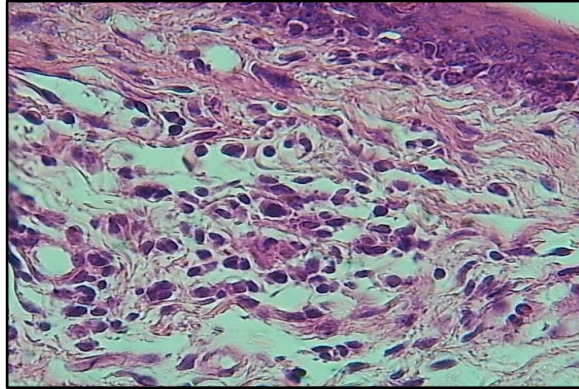


Figure 12: A histopathological section in the eyelid (third group, right eye) shows mild thickening associated with fibrovascular stroma with edema and moderate mononuclear cell infiltration. H&E stain, 400x

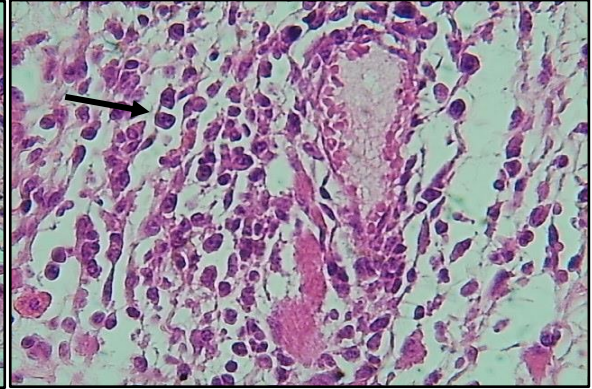


Figure 13: A histopathological section of the eyelid (third group, left eye) shows severe vascular congestion, edema, and marked leukocyte infiltration (black arrow). H&E stain, 400x

Further examination of the eyeball showed the presence of glandular tissue and lacrimal sac tissue, and all the animals showed pronounced vascular congestion, edema, hemorrhage, and a significant infiltration of mononuclear leukocytes (Figure 14 A, B). In contrast, the conjunctiva in the same eye revealed *C. auris* infiltration in the epithelia and glands, which showed a hyperplastic appearance with congestion of blood vessels and infiltration of inflammatory cells in the interstitial tissue (Figure 15 A, B).

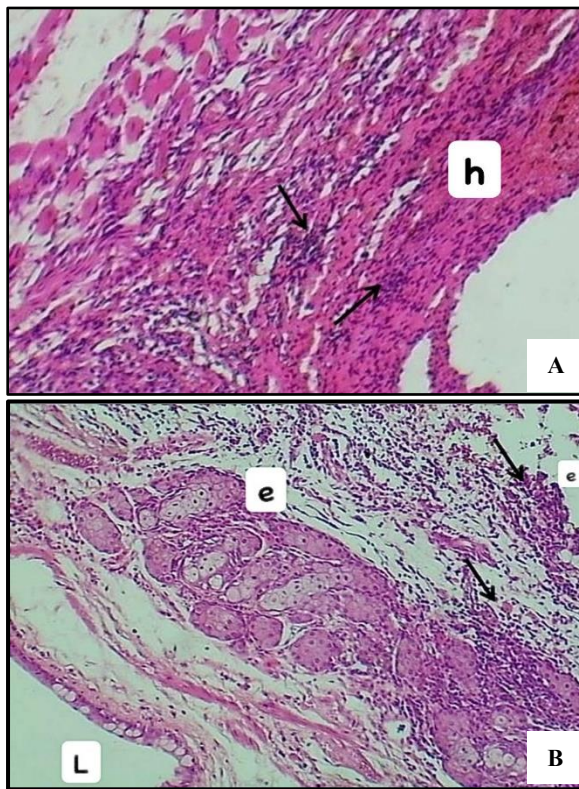


Figure 14: A histopathological section of the eyelid (third group, left eye) shows severe thickening, marked infiltration of multiple mononuclear cells infiltration (black arrows), along with hemorrhage (h) (A), (B) shows prominence of hyperplastic glands with marked thickening associated with vascular congestion and edema (e) and marked infiltration of leukocytes (black arrows). H&E stain, 100x

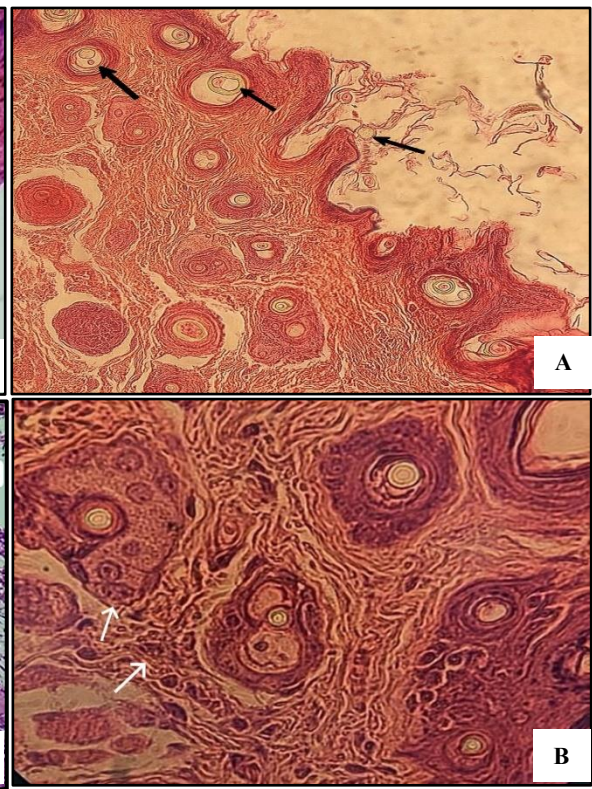


Figure 15: A histopathological section of conjunctiva (third group, left eye) shows *Candida* infiltration into the epithelia and glands (black arrows), congestion of blood vessels with infiltration of inflammatory cells (A), (B) shows scattered cells in the stromal tissue (substantial propria) with *Candida* inside the glands that appear hyperplastic (white arrow). H&E stain, 400x

Discussion

This research specifically compared the clinical and histopathological outcomes in both immunocompetent and immunosuppressed mice after inducing experimental eye infection with *C. auris*. The findings confirm that *C. auris* can establish an infection on the ocular surface of immunocompetent hosts. Moreover, the research revealed the much more severe manifestation of the disease in the state of immune suppression due to dexamethasone, this also corresponds with the findings of Rasheed *et al.* (2020).

Clinically, no changes were seen in the negative control (first group), and no effects were seen in the eye tissue, meaning that there were no inflammatory and degenerative conditions. The second group (the positive control) showed clinical and histopathological lesions similar to those of Khan *et al.* (2018), Shenoy *et al.* (2019), and Centers for Disease Control and Prevention (2024). This mild, localized infection pattern implies that although *C. auris* had the necessary virulence factors, e.g., adhesion and colonization capabilities, to cause infection, the local immune response in the immunocompetent host was largely successful and quite effective in limiting the yeast, preventing further tissue penetration or complete-blown keratitis (Phongkhun *et al.*, 2023; Sharma & Kadosh, 2023). This reduced virulence in immunocompetent models is in line with previous studies that find that *C. auris* can express weaker virulence factors than other species, such as *C. albicans*, in some infection environments (Abe *et al.*, 2018; Du *et al.*, 2020; Satala *et al.*, 2022). The fact that the pathological changes would be confined to the eyelid, conjunctiva, and lacrimal sac tissues (the site of application) further confirms that there is an effective host defense in this group, and this aligns with the findings of Douglas and McCourtie (1983).

Hyperplasia was found in the second group and was detected in a few regions of the tissues of the eye. In this condition, there was also mucoid degeneration of the conjunctival glands and the goblet cells, the inflammatory cells, and *Candida* debris in the interstitial tissue. These findings provide grounds according to which the immune system of a host was, to some degree, controlling and limiting the infection, which also seems to confirm the results of Abe *et al.* (2018).

This study revealed a significant increase in the level of severity of the disease in the immunosuppressed mice (third group) who were pre-treated with dexamethasone sodium. The symptoms appeared earlier, within five days of treatment and infection, and much more intense. Such symptoms were conjunctival hyperemia, purulent discharge, corneal opacities, and eye proptosis. The same signs were also observed by Breazzano *et al.* (2020). Kathuria *et al.* (2015) also stated that *C. auris* has been associated with serious eye infections, including panophthalmitis. Histopathology was clear evidence of the increased virulence on the basis of immunosuppression. The eyelid of the right eye was mildly thickened and had minimal infiltration of leukocytes, probably due to rubbing and licking by the mice, making *C. auris* to be transferred between the left (infected) and the right (uninfected) eye. However, the eyelid tissue was highly thickened in the left eye, which was the main area of infection. This was also characterized by significant vascular congestion, edema, hemorrhage, and severe infiltration of mononuclear leukocytes. Such a stormy, uncontrollable, inflammatory reaction of adnexa, gland tissue, and lacrimal sac is indicative of the extensive effects of immune deficiency on the pathogenicity of yeasts (Haseeb *et al.*, 2021).

The use of glucocorticoids, including dexamethasone, is also shown to have an effect of suppressing host defenses, making a person more vulnerable to yeast pathogens, as demonstrated by Aristimuno *et al.* (1993) and Hemady (1995). The treatment of mouse models of keratomycosis indicated that immunosuppressive agents increased both fungal persistence and the disease severity significantly (Abe *et al.*, 2018; Phongkhun *et al.*, 2023). The clinical progression of the corneal opacities and proptosis of the immunosuppressed group points to the fact that the infection has progressed to include a localized adnexal infection, possibly a life-threatening infection of the cornea and other deeper ocular structures. This acquisition is clinically significant for *C. auris* in high-risk patients (Wu *et al.*, 2002; Breazzano *et al.*, 2020). The pathological features of the fulminant inflammation response that did not manage to eliminate the pathogen are the severe edema, congestion, and the presence of pronounced infiltration of leukocytes along with hemorrhage (Egwuagu *et al.*, 2015).

Compared to the second group, the third group, which experienced immunosuppression prior to infection, had extensive invasion of eye structures by yeasts. The yeast was rich and entrenched in the conjunctival glandular tissue of the eyeball and the lacrimal sac. There was hyperplasia in this tissue, and congestion and inflammatory

cell infiltration of the interstitial space of blood vessels. The immunosuppression obviously promoted the growth of yeast and tissue invasion, leading to intensive necrosis, tissue disorganization, and massive loss of inflammatory cells. The results are consistent with those of García-Bustos *et al.* (2022). The marked disparity between the second and the third groups is that host immunity plays a role in limiting *Candida* in the ocular tissues. In the presence of normal immune defenses, the growth of *Candida* is superficial and localized. Nevertheless, when a person is immunocompromised, the yeast may invade deeper tissues without any complications and cause devastating pathology. These findings align with the evidence provided by Phongkhun *et al.* (2023), who state that *C. auris* is an opportunistic pathogen, so that it can use altered host defenses and develop systemic or invasive infections.

Conclusions

The current study was able to generate a mouse model of *C. auris* ocular infections. It proved that systemic immunosuppression is one of the significant factors in terms of clinical severity and histopathological damage of the infection. The results demonstrate that the immune condition of the host plays a pivotal role in deciding on the outcome of the disease caused by *C. auris*.

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Authors' Contributions

S.H.A. was responsible for sample collection, laboratory work, experimental infection, data analysis, and drafting the manuscript. Z.A.A. oversaw and made the study design and contributed to manuscript revision.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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المظاهر السريرية والمرضية لعدوى *Candida auris* في عيون الفئران

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المستخلص

الخلفية: تُعد عدوى الخميرة في العين سبباً رئيسياً لفقدان البصر، لا سيما في المناطق النامية. يمكن أن تؤثر هذه العدوى على مواقع تشريحية مختلفة: إما حول العين أو داخلها. على الرغم من أن عدوى الخميرة العينية أقل شيوعاً من الأنواع البكتيرية، إلا أنها قد تؤدي إلى مضاعفات خطيرة، لا سيما لدى الأشخاص الذين يعانون من نقص المناعة والذين يستخدمون العدسات اللاصقة بإفراط. تُمكن عوامل الضراوة المختلفة الفطريات مثل المبيضات أورييس من إصابة العيون، متأثرة بالعوامل البيئية والتشريحية. هدف البحث إلى تقييم ضراوة وإمراضية عزلة بكتيريا *C. auris* عن طريق إحداث عدوى عينية تجريبية لدى الفئران. **المنهجية:** استُخدمت سلالة *C. auris* PV715838.1 لإصابة الفئران تجريبياً. حُصرت جرعة العدوى بتنشيط مستعمرات *C. auris* من عزلات سابقة وزراعتها في أجار الدم وأجار BHI، مما أدى إلى تركيز 1×10^8 خلية/مل. استُخدم ثلاثون فأراً من سلالة BALB/C في البحث، مُقسّمين إلى ثلاث مجموعات: مجموعة ضابطة سلبية، ومجموعة ضابطة إيجابية تلقت قطرات عين تحتوي على *C. auris*، ومجموعة عولجت بالديكساميثازون قبل الإصابة. بعد ثلاثة أسابيع، أُعدمت الفئران قتلًا رحيمًا، وُجمعت أنسجة عيونها للفحص النسيجي المرضي، والذي تضمن تقطيعها وتلوينها بالهيماتوكسيلين والإيوزين. **النتائج:** في المجموعة الضابطة السلبية، بقيت جميع الفئران بصحة جيدة ولم تظهر عليها أي علامات إصابة عينية. في المقابل، ظهرت على المجموعة المصابة آفات عينية خفيفة إلى متوسطة بعد التطعيم بفترة وجيزة، شملت احمراراً في الملتحمة وعتامة طفيفة في القرنية. أما المجموعة المصابة المثبطة مناعياً، فقد أظهرت آفات عينية أكثر شدة، اتسمت باحتقان شديد في الملتحمة وعتامة كثيفة في القرنية، والتي تطورت إلى تقرح. ظهرت هذه الأعراض بسرعة أكبر وكانت أكثر شدة مقارنةً بالمجموعة المصابة غير المثبطة مناعياً. **الاستنتاجات:** يوضح هذا البحث أن فطريات المبيضات أورييس يمكن أن تسبب التهابات عينية لدى الفئران، وأن شدة الآفات ترتبط ارتباطاً وثيقاً بالحالة المناعية للمضيف.

الكلمات المفتاحية: *C. auris*، علم الأمراض النسيجي، الفئران، العين، العدوى