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Isolation and Molecular Identification of *Candida auris* in Ocular Infections in Humans, Dogs, and Pet Birds

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Abstract | This research aimed to isolate and molecularly detect *Candida auris* from ocular infections in both humans and pet animals (specifically dogs and birds). *Candida auris* is particularly concerning due to its high resistance against multiple antifungal drugs. This is the first reported case of a *C. auris* eye infection in humans and pet animals. The research involved 400 eye swabs from 100 individuals suspected of having ocular yeast infections, as well as 50 dogs and 50 pet birds presenting with ocular disorders believed to be caused by fungal infections. The cultural isolation rate of *Candida* species from the primary isolates was 9.25%, with 27% of those identified as *C. auris* using the DL 96II microbial ID/AST system. Furthermore, all verified *C. auris* isolates tested positive for the 5.8S rRNA gene using conventional PCR. These finding highlight the prevalence of *C. auris* in the country and may pose a zoonotic risk to public health.

Keywords | *Candida auris*, Eye swab, Humans, Dog, Bird, The DL 96II microbial

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INTRODUCTION

Candida auris is an emerging multidrug-resistant pathogen that was first isolated in 2009 from the external ear canal of an inpatient in a Japanese hospital (Satoh *et al*., 2009). *Candida auris* is a biofilm-forming, and thermo-resistant yeast that can grow at temperatures ranging from 30°C to 42°C and is tolerant of salinity levels of up to 10% (Welsh *et al*., 2005). This adaptability enables *C. auris* to colonise various medical equipment, plastic surfaces, and healthcare environments, making it a highly invasive pathogen (Osei Sekyere, 2018). Panophthalmitis with *Candida auris* may occur in an immunocompromised patient without a history of trauma, and the infection may have a fulminant course, resulting in loss of vision and structural integrity of the eye (Keighley *et al*., 2019).

Furthermore, *Candida auris* exhibits significant resistance to various antifungals against the three main antifungal classes: Azoles, polyenes, and echinocandins (Rudramurthy *et al*., 2017). It is associated with high mortality rates, particularly in immunocompromised patients with multiple comorbidities, such as diabetes mellitus, renal failure, and cardiovascular disease. Therefore, the rapid identification and characterisation of *Candida auris* are crucial for optimising clinical outcomes and containing its spread in healthcare settings and worldwide (Osei Sekyere, 2018).

Diagnosing *Candida auris* can be challenging primarily due to its frequent misidentification (Mizusawa *et al*., 2017). One of the main reasons for this issue is the absence of this specific species in many biochemical databases. Most

commercial diagnostic tools that are readily available, such as VITEK, API 20C-AUX, Auxa-Colour 2, BD Phoenix, and MicroScan, often yield misleading results. Consequently, *C. auris* is frequently misidentified as other *Candida* species. This misidentification can result in delays in appropriate management and treatment (Kathuria *et al.*, 2015; Kullberg and Arendrup, 2015).

We aim to detect, isolate, and characterize *Candida auris* from ocular infections in both humans and pet animals (specifically dogs and birds) to assess their prevalence and potential to infect humans and animals.

MATERIALS AND METHODS

Sample collection

This research was conducted in Baghdad from October 2024 to February 2025, involving 200 eye samples from 100 individuals suspected of ocular yeast infections, 100 eye swabs from 50 pet birds, and 100 eye swabs from 50 dogs presenting ocular disorders clinically believed to be fungal infections. Subsequently, the samples were transported aseptically and cultured by inoculating them onto Sabouraud dextrose agar (SDA) enriched with chloramphenicol as described before (Minnat and Khalaf, 2019; Mahmoud and Yassein, 2024).

Traditional laboratory diagnosis

The diagnosis of the yeast fungus growth was confirmed through a slide smear stained with Gram stain and lactophenol cotton blue (Othman *et al.*, 2018), urease test (Alwan and Aziz, 2012), and cultures on Hicrome *Candida* Differential Agar HICROM agar (Al-Dahlaki and Al-Qaysi, 2023), Cornmeal agar (CMA) (Tille, 2021), and Brain Heart Infusion agar (BHIA) with 0.1% cycloheximide (Dal Pizzol *et al.*, 2021; Kamal and Al-Haddad, 2022; Kamal *et al.*, 2025).

Biochemical diagnosis

The accurate diagnosis of isolated yeasts was done by the DL 96II microbial ID/AST system by preparing a yeast suspension using sterile saline 0.9% at the specified concentration, typically around 10^6 CFU/mL (1 McFarland standard). Inoculate the DL-96 FUNGUS Test Cards containing 96 wells pre-loaded with desiccated reagents for fungus identification and antifungal susceptibility testing. These test cards include substrates for identification, such as sugar fermentation tests, and antifungal agents to evaluate the yeast's susceptibility.

Molecular identification

DNA extraction

Yeast DNA extraction was performed according to the company's instructions for the FavorPrep Mini Kit for Fungal/Yeast Genomic DNA Extraction.

Amplification of PCR

The primers used for the amplification targeted the 5.8S rRNA gene to identify different species of yeast. The expected fragment lengths range from 400 to 1000 base pairs, and further information on the primers can be found in Table 1.

The amplification procedure commenced with an initial denaturation cycle at 95 ˚C for 5 minutes. This was followed by 35 cycles consisting of denaturation at 95 ˚C for 45 seconds, primer annealing at 55˚C for 1 minute, and chain extension at 72˚C for 1 minute. Finally, a 72 ˚C extension step was performed for 5 minutes (Tamura *et al.*, 2013).

After amplifying the PCR products, they were separated using agarose gel electrophoresis with a 1.5% agarose concentration. The electrophoresis was conducted at 70V and 65A for 1 hour. The DNA was visualised using a UV transilluminator. Subsequently, the amplicons were stored at -20˚C for further examination (Sneath and Sokal, 1973; Tamura *et al.*, 2004).

Statistical analysis

The Statistical Packages of Social Sciences, SPSS (2019) program was used to detect the effect of different groups/factors on study parameters. Chi-square test was used to significantly compare the percentages (0.05 and 0.01 probability) in this study.

RESULTS AND DISCUSSION

The overall isolation rate of yeast species using the primitive isolation technique in the laboratory was 9.25% (37 isolates) (Table 2). The results indicated that the cultural isolation rate of yeast species varied among different sources: 8% (16 isolates) from human eye swabs, 14% (14 isolates) from dog eye swabs, and 7% (7 isolates) from bird eye swabs.

Table 1: The sequence of primers used in this research.

Primer	Sequence	Primer sequence	Tm (°C)	GC%	Size of product (bp)
small subunit ribosomal RNA gene	F	5`- CTTGGTCATTTAGAGGAAGTAA -3`	53.21	36.36	400-1000
	R	5` TCCTCCGCTTATTGATATGC-3`	55.09	45.00	

Table 2: Prevalence of yeasts in samples analysed in this study.

Host	Total No.	No. of Primary isolates (%)
Human	200	16 (8.00%)
Dogs	100	14 (14.00%)
Birds	100	7 (7.00%)
Total	400	37 (9.2%)
P-value	---	0.2233 NS
NS: Non-Significant.		

Traditional laboratory differentiation of yeast species

The observed unidentified yeast colonies on Sabouraud dextrose agar displayed a creamy to pale beige consistency, featuring smooth, shiny to slightly matte colonies (Figure 1). The present findings align with those documented by Borman *et al.* (2021).

Figure 1: The suspected yeast isolates on SDA at 30 °C, after 2-3 days of incubation.

Lactophenol, cotton blue, and Gram stains were used to identify suspected yeast species cells. While the lactophenol cotton blue showed cylindrical, spherical, and ovoid cells, the gram stain showed gram-positive cells (Figure 2). These findings correspond with those documented by Tap *et al.* (2018).

The urease test is used to distinguish *Candida* species from other types of yeast. The isolated suspected yeasts could break down urea into ammonia and carbon dioxide with the enzyme urease, leading to a rise in the environment's alkalinity (Faris *et al.*, 2006); thus, twelve suspected yeast isolates showed a positive reaction (indicated by pink colouration) in the urease test, which were categorised into six from humans, five from dogs, and one from birds, as shown in Table 3 and Figure 3. These results align with those reported by Lee *et al.* (2011), who found that *Candida* species had a negative urease test.

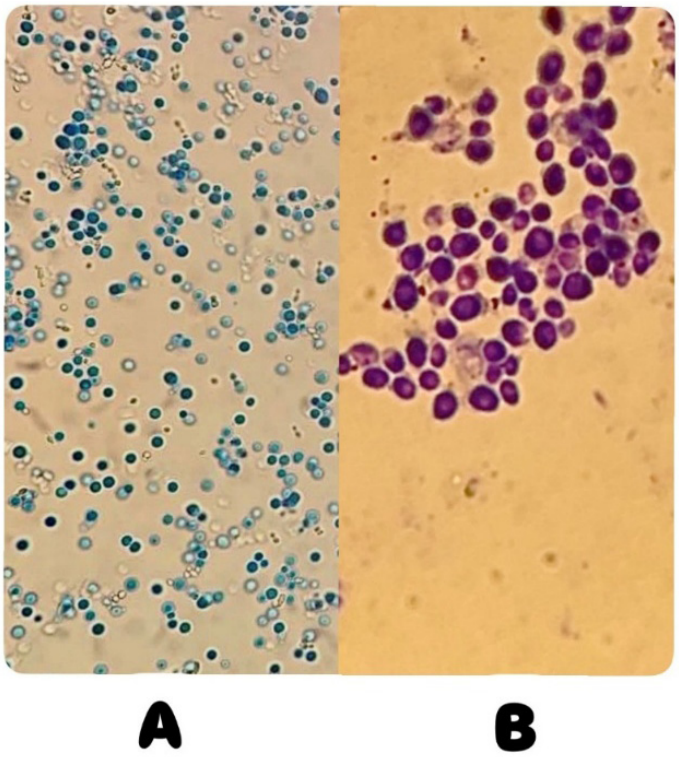

Figure 2: Wet mount smears of suspected yeast isolates (A) lactophenol cotton blue stain at 40x, (B) Gram stain at 100x.

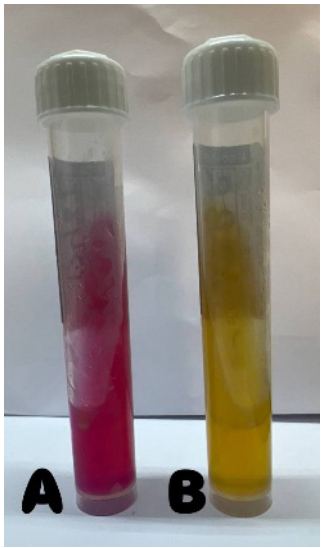

Figure 3: The suspected yeast isolates examined by the urease test after 3 days of incubation at 30°C, where (A) represents a positive reaction and (B) represents an adverse reaction.

HiCrom agar is a specialised medium designed to differentiate between several species of *Candida* (Borman *et al.*, 2021). The suspected yeast isolates exhibit distinct growth patterns, with *Candida auris* appearing in a pale pink colour. This highlights the importance of media colouration in diagnosing fungal infections, as it facilitates quicker identification (Borman *et al.*, 2021), as shown in Table 3 and Figure 4.

Figure 4: Suspected *Candida auris* isolates demonstrated pale pink growth on HCDA following 48 hours of incubation at 30 °C.

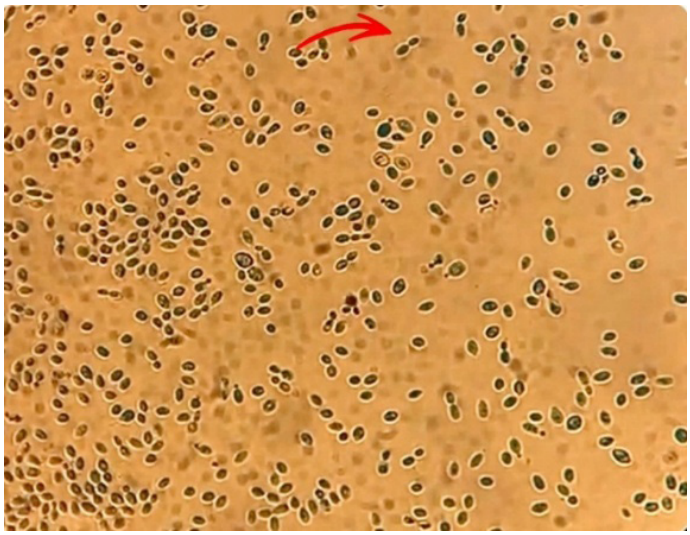

Figure 5: Microscopic appearance of suspected yeast inoculated at 30 °C on CMA for 48-72 hours, stained with lactophenol cotton blue at 40X. The arrow indicates a budding cell.

Sixteen suspected yeast isolates, including six from humans, eight from dogs, and two from birds. All these isolates showed negative results for oval to globose budding yeast-like cells and pseudo-hyphae when cultured on CMA media. These findings are consistent with those reported by Tap *et al*. (2018) and Ding *et al*. (2019), as shown in Table 3 and Figure 5.

BHIA with cycloheximide (a chemical that inhibits yeast protein synthesis) can help distinguish between different fungi (Walsh *et al*., 2018). It can be an effective tool in fungal identification by preventing the growth of harmful yeasts, such as *Candida auris* and *Cryptococcus neoformans* (Lee *et al*., 2011). However, Table 3 shows that sixteen yeast isolates can still grow in media with cycloheximide, which were categorised into six from humans, six from dogs, and four from birds, as illustrated in Figure 6.

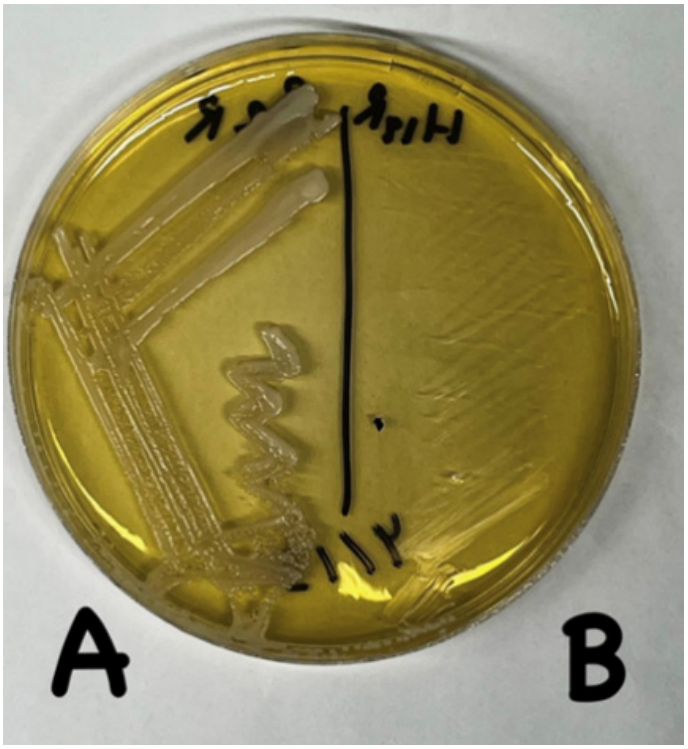

Figure 6: (A) revealed the suspected yeast growth on BHIA with cycloheximide, incubated at 25°C for 24-48 hours. At the same time, no yeast growth was observed in (B).

The DL 96 microbial identification/ antimicrobial susceptibility testing (ID/AST)

Results of microbial identification

The DL 96II Microbial ID AST analysis system identified ten (27%) of the yeast isolates out of 37 isolates as *Candida auris*, which were obtained according to routine laboratory techniques and were divided into three (18.75%) isolates from humans, five (35.7%) isolates from dogs, and two (28.6%) isolates from pet birds, as shown in Table 4.

Table 3: Laboratory differentiation of yeast species based on the Urease test, HiCrom agar, Cornmeal agar, and BHIA with cycloheximide.

Sources of the sample	No. of samples	Urease test		HiCrom agar					Cornmeal agar		BHIA with cycloheximide	
		+	-	Blue	Cream	Purple	Light pink	Green	+	-	+	-
Humans	16	6	10	1	7	2	6	-	10	6	6	10
Dogs	14	5	9	-	3	2	6	-	6	8	6	8
Birds	7	1	6	1	1	1	2	2	5	2	4	3
Total	37	12	25	2	11	5	14	2	21	16	16	21

Table 4: Appearance of *Candida auris* among hosts distribution according to the DL 96II system.

Host	Total No.	No. of *C. auris* isolates (%)
Human	16	3 (18.75%)
Dogs	14	5 (35.71%)
Birds	7	2 (28.57%)
Total	37	10 (27.03%)
P-value	---	0.1772 NS
NS: Non-Significant.		

The isolates were categorised into three groups: those isolated from humans, which aligns with a case reported by Shenoy *et al.* (2019) detailing a 30-year-old immunocompetent man who developed pan ophthalmitis caused by *Candida auris*. Additionally, five isolates were obtained from dogs, which corresponds to a recent study conducted in Kansas by White *et al.* (2024), where *Candida auris* was isolated from the oral cavity of only one out of 251 dogs. Furthermore, two isolates of *Candida auris* were found in birds, marking the first time this yeast has been isolated from the birds' eyes. This may be attributed to the unique ocular anatomy of birds and their stronger immune defences. However, infections in birds remain under-researched, whereas Casadevall *et al.* (2019) proposed that birds may be possible intermediate hosts in the dissemination of *C. auris* ancestors.

Results of Sequencing and Genetic Analysis

The cultural and microscopic characteristics of isolates were described by Borman *et al.* (2021). While the results from the DL96 Microbial Identification were consistent, a conventional PCR assay was conducted to confirm their molecular identity. Sequencing and genetic analysis revealed that three isolates (18.75%) were from humans, which were recorded in NCBI GenBank with accession numbers PV715816.1, PV715817.1, and PV715818.1, as shown in Figure 7. Additionally, five isolates (35.7%) were from dogs, recorded in NCBI GenBank with accession numbers PV715827.1, PV715828.1, PV715829.1, PV715830.1, and PV715831.1, as shown in Figure 8. Finally, two isolates (28.6%) from pet birds are recorded in NCBI GenBank with accession numbers PV715838.1 and PV715839.1, as shown in Figure 9. All these isolates were diagnosed as *C. auris*, as outlined in Table 5.

PCR results revealed the identification of *C. auris* in 10 isolates from 37 isolates. Yeast species identification based on the 5.8S-ITS region (Table 5). The 5.8S–ITS region appears to help detect genetic variability among *Candida* species, which is valuable for taxonomic purposes and for species identification consistent with Guillamón *et al.* (1998), De Llanos Frutos *et al.* (2004), Mahdi *et al.* (2016), and Habib *et al.* (2016).

Table 5: Results of sequencing and genetic analysis of *Candida auris.*

Host	Total No.	Sequenced and genetic analysis of *C. auris* (%)
Human	16	3 (18.75%)
Dogs	14	5 (35.71%)
Birds	7	2 (28.57%)
Total	37	10 (27.03%)
P-value	--	0.1772 NS
NS: Non-Significant.		

In Kuwait, Khan *et al.* (2018) have identified *C. auris* in 1 of 158 human (0.63%) eye swabs from a case of endophthalmitis by both PCR sequencing of the internal transcribed spacer (ITS) region and the D1\D2 domains of ribosomal DNA (rDNA). The researchers suggested that the frequency of *C. auris* isolation significantly increased between 2014 and 2017.

While other researchers were able to establish a diagnosis of *C. auris* by recognising the D1\D2 region of the 28S ribosomal DNA, the ITS regions of ribosomal DNA, and real-time polymerase chain reaction (PCR) assays, all these techniques are sensitive and specific methods for directly identifying *C. auris* nucleic acids in clinical samples (McCarty *et al.*, 2021; Lionakis and Chowdhar, 2024; Bhargava *et al.*, 2025).

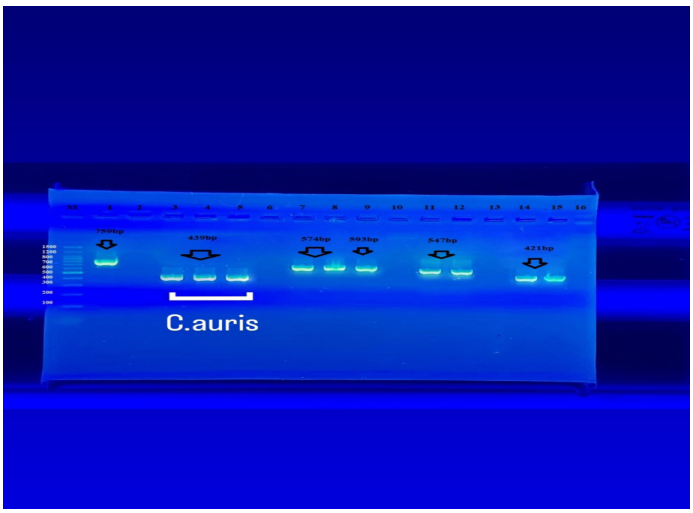

Figure 7: Gel electrophoresis for PCR product of 5.8S ribosomal RNA gene (Agarose 1.5% at 5 volt/cm², 90 min.), M: DNA ladder (100-1500 bp). Visualised under UV light after staining with Red Safe, (3-5) represents *C. auris* from a human eye swab; the product length is 439 bp.

White *et al.* (2024) detected *C. auris* in the oral cavity of a dog in Kansas, United States, by use of the ITS regions of ribosomal DNA. In Delhi, Wang (2023) isolated *C. auris* in 4 of the 87 dogs (4.5%) from their ears and skin surface using ITS meta-barcode sequencing.

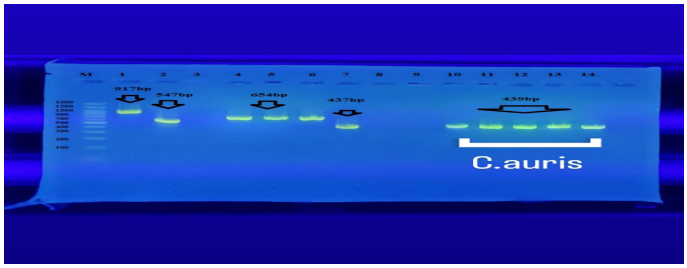

Figure 8: Gel electrophoresis for PCR product of 5.8S ribosomal RNA gene (Agarose 1.5% at 5 volt/cm^2, 90 min.), M: DNA ladder (100-1500 bp). Visualised under UV light after staining with Red Safe, (10-14) represent *C. auris* from a dog's eye swabs; the product length is 439 bp.

In this research, *Candida auris* was identified for the first time as a causative agent of eye infections in pet birds in Baghdad, Iraq, by using the 5.8S–ITS region, which successfully detected two isolates from the eyes of the birds, as illustrated in Figure 9.

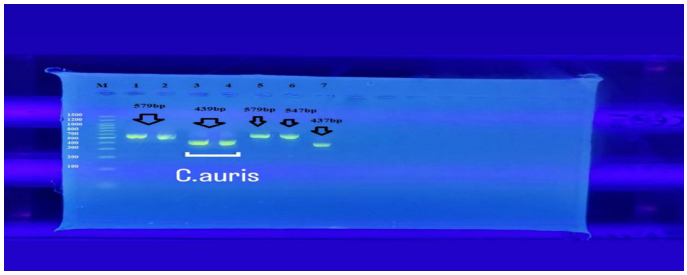

Figure 9: Gel Electrophoresis for PCR Product of 5.8S ribosomal RNA gene (Agarose 1.5% at 5 volt/cm^2, 90 min.), M: DNA ladder (100-1500 bp). Visualised under UV light after staining with Red Safe, (3,4) represents *C. auris* from bird-eye swabs; the product length is 439 bp.

In conclusion, there can be difficulties in identifying unusual yeast species like *C. auris*, false identification at the genus level, or the identification of strains using phenotypic methods, even with the use of manual commercial kits. On the other hand, conventional methods for identifying yeast species are used, and results can be available in three to five days. Therefore, molecular techniques such as PCR can provide a valuable alternative method that offers high specificity and sensitivity, yielding results more rapidly than the conventional methods currently used in hospitals and laboratories. In addition, identification of the species level of yeast is essential, especially for species that are intrinsically resistant to antimicrobial drugs.

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NOVELTY STATEMENT

This manuscript presents the first reported case of a *C. auris* eye infection in humans and pet animals. Diagnosis was made by the DL 96 II microbial ID/AST system and conventional PCR. These findings highlight the prevalence of *C. auris* in the country and may pose a zoonotic risk to public health.

AUTHORS' CONTRIBUTION

SHA: Responsible for sample collection, laboratory work, experimental infection, data analysis, and drafting the manuscript. ZAA: oversaw and made the study design and contributed to manuscript revision.

Generative AI and AI-assisted technology statement

The author(s) declare that no generative AI was used in the creation of this manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

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