Comparative Evaluations of the Pathogenesis of Candida auris Phenotypes and Candida albicans Using Clinically Relevant Murine Models of Infections

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ABSTRACT The newly emerged Candida species Candida auris is associated with an exponential rise in life-threatening invasive disease in health care facilities worldwide. Unlike other species, C. auris exhibits a high level of transmissibility, multidrug resistance, and persistence in the environment, yet little is known about its pathogenesis largely due to limited data from animal models. Based on in vitro biofilm evaluations and confocal laser scanning microscopy, C. auris phenotypes with different biofilm-forming abilities were identified, indicating potential clinical implications. Using clinically relevant murine models of implanted catheter, oral, and intraperitoneal infections, we comparatively evaluated the host site-specific pathogenic potential of C. auris phenotypes and Candida albicans. Based on the results of microbial recovery and scanning electron microscopy analysis of explanted catheters, compared to C. albicans, C. auris more avidly adhered and formed biofilms on catheters. However, although C. auris adhered to oral tissue ex vivo, unlike C. albicans, it failed to colonize the oral cavity in vivo, as demonstrated by microbial recovery and tissue histopathology analysis. In contrast, recovery from peritoneal lavage fluid and kidneys during time course experiments demonstrated that C. auris persisted longer in the peritoneal cavity and kidneys. Although there were clear niche-specific differences in pathogenic features between C. auris and C. albicans, no significant differences were noted between the C. auris phenotypes in vivo. The combined findings highlight unique niche-specific pathogenic traits for C. auris warranting further investigations. Understanding the factors contributing to the rise of C. auris as a nosocomial pathogen is critical for controlling the spread of this species.

IMPORTANCE The newly emerged Candida species C. auris has been associated with an exponential rise in invasive disease in health care facilities worldwide with a mortality rate approaching 60%. C. auris exhibits a high level of transmissibility, multidrug resistance, and persistence in hospital environments, yet little is known about its pathogenesis largely due to limited data from animal studies. We used clinically relevant murine models of infection to comparatively evaluate the host niche-specific pathogenic potential of C. auris and C. albicans. Findings demonstrated that C. auris adheres more avidly, forming robust biofilms on catheters implanted in mice. However, although C. auris adhered to oral tissue ex vivo, it failed to colonize the oral cavity in vivo. In contrast, in the intraperitoneal infection model, C. auris persisted longer in the peritoneal cavity and kidneys. Understanding the host-pathogen factors contributing to the rise of C. auris as a nosocomial pathogen is critical for controlling the spread of this species.

KEYWORDS fungal pathogens, Candida auris, Candida albicans, animal models, biofilm formation, biofilms, host-pathogen interactions
*Candida auris* has inexplicably and simultaneously emerged on six different continents as a nosocomial pathogen causing outbreaks in health care facilities in more than 40 countries (1–5), with mortality rates as high as 60% (6, 7) (https://www.cdc.gov/fungal/diseases/candidiasis/tracking-c-auris.html). *C. auris* exhibits several concerning features compared to other *Candida* species; the most surprisingly feature is the efficient person-to-person transmission (8). Typically, infections caused by *Candida* arise from the patient’s own microbiome; however, there is no evidence that *C. auris* can colonize the gastrointestinal tract or the oral cavity (9, 10). In addition to its aptitude to colonize skin, survive for weeks on nosocomial surfaces, and resist common disinfectants, *C. auris* exhibits high levels of drug resistance. More concerning however, is its unique ability to develop resistance to all main classes of antifungals (azoles, polyenes, echinocandins), severely limiting treatment options (7, 10, 11). In fact, the multidrug resistance pattern has been observed in around 40% of clinical isolates (7, 10). Compounding its high transmissibility and multidrug resistance, misidentification by available systems has resulted in delay in detection, further complicating clinical management (12).

While much remains unknown about its biology, *in vitro* studies demonstrated that *C. auris* expresses several key virulence factors common to *Candida*, such as phospholipases, proteinases, secreted aspartic proteases, adhesins, and the ability to form biofilms (13–16). One interesting observation is an aggregative phenotype where daughter cells fail to be released after budding, causing isolates to grow in clumps (14, 17, 18). This aggregative form was shown to be linked to transcriptional changes in genes involved in cell adhesion to surfaces (1), inducible by exposure to antifungals *in vitro* (19), and less virulent than the nonaggregative phenotype in invertebrate infection models (14, 17). Although yet to be investigated in vertebrate animal models, this unique growth feature could have potential clinical implications.

Unlike *Candida albicans*, *C. auris* does not undergo morphological switching between yeast and hyphal forms (5, 17), and the lack of filamentation may explain the distinct niches of colonization between the species; while *C. albicans* can colonize and infect mucosal surfaces, *C. auris* primarily colonizes skin (13, 16, 20, 21). However, occasional elongation of cells in *C. auris* into a filamentous and/or pseudohyphal form has been reported in response to temperature, cell cycle arrest, or depletion of Hsp90 (22, 23) and DNA damage from exposure to antimicrobial agents (24). Although *C. auris* does not form true hyphae, it can adhere and form robust biofilms on surfaces, albeit the biofilms are less complex than those formed by the hypha-producing *C. albicans* (13, 14, 25). Biofilm formation accounts for much of the antifungal tolerance among *Candida* species as a result of drug sequestration by the biofilm mannan-glucan polysaccharide matrix (26). While the molecular mechanisms governing drug resistance in *C. auris* are not fully characterized, a recent study demonstrated that the extracellular polysaccharide biofilm matrix sequestered nearly 70% of available triazole drug *in vitro* (27), clearly indicating a key role for *C. auris* biofilm formation in its high level of drug tolerance (7, 9, 25, 27).

Despite the gravity of this newly emerged nosocomial pathogen, few *in vivo* models of infections have been used to evaluate *C. auris*, and thus, a great deal is yet to be understood as to how *C. auris* colonizes and causes disease. Moreover, although indwelling venous catheters are considered important predictors of *C. auris* infections in hospitals, *in vivo* biofilm-associated infections remain understudied. Similarly, the ability of *C. auris* to colonize oral mucosal surfaces has not been observed clinically or investigated. Therefore, reliable animal models for *C. auris* candidiasis are critical to study the unique aspects of *C. auris* pathogenesis and host-pathogen interaction. To that end, in this study, murine models of oropharyngeal, intraperitoneal, and implanted catheter infections were used to comparatively investigate the pathogenic potential of two phenotypically different *C. auris* isolates. Importantly, to fully characterize the pathogenesis of *C. auris*, *C. albicans*, the species considered most pathogenic, was also included.
RESULTS

Strain-dependent variations in C. auris biofilm formation. Quantitative evaluation of biofilms demonstrated that all C. auris isolates formed significantly (P < 0.0001) less biofilms compared to C. albicans (Fig. 1A). However, a wide range of biofilm-forming abilities was noted among the isolates with isolate 0382 showing the highest activity and isolate 0387 showing the lowest activity. OD, optical density. Values are means plus standard errors of the means (error bars). ****, P < 0.0001.

Confocal laser scanning microscopy (CLSM) reveals variable spatial biofilm distribution. Based on fungal cell wall and mannan and glucan secreted polysaccharide fluorescent staining, C. albicans images revealed a dense biofilm with complex architecture consisting of hyphal and extracellular polysaccharides with significantly less dense biofilms formed by the C. auris isolates. However, biofilm of isolate 0382-HBF appeared homogeneous and more tightly packed than that formed by isolate 0387-LBF, which appeared patchy and heterogeneous (Fig. 2A to C).

Transmission electron microscopy (TEM) indicates variability in cell wall-outer fibrillar layer density. The overall cell structures of C. albicans and C. auris were comparable consisting of multilayer cell wall with an outer fibrillar layer. However, noticeable differences in fibrillar layer density and thickness were observed between the C. auris isolates in biofilm-associated (Fig. 1C), as well as planktonic cells (Fig. S2), with 0382-HBF cells exhibiting a longer fibrillar layer compared to the shorter but more dense layer seen in 0387-LBF cells (Fig. 1C and Fig. S2).

Significantly higher C. auris recovery from infected catheters compared to C. albicans. Based on assessment of microbial recovery from explanted catheters, for both C. auris isolates, CFU values were significantly higher than those for C. albicans (P = 0.0003 for isolate 0382-HBF; P < 0.05 for isolate 0387-LBF) (Fig. 3D). However, although slightly higher for isolate 0382-HBF, the difference between the C. auris isolates was not significant (median values of 2 × 10^5 and 1 × 10^5 CFU/ml, respectively).

Comparable levels of biofilm formation for C. auris and C. albicans within catheters by scanning electron microscopy (SEM) analysis. Representative images...
of catheter lumens revealed a robust and comparable ability for both C. auris isolates and C. albicans to form biofilms with extracellular matrix consisting of yeast cells for C. auris and primarily hyphae for C. albicans (Fig. 4). Influx of host immune cells can be seen in all samples (Fig. 4).

**C. auris isolates do not colonize oral tissue in vivo.** CFU recovery from tongues explanted over time demonstrated increasing levels of C. albicans recovery, whereas no C. auris was recovered at any of the time points sampled (Fig. 5C). Histopathology evaluation of tongue tissue revealed extensive C. albicans hyphal penetration of the epithelium with massive influx of inflammatory cells concomitant with the presence of lesions consistent with clinical candidiasis (Fig. 5B). In contrast, for both C. auris isolates, tongues appeared healthy with no fungal presence (Fig. 5A and B). Similarly, SEM revealed a thick C. albicans matrix covering the dorsum of tongues (Fig. 6B) with hyphae penetrating epithelial layers causing extensive tissue damage (Fig. 6C). In contrast, no C. auris was seen in any sample (Fig. 6A). To demonstrate that lack of C.
C. auris recovery from the oral infection model was not due to experimental variations in the inoculation method, in vitro experiments were performed to demonstrate comparable levels of uptake and release of cells from the calcium alginate swabs used for inoculating animals (Fig. S3).

C. auris isolates avidly adhere to tongue tissue ex vivo. In stark contrast to the in vivo model, microbial recovery from in vitro-infected excised tongues demonstrated avid C. auris adherence to tissue comparable to that of C. albicans (Fig. 5D). Interestingly, compared to C. albicans, C. auris recovery was higher (P < 0.05) when tongues were inoculated on the dorsum spiny surface, particularly for isolate 0382-HBF (<0.0001) (Fig. 5D, left), whereas recovery was comparable for all three when tongues were inoculated sublingually.

C. auris isolates are more adept at persisting and disseminating in an intra-peritoneal infection model. Based on CFU/milliliter peritoneal lavage, compared to C. albicans, C. auris recovery was higher and more consistent. Time course experiments indicated C. albicans clearance from the intraperitoneal cavity 2 to 4 days postinoculation (Fig. 7A); in contrast, C. auris persisted for up to 7 days postinoculation. Similarly, C. auris was recovered from kidneys after 4 days, whereas no C. albicans was recovered after 4 days (Fig. 7B, P > 0.05 for day 4). Interestingly, C. auris 0382-HBF had the highest burden and dissemination potential in the first 4 days of infection (Fig. 7B).

DISCUSSION

The multidrug-resistant species C. auris has been associated with an exponential emergence of outbreaks with life-threatening invasive disease (28, 29). In fact, C. auris is the first fungal pathogen categorized as a public health threat (30) due to its capacity to readily colonize skin and persist in abiotic surfaces of the health care environment allowing for high level of transmissibility between patients (31–34). Although relatively little is known about how C. auris colonizes and causes disease, it is quite evident that this novel pathogen is strikingly distinct among yeasts (35, 36). One unique growth feature in some isolates is cell aggregation (17) which was associated in vitro with differences in drug susceptibility, biofilm formation, and adhesin expression compared to nonaggregative isolates (1, 11, 14, 17, 32). Since previous studies have reported variable results for the biofilm-forming abilities of these isolates (14, 17, 18), we comparatively evaluated the 10 C. auris isolates in the CDC panel and selected the highest (0382-HBF) and lowest (0387-LBF) biofilm-forming isolates as phenotypic representatives for subsequent studies. Specifically, fluorescent confocal microscopy demonstrated a dense multilayer and homogeneous biofilm for isolate 0382-HBF, as was described for the “aggregative” phenotype, whereas isolate 0387-LBF formed a sparse biofilm with sporadic clumps of cells reported for the “nonaggregative” phenotype (18). A common observation, however, was the pronounced presence of secreted extracel-
FIG 4 Representative low- and high-magnification scanning electron microscopy images of biofilms formed within catheters implanted in mice. Images of 72-h biofilms formed in the lumens of infected catheters harvested from mice demonstrating the formation of sheets of thick and mature biofilms for all isolates. *C. albicans* biofilm consisted of a matrix of hyphae and extracellular polysaccharides, and *C. auris* isolates formed robust multilayer biofilms consisting of yeast cells and extracellular polysaccharide matrix. SN influx of immune cells can be seen in all samples (white arrows). Bars, 200 μm (A and C), 50 μm (B, D, and F), 40 μm (E), 20 μm (G and I), 10 μm (H, J, and L), and 5 μm (K).
ular polysaccharides, which was not surprising, as large amounts of extracellular matrix were reported for *C. auris* biofilms *in vitro* and *in vivo* (27). These spatial-structural differences may have clinical and therapeutic implications for biofilm-associated infections, cells may easily be released from less-compact biofilms leading to disseminated disease, whereas a dense biofilm is associated with drug resistance as was described for *C. albicans* (37, 38).

To investigate the virulence of *C. auris*, several studies used the invertebrate model *Galleria mellonella*, and similar to the *in vitro* studies, findings have been inconsistent.
In comparing *C. auris* to *C. albicans*, one study reported similar levels of virulence, while another study found the nonaggregating phenotype to be significantly more pathogenic than *C. albicans* (14). In another study, *C. auris* aggregative isolates were found to be less pathogenic than the nonaggregative isolates (17). So far, a limited number of animal studies have been performed to evaluate *C. auris*, with the majority using the intravenous systemic model (39–41), the most recent of which found *C. auris* to be less virulent than *C. albicans* (39). Therefore, our study was designed to comparatively evaluate the *C. auris* phenotypes and *C. albicans* using three other clinically relevant murine models of infection.

To investigate biofilm-associated infections, we used the mouse subcutaneous catheter model, a feasible and practical model to study catheter and indwelling device infections. A previous in vitro study evaluating *C. auris* adherence and biofilm formation on catheters found *C. auris* to adhere less to silicon elastomer catheters than *C. albicans*.

**FIG 6** Low- and high-magnification scanning electron micrographs of the dorsum (surface) of tongues from infected mice. (A) Representative image of surface of tongue of *C. auris*-infected animals. No visible colonization of *C. auris* of either isolate was seen on the surface of any of the tongues examined. (The image shown for isolate 0382-HBF is representative of both isolates.) (B) In contrast, a thick biofilm is seen covering the surfaces of tongues from *C. albicans*-infected animals with clinical candidiasis. (C and D) Upon higher magnification, matrix consisting of *C. albicans* hyphae could be seen with the hyphae (white arrows) penetrating the epithelial tissue, causing extensive tissue damage.
(13, 42); however, based on microbial recovery from explanted catheters, we found recovery of both C. auris isolates to be significantly higher than that of C. albicans when biofilms were formed in vivo. Although surprising, the seemingly superior adherence of C. auris to catheter material may explicate the emergence of C. auris as a nosocomial pathogen, given that catheter infections are considered a predictor for invasive C. auris infection in hospitalized patients (10, 29). The ability of C. auris to adhere to catheters and form biofilm in vivo was also demonstrated by SEM analysis revealing a massive amount of extracellular biofilm matrix, consistent with what was observed in a rat model of central-venous-catheter infection (27).

C. albicans is a common colonizer of the oral mucosal surfaces and the causative agent of oral candidiasis (43, 44); however, the ability of C. auris to adhere and colonize oral mucosal tissue has not been investigated. Using a modification of our established mouse model (45), we performed comparative time course studies to monitor colonization and disease development over time. Our findings demonstrated that both C. auris strains were cleared from the oral cavity within 4 h of inoculation, whereas C. albicans persisted, causing advanced clinical candidiasis. As hyphae are responsible for tissue penetration and mucosal infection, C. auris was not expected to cause clinical disease; however, the complete inability to colonize oral tissue was somewhat surprising. To explore whether this was due to deficiency in tissue adherence, we performed ex vivo experiments on excised mouse tongues. Surprisingly, in contrast to the in vivo findings, both C. auris isolates were recovered in higher numbers than C. albicans. C. albicans adherence to surfaces is mediated by glycosylphosphatidylinositol (GPI)-linked cell wall adhesins, most notably the ALS (agglutinin-like sequence) family of glycoproteins, with Als3 being the key member for tissue adherence (46). Interestingly, in a C.

![Graph A](image1.png)

**FIG 7** Evaluation of persistence and dissemination in a mouse model of intraperitoneal infection. (A) Microbial recovery from intraperitoneally infected mice sampled over 7 days demonstrated significantly higher and more persistent recovery for C. auris from intraperitoneal lavage fluid compared to C. albicans which was cleared from the cavity by day 4. (B) Comparable to recovery from lavage, C. auris was recovered from kidneys up to day 7, whereas C. albicans was effectively cleared by day 4. CFU for C. auris 0382-HBL were consistently higher at all time points sampled. Error bars represent standard errors of the means. ***, P < 0.001; ****, P < 0.0001.
auris transcriptome analysis of in vitro-grown biofilms, Kean et al. (47) found only ALS1 and ALS5. However, using bioinformatic and structural homology modeling, Singh et al. (48) found protein homologs of Ca-Als3p for C. auris and demonstrated in vivo humoral and cell-mediated protection upon vaccination with anti-Als3p antibodies. Nevertheless, the lack of key adhesins may explain the inability of C. auris to colonize the oral cavity. Alternatively, although speculative at this juncture, the discrepancies in C. auris tissue adherence under ex vivo and in vivo conditions suggest a key role for the host in controlling C. auris colonization. The contribution of host immune factors cannot be disregarded, as in vitro studies have indicated significant differences in host response to C. auris isolates. Using the zebrafish model, Johnson et al. (49) demonstrated that C. auris cells are able to avoid neutrophil recognition and survive in neutrophils upon phagocytosis. Interestingly, Pathirana et al. (18) found that strain 0382-HBF is able to survive inside neutrophils significantly better than C. albicans, whereas strain 0387-LBF was susceptible to neutrophil killing. Additionally, unique fungal recognition and/or host profiles to different C. auris phenotypes were also reported using an in vitro skin wound model (1).

Using the mouse model of intraperitoneal infection, previous studies showed C. albicans is effectively cleared from the peritoneal cavity of immunocompetent mice (50). Similarly, in our hands, C. albicans was cleared 2 to 4 days postinfection. In contrast, C. auris isolates persisted and were recovered from kidneys for up to 7 days postinfection. Although warranting further investigations, these preliminary findings are in line with in vitro findings demonstrating that human neutrophils preferentially engage and kill C. albicans over C. auris (49) and that innate immune recognition of C. auris and phagocytosis are different from those for C. albicans (51). Interestingly, Torres et al. (41) demonstrated that neutrophil-depleted mice succumb to systemic C. auris infection, while very small amounts of C. auris persisted in the immunocompetent infected control without causing disease, suggesting a potential opportunistic resurgence upon a change in immune status (41). Combined with our studies, these studies strongly suggest that C. auris employs unique strategies to interact with and infect the host in various niches.

In summary, in this study, we used three different animal models to comparatively evaluate the host site-specific pathogenic potential of C. auris. The findings from the catheter infection model demonstrated the avidity of C. auris to adhere and form robust biofilms on abiotic surfaces supporting the strong association of C. auris systemic diseases with the presence of indwelling catheters. Importantly, we demonstrate for the first time the inability of C. auris to colonize the oral cavity in a host, attributing a potential role for efficient host immune clearance on mucosal surfaces. In contrast, in the intraperitoneal infection model, C. auris persisted longer in the peritoneal cavity and kidneys. Interestingly however, although there were clear niche-specific differences in pathogenic features between C. auris and C. albicans, overall we did not observe significant differences between the C. auris phenotypes in the animal models. One limitation of the study is that only the C. albicans standard strain SC5314, which displays high virulence and biofilm formation, was used for comparison. Nevertheless, the combined findings warrant further in-depth analysis into the unique virulence traits of C. auris and the niche-specific host-pathogen interactions. A clear understanding of the various host and pathogen factors that have contributed to the rise of C. auris as a nosocomial pathogen is critical for developing new strategies to prevent and control the spread of this multidrug-resistant pathogen.

MATERIALS AND METHODS

In vitro Biofilm Formation. The reference strain C. albicans SC5314 (52) and the C. auris CDC panel containing 10 C. auris isolates (Antibiotic Resistance [AR] Isolate Bank number 0381-0390) were used in this study. Isolates were grown overnight in yeast peptone dextrose broth (YPD) (Difco Laboratories) at 30°C, washed in phosphate-buffered saline (PBS), and resuspended in RPMI 1640-HEPES (Invitrogen) medium (1 × 10⁶ cells/ml). For comparative evaluation of biofilm formation, C. auris isolates and C. albicans biofilms were grown by seeding 100 µl of cell suspensions in flat-bottom 96-well polystyrene microtiter plates. Following incubation at 37°C for 24 h, the wells were washed with PBS and biofilms were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-
tetrazolium (MTS) metabolic assay (Promega) per the manufacturer's recommendation. Color intensity was measured at 490 nm using a cell imaging multimode reader (Cytation 5; BioTek). Biofilms were also simultaneously evaluated by phase-contrast imaging. Based on initial evaluations, two C. auris strains representing “high biofilm former” (0382-HBF) and “low biofilm former” (0387-LBF) phenotypes were selected for subsequent experiments. Both isolates belong to clade II (East Asian) and were isolated in Pakistan; isolate 0382 was recovered from a burn wound, and isolate 0387 was recovered from blood.

Confocal laser scanning microscopy (CLSM) evaluation of in vitro-grown biofilms. C. auris 0382-HBF and 0387-LBF and C. albicans biofilms were grown in 24-well glass-bottom microtiter plates for 24 h as described above; biofilms were stained with a concanavalin A-conjugated to Alexa Fluor 647 (Invitrogen) (50 μg/ml) for 45 min at 37°C and 0.1% calcofluor stain (Sigma-Aldrich) for 10 min at room temperature. Biofilms were visualized using an inverted confocal laser scanning microscope (T2i; Nikon), and images were analyzed using Imaris 9.3 Arena software and ImageJ.

Transmission electron microscopy evaluation of cell ultrastructure. Cells harvested from biofilms and planktonic cultures were fixed and embedded in agarose, and blocks were postfixed with 1% osmium tetroxide—1.5% potassium ferrocyanide and then stained with uranyl acetate. Specimens were dehydrated in ethanol and embedded in Spurr resin. Ultrathin sections (70 nm) were examined.

Animal studies. All animal experiments were conducted at the AAALAC-accredited Animal Facility of the University of Maryland, Baltimore, and were approved by Animal Care and Use Committee. Three-month-old female C57BL/6 mice (oral model) and BALB/c mice (catheter and intraperitoneal models) were purchased from Envigo. Mice were housed at a maximum of five per cage, weighed, and closely monitored for any signs of distress throughout experimental periods.

Mouse infection model. The model previously described by Kucharíková et al. (53) was used with modifications. Fragments (0.5 cm) of polyurethane triple-lumen central venous catheters (Jorgensen Laboratories) precoated overnight with fetal bovine serum (Gibco) were incubated with 1 × 10^8 cells/ml cell suspensions in RPMI for 1.5 h at 37°C, rinsed, and kept on ice until implanted. BALB/c mice were anesthetized with 0.5 ml intraperitoneal injections of tribromoethanol (TBE) solution (250 mg/kg of body weight; Sigma-Aldrich); the dorsum was shaved, a small incision was made aseptically, and a subcutaneous tunnel was created allowing for insertion of five to eight pieces of preincubated catheters (Fig. 3A to C). Incisions were sealed using 3M Vetbond tissue glue, and lidocaine analgesic gel was applied. Biofilms were allowed to form within catheters for 72 h, and then animals were euthanized by CO2 inhalation followed by cervical dislocation. Catheters were harvested individually, aseptically fragmented, and sonicated in sterile PBS to detach biofilms. Cell suspensions were diluted and plated in triplicate on chromogenic medium CHROMagar (DRG International) for CFU enumeration. A total of seven mice were included for each group, and a total of 25 to 35 catheters were analyzed individually. Experiments were performed on four separate occasions, and the data were combined.

Scanning electron microscopy of explanted catheters. From each group, representative catheters were processed for scanning electron microscopy (SEM) to visualize in vivo-grown biofilms. Catheters were cut longitudinally to expose the lumen, fixed in 2% paraformaldehyde—2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, serially dehydrated in ethyl alcohol (30 to 100%), and critical point dried. Samples were coated with carbon and observed with Quanta 200 SEM (FEI Co.), and images were processed using Adobe Photoshop software.

Mouse model of oropharyngeal candidiasis. The established mouse model of oropharyngeal candidiasis (54) was used as we previously performed (45). C57BL/6 mice were immunocompromised by subcutaneous administration (0.2 ml; 250 mg/kg) of cortisone-acetate (Sigma-Aldrich) in the neck dorsum every other day starting 1 day preinfection. Animals were divided into three groups for infection with (i) C. albicans, (ii) C. auris 0382-HBF, and (iii) C. auris 0387-LBF. On the day of infection, mice were anesthetized with TBE and then orally infected by placing calcium alginate swabs (Fisher Scientific, Waltham, MA) saturated (10 min, room temperature [RT]) with cell suspension (2 × 10^7 cells/ml). Swabs were kept sublingually for 45 min, and the animal’s body temperature was maintained at 37°C using a heat lamp. Animals were monitored until they recovered from anesthesia and daily for any clinical signs of distress. Animals were euthanized at different time points (4, 24, 48, and 96 h postinfection), and their tongues were harvested, weighed, homogenized, and cultured in triplicate on CHROMagar for CFU enumeration (CFU/gram tissue weight). A total of three or four mice were included in each group per time point; experiments were performed on two separate occasions, and results were combined.

Histopathology and SEM of infected tongues. Representative tongues from each group were fixed in 10% formalin and embedded in paraffin, and xylene-deparaffinized sections were stained with periodic acid-Schiff (PAS); slides were examined by light microscopy. Representative tongues were also processed for SEM as described above.

Tongue ex vivo model of infection. An ex vivo infection model was used as we previously described (55) to specifically evaluate tissue adherence abilities in the absence of host immune factors. Tongues excised from healthy euthanized mice were weighed and infected in vitro mimicking in vivo procedure; top or bottom surfaces of tongues were streaked with saturated swabs which were then left under the tongues for 45 min at 37°C. Tongues were rinsed, homogenized in PBS, and plated for CFU enumeration.

Mouse model of intraperitoneal infection. BALB/c mice were injected intraperitoneally with 0.2 ml of cell suspensions (3.5 × 10^7 cells/ml), and groups of animals were euthanized at different time points: 1, 2, 4, and 7 days postinfection. Peritoneal cavities were lavaged by injection of 3 ml sterile PBS followed by gentle massaging; fluid was carefully recovered with a syringe. Kidneys were harvested, weighed, and homogenized for evaluation of disseminated disease. Lavage fluid and kidney homogenates were diluted.
and plated for CFU enumeration. A total of 20 mice were included in each group. The experiments were performed on three separate occasions, and the results were combined.

**Data analysis.** Statistical analysis was performed using GraphPad Prism 8.0 software. The standard error of the mean was used in all graphs; one-way analysis of variance (one-way ANOVA) was used with Tukey’s post hoc test. P values of <0.05 were considered significant.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**FIG S1**, TIF file, 0.9 MB.

**FIG S2**, TIF file, 2.5 MB.

**FIG S3**, TIF file, 0.1 MB.

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We declare that we have no conflicts of interest.

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