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First Detection of Candidozyma (Candida) auris in Wastewater in Türkiye: Evidence for Environmental Circulation under a One-Health Framework

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Abstract

Background

Candidozyma (Candida) auris is an emerging multidrug-resistant fungal pathogen with expanding ecological presence beyond healthcare settings. Wastewater-based surveillance (WBS) has recently become an important tool for early detection of community-level circulation. However, no environmental monitoring data have previously been reported from Türkiye. This study aimed to investigate the presence of *C. auris* in hospital-associated wastewater and assess its potential role as an environmental reservoir.

Methods

Between January and July 2025, 46 wastewater samples were collected twice weekly from the main outlet of a tertiary hospital. Samples underwent concentration, culture on selective chromogenic agar, and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) confirmation. DNA extracted from Sterivex filters was tested using the species-specific CauF/CauR qPCR assay. All runs included appropriate positive, negative, and no-template controls. The Bio-speedy *Candida auris* Clade Detection qPCR Kit (Bioeksen R&D Technologies Inc., Türkiye) was used for clade identification of the isolates. Antifungal susceptibility testing of the recovered *C. auris* isolates was performed using the EUCAST reference microdilution method, and minimum inhibitory concentrations (MICs) were interpreted according to EUCAST guidelines.

Results

C. auris DNA was detected in three wastewater samples (Ct 31–36), and two culture-confirmed isolates were recovered and stored. The repeated qPCR positivity across different sampling dates suggests intermittent yet detectable shedding into the sewage system. Importantly, no outbreak was ongoing in the hospital, with only sporadic clinical cases reported, indicating that even low-level colonization may generate measurable environmental signatures. All isolates were evaluated as Clade-1. Antifungal susceptibility testing revealed high fluconazole MICs (> 64 mg/L) in both *C. auris* isolates, consistent withno activity of fluconazole. In contrast, low MIC values for echinocandins (anidulafungin 0.06–0.25 mg/L; micafungin 0.125–0.25 mg/L) indicated favorablein vitro activity of this antifungal class against the tested isolates.

Conclusion

This study provides the first culture-confirmed detection of *C. auris* in wastewater in a tertiary hospital in Türkiye, demonstrating organism persistence under local wastewater conditions. These findings

highlight the value of WBS as an early-warning component of fungal surveillance and underscore the need to integrate environmental monitoring into One Health strategies for *C. auris*.

1. Introduction

Wastewater-based surveillance (WBS), originally accelerated during the COVID-19 pandemic, has emerged as a promising strategy to detect emerging biological threats at a population scale. Wastewater surveillance of *Candidozyma* (formerly *Candida*) *auris* has gained substantial importance in recent years, providing One Health-aligned insights that help trace both the organism's ecological niches and life cycle dynamics, as well as the pathways and hotspots of infection transmission. (Babler et al 2023) This fungal pathogen having multdrug resistance potential leads toinvasive and healthcare-associated infections with alarming mortality rates. Its environmental resistanceincluding thermotolerance, halotolerance, biofilm formation, and persistence on abiotic surfaces suggests a capacity for survival beyond clinical environments. Although it was first isolated from hospital environments, increasing reports indicate that *C. auris* may also exist in natural ecosystems such as salt marshes, sandy beaches, and estuarine waters, raising questions about its broader ecological niche. (Chang 2025)

Several studies across the United States and the United Kingdom have demonstrated that *C. auris* nucleic acids and even viable isolates can be recovered from hospital effluents, ward wastewater, and municipal wastewater treatment plants. Wastewater monitoring in Baltimore detected *C. auris* DNA in 11.8% of influent samples over 47 weeks, (Nwaubani et al 2024) while community-scale studies in Nevada reported 79% sample positivity across healthcare-linked sewersheds. (Barber 2023) Similarly, ward-level wastewater sampling during a hospital outbreak revealed qPCR sensitivity of 100% compared with clinical colonization screening. (Rossi 2023) More recently, nationalscale surveillance identified *C. auris* genomic material in wastewater solids from 34% of U.S. wastewater treatment plants, strongly associated with older populations and higher densities of hospitals and nursing homes. (Zulli 2024)

WBS is an established population-level monitoring approach that relies on the systematic analysis of untreated wastewater to capture aggregated biological signals, including pathogens, antimicrobial residues, and disease-associated biomarkers. (Tlhagale et al., 2022; Kocamemi et al., 2024) By integrating clinical activity with environmental compartments, WBS offers a unique opportunity to detect emerging threats beyond the limits of patient-based surveillance alone. In Türkiye and neighbouring regions, rising rates of fluconazole-resistant and echinocandin-tolerant *Candida auris* have been reported, yet environmental surveillance frameworks capable of tracking this pathogen outside healthcare settings remain absent. Considering (i) the pathogen's surface persistence, (ii) shedding via colonized patients, (iii) its potential aquatic origin hypotheses, and (iv) wastewater transmission models developed internationally, systematic investigation of *C. auris* in wastewater streams may provide early warning signals for silent spread, bridge clinical blind spots, and illuminate environmental adaptation.

This study represents the first structured attempt to detect *C. auris* in healthcare-associated wastewater sample in Türkiye. We combine optimized culture-based isolation, ITS2-targeted qPCR assays, and genomic comparison to elucidate detection rates, sample-specific signal dynamics, and potential reflection of clinical epidemiology. This work provides foundational knowledge for integrating WBS into fungal surveillance frameworks and informs One Health understanding of *C. auris* ecology.

2. Materials and Methods

2.1. Study Design and Sampling Framework

This study was conducted as part of a multi-partner research initiative on *C. auris* (124S746). Within this framework, wastewater-based environmental surveillance was carried out between January and July 2025. Composite wastewater samples were collected twice weekly from the primary effluent discharge point of Gazi University Hospital in Ankara. In total, 46 wastewater samples were obtained from this hospital-associated wastewater system during the surveillance period (8 January-24 July 2025). The sampling location was selected due to its direct connection to a tertiary healthcare facility and its suitability for assessing pathogen shedding into wastewater and its potential environmental dissemination.

2.2. Wastewater Collection and Transport

Wastewater samples (50–100 mL) were aseptically collected from the main hospital wastewater effluent of Gazi University Hospital in Ankara using sterile polypropylene containers during morning flow hours. Samples were immediately stored on ice (4–8°C), transported to the laboratory within 2–6 hours, and processed under Biosafety Level 2 precautions with institutional biosafety authorization. To maximise recovery efficiency, two complementary concentration approaches were used: 50 mL aliquots were centrifuged at 4,000 × g for 30 minutes and the resulting pellets were resuspended in 1–2 mL sterile phosphate-buffered saline (PBS, pH 7.4). Concentrates obtained from both workflows were pooled for downstream culture-based analysis, and sterile water field blanks processed in parallel were included as negative controls.

2.3. Culture-Based Detection and Antifungal Susceptibility of *Candidozyma auris*

Concentrated suspensions were streaked onto selective chromogenic agar plates (CHROMAgar *Candida* Plus™, CHROMagar, France and Brilliance™ Candida Agar, Oxoid), previously adopted in the project for phenotypic identification of *C. auris* isolates. Plates were incubated aerobically at 37°C for 48 hours. Colonies exhibiting chromogenic colour change consistent with *C. auris* morphology were subcultured on Sabouraud dextrose agar (SDA) for purification. Pure colonies were stored in 20% glycerol at 80°C. Environmental isolates with presumptive identification of *C. auris* were subjected to matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), following the same analytical workflow used for clinical isolates in the study. Antifungal susceptibility testing of the recovered *C. auris*

isolates was performed using the EUCAST reference microdilution method (Guinea et al., 2023), and minimum inhibitory concentrations (MICs) for amphotericin B, azoles, echinocandins, and flucytosine were determined in accordance with EUCAST guidelines (version 12.0, 2025 and Arendrup et al., 2026).

2.4. DNA Extraction and Molecular Confirmation

To obtain DNA for metagenomic analyses, extraction was performed from filtered water samples under sterile and carefully controlled laboratory conditions. Field-collected samples retained on 0.22 µm Sterivex filters were brought to room temperature upon arrival at the laboratory. Each Sterivex cartridge was aseptically opened to avoid contamination, the filter membrane was cut and trimmed to appropriate dimensions as described in the kit protocol, and the pieces were directly transferred into the isolation tubes of the NucleoSpin eDNA Water Kit (Macherey-Nagel, Germany). DNA extraction was then carried out strictly following the manufacturer's instructions. Following isolation, DNA concentration and purity were assessed using a SPECTROstar Nano spectrophotometer (BMG Labtech, Germany). These measurements were performed to evaluate extraction efficiency and to confirm the suitability of DNA samples for downstream metagenomic analyses. Species confirmation was performed by ITS region amplification using species-specific detection of *C. auris* was performed using the primer pair CauF (5'-CGCACATTGCGCCTTGGGGTA-3') and CauR (5'-GTAGTCCTACCTGATTTGAGGCGAC-3'), as originally described by Kordalewska et al. (2017). qPCR amplifications were performed using the SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix (Bio-Rad, USA) on a CFX Connect Real-Time PCR Detection System (Bio-Rad, USA). The analytical conditions were optimized to minimize the impact of potential PCR inhibitors originating from environmental samples, aided by the high inhibitor tolerance of the reaction mix. To verify reaction accuracy and monitor potential contamination, positive controls, negative controls, and no-template controls (NTC) were included in every run, and amplification curves were evaluated to confirm target specificity and reaction efficiency.

2.5. Clade detection

DNA extraction from two fresh cultures was carried out using the Bio-speedy Rapid Nucleic Acid Extraction Kit (Bioeksen R&D Technologies Inc., Türkiye) with the Zybio EXM3000 robotic system and the nine-minute B-200 protocol, in accordance with the manufacturer's recommendations. The identification, confirmation, and clade detection of *C. auris* isolates were performed using the BIO-RAD CFX96 real-time PCR platform (Bio-Rad Laboratories, USA) together with the Bio-speedy *Candida auris* Clade Detection qPCR Kit (Bioeksen R&D Technologies Inc., Türkiye).

3. Results

3.1. Detection of *C. auris* in Wastewater Samples

During the first 7-month sampling period of the ongoing One-Health surveillance project, *C. auris* was successfully isolated from two independent wastewater samples among 46 samples analysed. These findings represent the first culture-confirmed environmental detection of *C. auris* from hospital-

associated and/or municipal wastewater matrices in Türkiye. These isolates were identified using chromogenic medium growth characteristics followed by MALDI-TOF MS confirmation, consistent with the phenotypic identification pipeline applied to clinical isolates. All isolates were identified as Clade I based on the results of the qPCR-based clade detection assay.

Antifungal susceptibility testing of the two *C. auris* isolates revealed identical minimum inhibitory concentration (MIC) values for amphotericin B (0.5 mg/L), interpreted as intermediate according to EUCAST criteria. While both isolates demonstrated high MIC values for fluconazole (> 64 mg/L), consistent with lack of in vitro activity, EUCAST breakpoints are not established for fluconazole against *C. auris*,. Voriconazole MICs were 0.5 mg/L for both isolates, while itraconazole MICs were 0.125 mg/L and 0.06 mg/L, and posaconazole MICs were 0.06 mg/L and 0.016 mg/mL, respectively;There are yet no determined EUCAST clinical breakpoints for these triazoles against *C. auris* due to insufficient evidence.. In contrast, low MIC values were observed for echinocandins, with micafungin MICs of 0.25 mg/L and 0.125 mg/L, and anidulafungin MICs of 0.06 mg/L and 0.25 mg/L, all interpreted as susceptible. Flucytosine MICs were 0.25 mg/L and 0.125 mg/L, although no interpretive criteria were available. Overall, the antifungal susceptibility profile is consistent with known Clade I characteristics, showing high fluconazole MICs and preserved echinocandin activity in environmental isolates recovered from wastewater.

Of the 46 hospital wastewater samples analysed in 7 months period, three samples as 5580 (07 April 2025), 5581 (10 April 2025) and 5582 (30 April 2025) yielded amplification signals indicative of positivity using the CauF/CauR assay. The detection of *C. auris* genetic material in hospital-associated wastewater suggests that DNA originating from clinical sources may be introduced into sewage systems, where it can persist for detectable periods. The repeated detection across different sampling days indicates the presence of *C. auris* genetic material in hospital wastewater over a defined period, potentially reflecting ongoing clinical cases or transient carriage within the facility.

This study provides the first evidence of *C. auris* in wastewater in Türkiye, confirming that the pathogen is shed into sewage compartments and can be successfully recovered, archived and phenotypically characterised. To the best of our knowledge, this report constitutes the first wastewater-based identification of *C. auris* on a transcontinental corridor between Europe and Western Asia. Collectively, these findings highlight wastewater as a relevant environmental interface for the epidemiological surveillance of *C. auris* and point to the potential for unrecognised environmental circulation beyond clinical settings. From a national perspective, this finding establishes wastewater as a previously unexplored environmental compartment for *C. auris* surveillance in Türkiye, underscoring the need to integrate non-clinical matrices into routine monitoring frameworks. Within a One Health context, the detection of *C. auris* at the environment–human interface highlights the interconnectedness of clinical activity and environmental dissemination pathways, reinforcing wastewater-based surveillance as a valuable early warning and risk assessment tool. Collectively, these observations support the inclusion of environmental reservoirs in future strategies aimed at understanding and mitigating the spread of emerging fungal pathogens.

Discussion

This study represents the first culture-confirmed detection of *C. auris* in wastewater in Türkiye, expanding the global map of *C. auris* environmental circulation beyond North America and Western Europe. The recovery of viable isolates from municipal WBS underscores that *C. auris* can persist and potentially disseminate through wastewater systems under local climatic and infrastructural conditions. Our findings demonstrate that the shedding of *C. auris* into wastewater is not restricted to outbreak-associated hospital effluents but may also occur at the community level, potentially reflecting unrecognised reservoirs or low-level colonisation within the population. This interpretation is further supported by the absence of an active outbreak at the hospital during the sampling period, despite the presence of sporadic clinical cases, suggesting that even intermittent or low-intensity transmission can generate a detectable environmental signal in wastewater.

The detection of *C. auris* in Türkiye aligns with previous international reports that have demonstrated the feasibility and epidemiological value of wastewater surveillance for fungal pathogens. In the United Kingdom, Davidson et al. (2025) successfully identified *C. auris* in hospital ward wastewater, with PCR sensitivity reaching 100% and culture sensitivity 75%, and showed near-complete genomic similarity (< 10 SNPs) between patient and wastewater isolates. Similarly, studies in the United States have repeatedly confirmed *C. auris* presence in municipal and hospital wastewater: Rossi et al. (2023) in Nevada, Nwaubani et al. (2024) in Baltimore, and Babler et al. (2023) in Florida all reported successful recovery of viable or molecularly confirmed *C. auris* from wastewater samples. Detection rates in these studies ranged from 10–12% in city-scale sampling to 79–94% in healthcare-associated sewers, with genetic or spatial links to ongoing clinical outbreaks.

Our culture-based confirmation is particularly significant because most earlier studies primarily relied on molecular assays such as qPCR or amplicon sequencing. While Zulli et al. (2024) demonstrated *C. auris* nucleic acids in 34% of 190 U.S. wastewater treatment plants, and Chang et al. (2025) identified antifungal-resistant variants through wastewater genomics, only a handful of reports worldwide have achieved successful isolation of viable *C. auris* from environmental samples. The present study addresses a critical geographic and methodological gap by demonstrating that *C. auris* can remain culturable in wastewater under conditions representative of Türkiye, a country characterised by pronounced climatic heterogeneity, mixed sewer infrastructures, and uneven wastewater treatment coverage.

Another notable observation is that *C. auris* could be recovered despite expected dilution, fluctuating flow rates, and exposure to physicochemical stressors such as temperature, salinity, and disinfectants. This finding reinforces the organism's known thermotolerance, halotolerance, and environmental persistence, which have been described previously in *in vitro* studies and field isolates from coastal ecosystems. The ability to survive under such stress conditions supports hypotheses proposed by Babler et al. (2023) and Angeles et al. (2020) suggesting that environmental antifungal residues originating from pharmaceutical use and other anthropogenic inputs, together with associated selective

pressures in aquatic environments, may promote the adaptation and persistence of *C. auris* beyond healthcare settings. Comparing Türkiye's wastewater detection with global data, our results suggest that *C. auris* may already be circulating silently in the environment, preceding or complementing clinical detections. Similar early-warning capacity was demonstrated by Chavez et al. (2024), who identified *C. auris* in wastewater weeks before clinical cases appeared in Utah. Hence, the current detection provides the first evidence that wastewater surveillance can play a comparable sentinel role within the Turkish context a region bridging Europe, the Middle East, and Central Asia, and therefore of strategic importance for transcontinental fungal spread.

The antifungal susceptibility profile of the environmental *C. auris* isolates demonstrated high MIC values for fluconazole and preserved susceptibility to echinocandins, which is consistent with previously reported Clade I characteristics. Notably, the detection of isolates with clinically relevant resistance traits in wastewater, even in the absence of an ongoing outbreak, suggests that antifungal-resistant *C. auris* may persist and circulate at low levels in environmental reservoirs. These findings support the potential role of wastewater systems as silent reservoirs and early warning platforms for antifungal resistance within a One Health framework. These results emphasize the interconnectedness of human, environmental, and healthcare domains. The presence of *C. auris* in wastewater demonstrates that colonized individuals contribute to environmental dissemination, potentially reintroducing the pathogen into healthcare or community settings. Coupled with Türkiye's increasing reports of fluconazole-resistant clinical isolates (Ağalar et al 2025), the establishment of a wastewater-based surveillance (WBS) framework may allow earlier detection of emerging antifungal resistance, especially for echinocandins and provide data to support infection control and environmental remediation strategies.

Conclusion

In conclusion, this study confirms the **first environmental isolation of C. auris from hospital wastewater in Türkiye**, bridging a critical knowledge gap between clinical observations and environmental occurrence. Our findings corroborate international evidence that wastewater systems can serve as sensitive and scalable indicators of *C. auris* circulation, capable of detecting the pathogen even in the absence of reported clinical outbreaks. Integration of WBS into Türkiye's fungal and antimicrobial resistance monitoring systems will be vital to detect emerging threats promptly, to understand regional transmission dynamics, and to align national health security measures with global One Health strategies.

Declarations

Author Contributions: S.E.; methodology, investigation, data curation, A.G..; methodology, investigation, data curation, as being TUBITAK STAR grantee of the project, E.K..; methodology, investigation, data curation, E.A.S..; methodology, validation, formal analysis, investigation, data curation, H.B.E.; methodology, formal analysis, investigation, data curation, B.K.; formal analysis, data curation, D.G.; formal analysis, data curation, S.A.A.; formal analysis, data curation, G.E.; methodology, software, conceptualisation, formal analysis, data curation, I.C.; software, conceptualisation, formal analysis, data

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