Journal of Hospital Infection 167 (2026) 199–203

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Detection of *Candidozyma (*formerly *Candida) auris* from ward wastewater during an outbreak using culture and molecular methods

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ARTICLE INFO

Article history:
Received 12 August 2025
Accepted 21 October 2025
Available online 4 November 2025

Keywords:
Candidozyma auris
Candida auris
Wastewater
Outbreak
Ward wastewater
Surveillance

SUMMARY

Background: *Candidozyma (Candida) auris* is a multi-drug-resistant fungal pathogen associated with protracted, costly hospital outbreaks. Conventional patient-level screening is resource-intensive and may not be cost effective. Wastewater surveillance may offer a pragmatic, ward-level approach to early detection.

Aims: To determine whether *C. auris* can be reliably detected in ward wastewater during an ongoing hospital outbreak; to compare the performance of culture- and PCR-based wastewater methods with patient-level colonization screening.

Methods: Between August and September 2024, during an ongoing *C. auris* UK hospital outbreak, a point-prevalence survey was conducted alongside sampling of 12 corresponding ward sluices. Wastewater grab samples and environmental swabs from sluice sinks, macerators and handwash sinks were investigated by direct culture and enrichment broth, as well as AurisID® real-time PCR assay on the centrifuged pellet. Whole-genome sequencing was performed on matched patient and wastewater isolates to assess genetic relatedness.

Findings: Using the detection of ≥1 *C. auris* patient on a ward as the standard, sensitivity of culture was 75% (95% confidence interval (CI) 35–97%) and specificity 100% (95% CI 40–100%); AurisID® PCR sensitivity was 100% (95% CI 63–100%) and specificity 75% (95% CI 19–99%). Whole-genome sequencing analysis demonstrated clustering and clonality of wastewater and patient isolates (<10 single-nucleotide polymorphisms difference).

Conclusion: We report the first detection of *C. auris* from hospital ward wastewater using culture and PCR. Screening ward wastewater may offer a sensitive, cost-effective alternative to individual patient screening in low-prevalence settings, supporting earlier ward-level detection: prospective, longitudinal studies are needed.

This article is part of a special issue entitled: Healthcare water systems and wastewater disposal published in Journal of Hospital Infection.

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https://doi.org/10.1016/j.jhin.2025.10.024

Introduction

Candidozyma (formerly *Candida*) *auris* is a multi-drug-resistant yeast and a WHO critical fungal priority pathogen [1] that causes persistent colonization and treatment-refractory invasive infections, often associated with protracted and expensive outbreaks [2—4], particularly in intensive care units (ICUs). Reports of clinical and colonization cases have increased dramatically since its first US isolation in 2016, respectively doubling and tripling in 2019—21 [5]. Recently updated UK Health Security Agency guidelines recommend screening of high-risk patients using composite nose-axilla-groin swabs, but clinical (individual patient) screening in a low-prevalence setting may not be cost-effective [6]. The ward sluice room (dirty utility) processes patient excreta and grey water from bedside patient bathing in macerators and sluice sinks (hoppers). Given this, and the prodigious shedding of *C. auris* from patient skin into the environment, surveillance of wastewater from ward sluices (ward wastewater) may provide a novel, efficient and less expensive alternative to clinical screening. Wastewater-based *C. auris* surveillance using culture and molecular methods has been successfully performed in community wastewater from sewage treatment plants servicing healthcare facilities experiencing an outbreak in Nevada [7,8] and molecular methods are being used to track *C. auris* in 150 sites across the USA [9]. Here we report, for the first time, the detection of *C. auris* in ward wastewater from sluices during a UK hospital outbreak.

Methods

St Thomas's Hospital is a large central London hospital which experienced a protracted *C. auris* outbreak affecting 100 patients, including one candidaemia, between October 2023 and August 2024, despite infection prevention and control (IPC) efforts, including refurbishment of the sentinel ward. Most cases were identified by screening with very few positive invasive specimens. Whole-genome sequencing has confirmed that this reflects a clonal outbreak, and not the importation of diverse *C. auris*.

During August—September 2024, a point-prevalence survey (PPS) of patients on all nine inpatient wards on the affected wing was undertaken, in conjunction with ward wastewater testing of the 12 sluice rooms supplying them, using both culture-based and molecular methods. Grab samples of wastewater (50 mL) were collected into Falcon tubes using sterile tubing from the bowl of the sluice sink, the standing water remaining in the body of the macerator after a run and the P-trap of the handwash sink of ward sluices. A simultaneous Amies swab sample was taken, scrubbing below the waterline of the sluice sink, the inside of the macerator and beyond the plughole of the handwash sink for 30 s. Swab samples were processed immediately by direct inoculation on to chromogenic agar (CHROMagar™ Candida Plus, E&O Laboratories Ltd, UK) and into enrichment broth (salt-Sabouraud-dulcitol broth, SSDB). Wastewater samples were first centrifuged, and aliquots of the resulting pellet similarly inoculated on to chromogenic agar and into SSDB. Remaining pellet aliquots were frozen at -80 °C for subsequent PCR analysis. Culture plates were incubated at 37 °C and checked daily; enrichment broths were incubated at 40—42 °C (with shaking) for 5 days, followed by terminal sub-culture on to chromogenic agar. Yeasts were identified phenotypically, then sub-cultured on to Sabouraud dextrose agar with chloramphenicol (E & O Laboratories) for matrix-assisted laser desorption/ionization—time-of-flight (MALDI-ToF) identification (Bruker, USA). Any *C. auris* colonies identified were stored at -80 °C. Patient swabs were taken from nose, axilla and perineum and directly cultured on chromogenic agar (CHROMagar Candida), with phenotypic identification and confirmation using MALDI-ToF. High-quality DNA extracts were prepared from broth cultures of all saved culture-confirmed *C. auris* isolates from patients ($N = 4$) and wastewater ($N = 22$) using the MasterPure yeast DNA extraction kit (EpiCentre). Of the 12 *C. auris* colonized patients identified in the point prevalence screen, four patients had clinical isolates saved for genomic analysis. Whole-genome sequencing was undertaken on the MiSeq platform (Illumina, https://www.illumina.com/), and reads mapped to a reference *C. auris* genome (clade I, B8441) using Burrows—Wheeler Alignment tool, with single nucleotide polymorphism (SNP) calling using bcftools (v1.21) mpileup, call and filter. Isolates differing by <10 SNPs were deemed as closely/highly related.

Detection of *C. auris* by PCR on freeze-thawed pellets was performed using the aurisID® real-time PCR assay (IMMY, USA), following DNA extraction with the Monarch® Genomic DNA Purification Kit (New England Biolabs, USA) with internal controls and results confirmed by repeat testing. Antifungal susceptibility testing was performed using Sensititre™ YeastOne™ (Thermo Scientific™, USA). Resistance was interpreted based on tentative CDC *C. auris* breakpoints based on the Clinical & Laboratory Standards Institute (CLSI) method: minimum inhibitory concentration (MIC) fluconazole $\geq$32; amphotericin B $\geq$2; anidulafungin $\geq$4, micafungin $\geq$2; caspofungin $\geq$2.

Results

The PPS detected *C. auris* colonization in $\geq$1 patient on seven of nine wards, supplied by eight of 12 sluices (see Table I). Culture testing found *C. auris* in at least one site (sluice sink or macerator) in six of eight sluice rooms (sensitivity 75%), and all eight detected *C. auris* using the AurisID assay (sensitivity 100%). Two wards had no patients detected on PPS, supplied by four sluices; culture testing was negative (100% specificity); one sluice room had a positive PCR from the macerator (75% specificity). No hand sink samples were positive for *C. auris* using culture methods. To verify our findings, ward wastewater from an ICU sluice at hospital without an outbreak was tested; *C. auris* was not detected by culture (specificity maintained:100%) and PCR (specificity increased to 80%).

Phylogenetic analysis demonstrated clustering of isolates and clonality of *C. auris* in patients and wastewater with minor variation in the genomic content ($<$10 SNP difference)

Table I
Detection of *Candidozyma auris* from ward wastewater using culture and PCR

Ward	Ward type	*C. auris* colonized patients on PPS	Sluice	Culture			PCR		
				Sluice sink	Macerator	Combined	Sluice sink	Macerator	Combined
A	ICU	0/7	A1	N	N	N	N	N	N
			A2	-	N	N	-	N	N
B	ICU	1/11	B1	P	P	P	P	P	P
			B2	P	P	P	P	P	P
C	Emergency care	3/20	C	N	N	N	P	P	P
E	Cardiac surgery	1/17	E	P	N	P	P	P	P
F	ICU (ECMO)	0/8	F1	N	—	N	N	—	N
			F2	N	N	N	N	P	P
G	Cardiac	1/27	G	N	P	P	P	P	P
H	Cardiac surgery	3/26	H	N	P	P	P	P	P
I	Vascular surgery	2/23	I	P	P	P	P	P	P
J	HDU	1/14	J	N	N	N	P	P	P
				Sensitivity		75%			100%
				95% CI		35–97%			63–100%
				Specificity		100%			75%
				95% CI		40–100%			19–99%

P = *C. auris* found, N = negative. ECMO, extracorporeal membrane oxygenation; HDU, high-dependency unit; ICU, intensive care unit; PPS, point-prevalence survey. Column 3 shows number of *C. auris* colonized patients identified using culture-based methods out of number of patients tested per ward on the affected wing. Positive detections from individual sampling sites are detailed for both culture and PCR. Sensitivity and specificity for the detection methods are calculated comparing detection of *C. auris* in any wastewater sample against a standard reference of presence of ≥1 colonized *C. auris* patient on award, with 95% Clopper—Pearson confidence intervals (CIs). Sluice A2 did not have a sluice sink, Sluice F1's macerator was out of service.

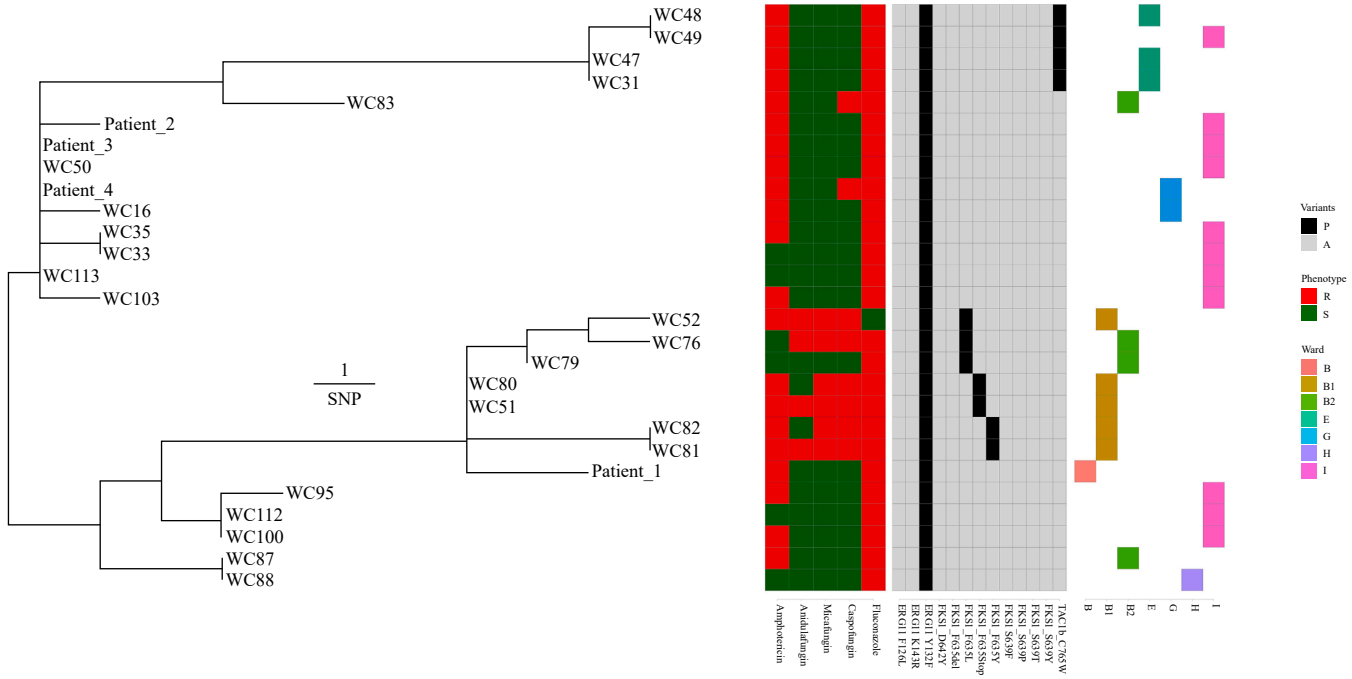

Figure 1. Phylogenetic relationships between *Candidozyma auris* isolates from four patient and 22 wastewater samples. Phylogenetic tree (panel 1, left) of *C. auris* isolates cultured from patients and wastewater (WC). All isolates were determined to be *C. auris* clade I. Panel 2: antifungal susceptibility (susceptible-green; resistant-red) for each isolate. Panel 3: presence (P) or absence (A) of mutations associated with echinocandin or fluconazole resistance. Panel 4 (right): Hospital wards from which the corresponding isolates originated. Sequence data were submitted to the European Nucleotide Archive database with accession number PRJEB95098.

(Figure 1). Clinical and wastewater isolates were almost universally fluconazole resistant (associated with *erg11* Y132F mutation). Six isolates (all from wastewater samples) showed phenotypic resistance to ≥1 echinocandin, all had SNPs in the *fks1* gene *F635L/Y/stop* (Figure 1); a further isolate (WC79) was phenotypically susceptible to echinocandins

(anidulafungin MIC 0.5 μg/mL, caspofungin MIC 1 μg/mL; micafungin MIC 0.5 μg/mL) but had an *fks1* F635L variant.

Discussion

Our study, undertaken using matched clinical and ward wastewater samples and isolates during a large UK hospital outbreak, demonstrates the ability to detect patient-related *C. auris* contamination of matched ward wastewater samples using either culture or PCR-based methods, with culture having the highest specificity and PCR the highest sensitivity. Sequencing confirmed that the *C. auris* clade I isolates obtained from patients and wastewater were highly genetically related (<10 SNPs difference). Whilst our study took place during an outbreak, testing ward wastewater may offer efficient screening of hospital environments for *C. auris* colonization, facilitating earlier detection and implementation of clinical screening and IPC measures at ward level, in order to prevent transmission to patients at high risk for development of invasive candidiasis (such as ICU patients), and to avert costly outbreaks. However, the cost effectiveness of this approach as well as appropriate *C. auris* prevalence setting/patient risk group remain to be determined in a prospective study.

Our study was limited by the small number of patients and samples tested during a short time period with a small number of control samples from a hospital ICU (St George's) without recent *C. auris* colonization or infections. A single sample taken from a macerator on a ward with no cases of *C. auris* colonization present was positive for *C. auris* using PCR. This may represent limitations in the sensitivity of culture-based clinical screening methods used (as the PPS was carried out using culture only), persistence of *C. auris* in ward wastewater following discharge of colonized patients, or a false-positive result (less likely in an outbreak context). *C. auris* is known to form biofilm on surfaces, a possible limitation of using this approach as a surveillance method in high *C. auris* prevalence settings [10]. Whilst it is technically possible that the organism may have spread to multiple wards via interconnected wastewater pipework — unlike biofilm-forming bacteria such as *Pseudomonas aeruginosa* which in our experience can thrive in and be transmitted from water and wet environments in hospital [11] — there is no evidence to date to support water as a niche or source of *C. auris* [12]. However, this is an important question which warrants longitudinal study.

This study provides proof-of-principle to support future studies of ward wastewater surveillance for *C. auris*, including prospective, longitudinal sampling of high-risk wards such as the ICU, establishing the optimal method for obtaining samples, testing and quantifying the *C. auris* burden (including the persistence of wastewater contamination following removal of colonized patients), the accuracy of detection methods in different contexts (in hospitals with and without *C. auris* colonized patients) and the cost-effectiveness of this approach — compared with patient-level screening — for preventing outbreaks. Research and evaluation of ward wastewater surveillance as a novel and pragmatic strategy is needed to address the urgent public health threat of *C. auris* and prevent further spread and outbreaks in low-burden settings such as the UK.

Acknowledgements

We thank Irene Monahan for her support with isolate identification; Michael Dibbens for his assistance during wastewater collections and the Infection Prevention and Control team at St Thomas' Hospital for organizing the point prevalence survey in the patients. We thank Gemma Johnson for her support with PCR and IMMY DX for supplying AurisID kits.

CRediT authorship contribution statement

H.C. Davidson: Writing — original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **A.-E. Griffin:** Writing — review & editing, Methodology, Investigation. **L. Symes:** Writing — review & editing, Methodology, Investigation, Conceptualization. **K.G. Laing:** Writing — review & editing, Supervision, Conceptualization. **A.A. Witney:** Writing — review & editing, Visualization, Supervision, Software, Formal analysis. **K. Gould:** Investigation. **P. Allebone-Salt:** Writing — review & editing, Methodology. **O. Abadioru:** Resources. **S.D. Goldenberg:** Methodology, Conceptualization. **J.A. Otter:** Supervision, Resources, Conceptualization. **R. Wake:** Writing — review & editing, Supervision, Conceptualization. **T. Bicanic:** Writing — review & editing, Supervision, Funding acquisition, Conceptualization.

Conflict of interest statement

H.C.D. has received speaker fees from Mundipharma/Napp UK and Gilead. T.B. has received advisory board and speaker fees from Mundipharma/Napp UK and Gilead Sciences and research grant funding from Gilead Sciences and Pfizer Inc. J.A.O. is a consultant to Gama Healthcare, Spectrum X, and Arka Healthcare, and co-founder of IPC Partners and received speaker fees from Solventum (previous 3M Healthcare) and Bode.

Funding sources

This work was funded by a fellowship in Invasive Fungal Disease from Gilead Sciences (Awarded to T Bicanic) and an internal pilot grant from Infection and Immunity Institute at CSGUL (awarded to H.C.D.).

Ethics statement

Ethical approval was not required for this study.

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