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

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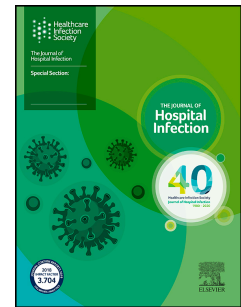
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Cover Page:

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

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Running title: Detection of *C. auris* in ward wastewater

Key words: *Candidozyma auris*, *Candida auris*, wastewater, outbreak, ward wastewater, surveillance, HCAI

Summary (75 words): We report the first detection of *Candidozyma* (formerly *Candida*) *auris* from ward wastewater using culture (sensitivity for detection of ≥ 1 *C. auris* patient on ward: 75%) and PCR (sensitivity: 100%). This proof-of-principle study was undertaken using matched clinical and ward wastewater samples during a large UK hospital outbreak. Sequenced wastewater and patient isolates were genetically identical (<10 SNPs different). Screening ward wastewater may be a

cost-effective alternative to clinical (individual patient) screening in low prevalence settings.

Text (1586 words):

Background:

Candidozyma (formerly *Candida*) *auris* is a multi-drug-resistant yeast and a WHO critical fungal priority pathogen[1] that causes persistent colonisation and treatment-refractory invasive infections, often associated with protracted and expensive outbreaks,[2–4] particularly in intensive care units (ICUs). Reports of clinical and colonisation 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.

The Study:

St Thomas's Hospital is a large central London hospital which experienced a protracted *C. auris* outbreak affecting 100 patients, including 1 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 (50ml) 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 hand wash 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 hand wash sink for 30 seconds. Swab samples were processed immediately by direct inoculation onto 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 onto 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 onto chromogenic agar. Yeasts were identified phenotypically, then sub-cultured onto Sabouraud dextrose agar with chloramphenicol (E & O Laboratories)

for 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* colonised patients identified in the point prevalence screen, 4 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 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 CLSI method: MIC fluconazole ≥ 32 ; amphotericin B ≥ 2 ; anidulafungin ≥ 4 , micafungin ≥ 2 ; caspofungin ≥ 2 .

Results:

The PPS detected *C. auris* colonisation in ≥ 1 patient on 7/9 wards, supplied by 8/12 sluices (see Table I). Culture testing found *C. auris* in at least one site (sluice sink or macerator) in 6/8 sluice rooms (sensitivity 75%), and all 8 detected *C. auris*

using the AurisID assay (sensitivity 100%). Two wards had no patients detected on PPS, supplied by 4 sluices; culture testing was negative (100% specificity); 1 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 single nucleotide polymorphism (SNP) difference) (Figure 2). 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* (Fig 2); a further isolate (WC79) was phenotypically susceptible to echinocandins (anidulafungin MIC 0.5 μ g/ml, caspofungin MIC 1 μ g/ml; micafungin MIC 0.5 μ g/ml) but had an *fks1* F635L variant.

Conclusions:

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 SNP difference). Whilst our study took place during an outbreak, testing ward wastewater may offer efficient screening of hospital environments for *C. auris* colonisation,

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* colonisation or infections. A single sample taken from a macerator on a ward with no cases of *C. auris* colonisation 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 done using culture only), persistence of *C. auris* in ward wastewater following discharge of colonised 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 colonised patients), the accuracy of detection methods in different contexts (in hospitals with and without *C. auris* colonised patients) and the cost-effectiveness of this approach - compared to 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.

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Conflict of interests:

HCD has received speaker fees from Mundipharma/Napp UK and Gilead. TB has received advisory board and speaker fees from Mundipharma/ Napp UK and Gilead Sciences and research grant funding from Gilead Sciences and Pfizer Inc. JAO 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.

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Ethical statement:

Ethical approval was not required for this study.

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Tables:

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

Ward	Ward Type	C. auris colonised 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%

Caption: P = *C. auris* found, N = negative

Column 3 shows number of *C. auris* colonised 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 (highlighted in green). Sensitivity and specificity for each detection method are calculated against a standard reference of presence of ≥ 1 colonised *C. auris* patient on a ward, with 95% Clopper-Pearson confidence intervals (CI). Sluice A2 did not have a sluice sink, Sluice F1's macerator was out of service.

Figure legends:

Figure 1: Title: Phylogenetic Relationships Between *C. auris* isolates from four patient and 22 wastewater samples

Caption: *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.*

