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RESEARCH

Prospective surveillance of *Candida auris* and assessment of diagnostic approaches in a Belgian hospital

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Abstract

Purpose *Candida auris* is an emerging multidrug-resistant fungal pathogen known for its ability to cause healthcare-associated outbreaks. Although only sporadic cases have been reported in Belgium, increasing incidence in Europe highlights the need for early detection strategies. This study aimed to evaluate *C. auris* colonisation among high-risk patients and assess three detection methods.

Methods A total of 100 patients were screened using two eSwabs® per patient from: (i) axillae and groins, and (ii) nose, throat and perineum. Culture on CHROMagar Candida plus was compared with two molecular assays: Altostar® Candida auris PCR, and AurisID. Given the overlap in risk factors for *C. auris* and other multi-drug resistant organisms, the feasibility of culturing *C. auris* from an enrichment broth containing TSB and 2.5% sodium chloride, often used for MRSA enrichment, was evaluated.

Results Analytical evaluation showed high specificity of the molecular assays with no cross-reactivity to clinically relevant non-target yeasts. Culture on CHROMagar Candida plus appeared more sensitive than both molecular methods. No *C. auris* carriage was detected among all screened patients.

Conclusion *Candida auris* was not detected in the screened population, indicating a very low or absent prevalence at present. However, surveillance is essential to ensure early recognition and address the risk of future spread.

Keywords Candida auris · Surveillance · CHROMagar candida plus · AurisID · Altostar *Candida auris* · Hospital epidemiology belgium

Introduction

Candida auris is an emerging pathogen associated with global outbreaks. It was first described in 2009 after being isolated from a Japanese patient's ear canal, and has since spread worldwide [1]. Whole genome sequencing initially identified four distinct clades of *C. auris* based on the number of single nucleotide polymorphisms (SNP's) between isolates. These clades are geographically linked with origins in South Asia (Clade I), East Asia (Clade II), South Africa (Clade III), South America (Clade IV) and Iran (Clade V). Recently, a sixth clade was identified in Singapore, with isolates differing more than 37,000 SNP's from other clades [2, 3].

Distinctive features of *C. auris* compared to other *Candida* species include its extensive antifungal resistance –almost always resistant to fluconazole, and elevated minimum inhibitory concentrations (MICs) for various antifungals including azoles, echinocandins, and amphotericin B - severely limiting treatment options. Panresistant *C. auris* strains have been documented [4–8]. Additionally, *C. auris* can colonize the skin, anterior nares and other body sites of asymptomatic carriers [9]. This colonisation can lead to contamination of surfaces such as floors, bed trays and sinks [6, 8]. Its resilience on dry surfaces for up to two weeks

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further contributes to its heightened potential for outbreaks in hospital and nursing home settings [6, 7, 10, 11]. These characteristics have resulted in *C. auris* being classified in the critical priority group of the World Health Organization (WHO) fungal priority pathogens list [12].

Risk factors for *C. auris* colonisation and infection include prolonged healthcare exposure, such as extended intensive care unit stays with mechanical ventilation, parenteral nutrition, and the presence of catheters; compromised immune systems; exposure to broad-spectrum antibiotics; and chronic conditions such as diabetes mellitus and chronic renal disease [6, 8, 13].

In 2018, the European Centre for Disease Prevention and Control (ECDC) highlighted the increasing risk of *C. auris* spread in European hospitals. From 2013 to 2021, *C. auris* was identified in 15 European countries, including Belgium, with Spain reporting the highest incidence [4, 6, 14]. Between 2019 and 2021, five European countries reported 14 *C. auris* outbreaks involving two or more epidemiologically linked cases, affecting a total of 327 patients. Interfacility transmission occurred in eight of these outbreaks, which proved difficult to control despite advanced infection control measures [6]. As of July 2024, only 16 cases have been identified in Belgium, with three cases unlinked to foreign hospital care. All strains, except for two, were typed as clade I (South Asia) [15]. A visualization depicting the origin of the *C. auris* strains isolated in Belgium is given in Fig. 1.

Given the multi-drug resistant nature of *C. auris*, preventing colonisation and infection is paramount. It is critical for medical laboratories to have tools to detect *C. auris*. Prompt detection of colonisation through rapid and reliable *C. auris* detection techniques is recommended. Identification can be achieved using either phenotypical- or molecular techniques.

In June 2024, the Belgian Superior Health Council issued 'recommendations for the diagnosis, prevention and management of *C. auris* infections'. The recommended screening sites for *C. auris* are quite broad, including the axillae, inguinal regions on both sides, naso-/oropharyngeal swab, suggesting that solo-location screenings such as rectal screening, currently used for detection of carbapenemase producing Enterobacterales (CPE), is not adequate [16]. *C. auris* exhibits a high salt tolerance, with Das et al.. (2021) reporting a tolerance up to 12.5% sodium chloride [17]. This characteristic led us to consider whether our screening process for *C. auris* could be streamlined in the future to match with that of methicillin-resistant staphylococcus aureus (MRSA), consisting of throat, nose, and perineum sampling. Sampling kits for MRSA (Copan, Brescia, Italy) contain tryptic soy broth (TSB) enriched with 2.5% sodium chloride.

At present, little is known about the epidemiology of *C. auris* in Belgium. Currently, our center's patient screenings primarily target multi-drug resistant Gram-negative bacteria and MRSA. Additionally, our current chromogenic *Candida* agar (BBL™ CHROMagar™ Candida, Becton Dickinson, New Jersey, United States) is not optimized for detecting *C. auris*. In this study, two commercially available realtime PCRs, AurisID (OLM diagnostics, Newcastle upon Tyne,

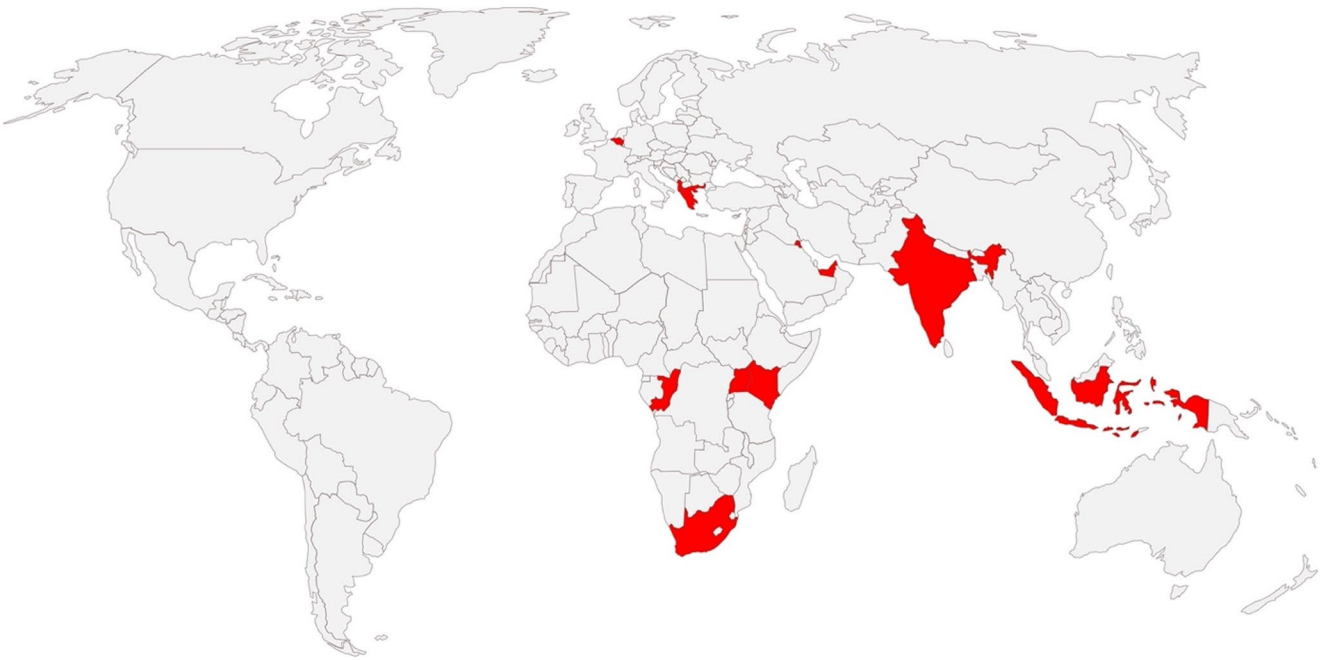

Fig. 1 visualization depicting the origin of *C. auris* strains isolated in Belgium. (world map by: www.freeworldmaps.net)

United Kingdom) and Altostar® *Candida auris* PCR (Altona diagnostics, Hamburg, Germany), and one chromogenic agar, CHROMagar Candida plus (CHROMagar, Paris, France), were evaluated for detecting and identifying *C. auris*. In addition, ITS rRNA sequencing was used to further assess the detection of *C. auris* in low-concentration samples.

Subsequently, high-risk patients in the intensive care and haematology units were screened using both real-time PCR tests and the chromogenic agar. This study is, to the best of our knowledge, the first to investigate the epidemiology of *C. auris* in high-risk patients in Belgium and includes the first clinical evaluation of the Altostar® *Candida auris* PCR. Additionally, we investigated whether *C. auris* can grow and survive effectively when sampled in TSB enrichment medium containing 2.5% sodium chloride.

Materials & methods

Epidemiological study and patient selection

Between June 2024 and October 2024, 100 high-risk patients admitted to the intensive care unit (ICU) or haematology ward at AZ Sint-Jan Hospital, a Belgian tertiary hospital, were included in the study. High-risk status was defined as:

(i) International patients or patients who received treatment in a foreign hospital ($n = 14$);
(ii) Extended ICU stay (>2 weeks) ($n = 51$);
(iii) Antifungal treatment received within 30 days prior to or during screening in the haematology ward ($n = 35$).

Screening was performed using two eSwabs® per patient: one for both axillae and groins, and another one for nose, throat, and perineum. All screening samples were processed in three ways: (i) culture on CHROMagar Candida Plus (10 µL inoculum); DNA extraction and amplification by (ii) Altostar® *Candida auris* PCR, and (iii) AurisID real-time PCR kits.

Evaluation of analytical performance

Specificity

Specificity was evaluated using 28 clinical yeast strains collected between April and July 2024, three external quality control strains conserved at −80 °C and five molecularly characterized *C. auris* strains. The molecularly characterized *C. auris* strains are clinical isolates obtained from UZ Leuven, the National Reference Center (NRC) for Mycosis, and will be further referred to as NRC strain 1–5 [18]. The strains included: 5 *C. auris* (NRC strain 1–5), 5 *Candida albicans*, 5 *Nakaseomyces glabratus*, 5 *Candida tropicalis*, 5 *Candida parapsilosis*, 5 *Pichia kudriavzevii*, 2 *Pichia norvegensis*, 1 *Meyerozyma guilliermondii*, 1 *Candida duobushaemulonii* and 2 *Candida haemulonii*.

Strains were cultured on Sabouraud (SAB) agar (Thermo Fisher Scientific, Waltham, USA), and identification was confirmed using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). A 0.5 and 0.25 McFarland (McF) suspension of each strain was prepared in sterile distilled water. Ten microliters of each suspension were cultured on CHROMagar Candida plus, and growth and colony colour/morphology were evaluated. DNA extraction was performed for each solution, and real-time PCR was performed by Altostar® *Candida auris* PCR and AurisID.

Analytical sensitivity

Five molecularly characterized *C. auris* stains (NRC strain 1–5) were used: clade I ($n = 3$) and clade III ($n = 2$). These strains were conserved at −80 °C and cultured manually on SAB agar. A 0.5 McF suspension approximating a concentration of 1×10^6 CFU/mL was made and serially diluted to to concentrations of 10 000 CFU/mL – 1000 CFU/mL − 400 CFU/mL − 100 CFU/mL – 80 CFU/mL – 16 CFU/mL [19]. Ten microliters of each dilution was cultured on CHROMagar Candida Plus. Growth was assessed as: + (growth in the first streak), ++ (growth in the first two streaks), +++ (growth until the third streak), and ++++ (growth until the fourth streak). DNA extraction was performed for each dilution, and real-time PCR assays were repeated in triplicate for concentrations near the suspected analytical sensitivity.

Feasibility of using TSB Salt enrichment broth (Copan) for C. auris detection

A 0.5 McF *C. auris* solution was prepared for five distinct strains (NRC strain 1–5), and diluted to approximately 10 000 CFU/mL, 1000 CFU/mL, 500 CFU/mL, 100 CFU/mL, and 50 CFU/mL. One swab from the TSB Salt enrichment broth™, often used for MRSA screening, was submerged in the solution and added to the enrichment broth in the tube. After 24 h of incubation at 37.5 °C, 10 µL was streaked on: (i) CHROMagar Candida plus (ii) SAB agar and (iii) BBL CHROMagar Candida. Growth was assessed after 48 h as negative or positive. Identification was confirmed using MALDI-TOF MS.

Molecular identification by real-time PCR

Automated DNA extraction was performed using the QIAsymphony SP (Qiagen, Hilden, Germany) instrument with the Virus/Pathogen kit (Qiagen) according to manufacturer's

recommendations, with 400 µL input and 110 µL elution volume. Yeast suspensions and screening samples were extracted within 12 h of collection, with extracts stored at −80 °C.

Primer/probe mixes were prepared following the manufacturer's protocols. The internal control for both real-time PCR kits could not be added as instructed due to automated DNA extraction, preventing checks for inhibition. To address this, we investigated whether the internal control could be spiked into the sample before extraction and the impact on PCR signals. A 0.5 McF *C. auris* solution was prepared from five distinct strains, then diluted to approximately 10 000 CFU/mL, 1000 CFU/mL, 500 CFU/mL, 100 CFU/mL, and 50 CFU/mL. These solutions were extracted in quintuplicate: (i) with AurisID internal control spiked prior to extraction (volume = one tenth of the elution volume) ($n = 2$), (ii) with Altostar® internal control spiked prior to extraction (volume = half of the elution volume) ($n = 2$) and (iii) without internal control ($n = 1$).

All real-time PCR runs were performed on the ViiA 7 real-time PCR system (Applied Biosystems, Thermo Fisher Scientific), adhering to manufacturer's recommendations. Each run included a positive and negative control provided by the kit.

Sequencing of ITS rRNA

Sequencing of internal transcribed spacer (ITS) rRNA was conducted for two dilutions from each strain with a low concentration (100 and 80 CFU/mL or 400 and 100 CFU/mL) to determine if *C. auris* reads could be detected in these samples. Amplification and nanopore sequencing of the target region was performed following the methodology described by Vanhee et al. (2024) [20] substituting the 16 s primers mentioned in the publication for ITS1F-Kyo2/LR3-I primers by Mafune et al. (2020) [21]. Sequences were obtained in FASTQ format which in turn were processed via amplicon sorter to generate a consensus sequence [22]. The identification tool of the Westerdijk Fungal Biodiversity institute was used to perform pairwise alignment of the consensus sequences with multiple fungal reference databases [23].

Culture-based identification of *C. auris*

Samples were cultured according to the manufacturer's recommendations and incubated at 37°C for 48 h. Plate readings were conducted at 24 and 48 h. Presumptive identification on CHROMagar Candida Plus was based on colony colour for *C. albicans*, *N. glabratus*, *P. kudriavzevii*, *C. tropicalis* and *C. auris*. Identification of all yeast isolates was confirmed by MALDI-TOF MS.

Ethics

The study was approved by the local Ethics committee of AZ Sint-Jan hospital (BUN number: B0492024000007). Informed consent was obtained from all patients or their legal guardians.

Results

Analytical performance of the assays

Evaluation of colour and colony morphology of non-*C. auris* yeast strains on CHROMagar Candida plus showed full concordance with the expected appearances described in the product insert.

Both real-time PCR assays (Altostar® *Candida auris* PCR and AurisID) were evaluated for specificity using genomic DNA extracted from non-*C. auris* yeast strains ($n = 31$) at two concentrations (0.25 and 0.5 McF). No cross-reactivity was observed in either assay.

The analytical sensitivity was assessed by serial dilution and extraction of five distinct *C. auris* strains. Results of the comparison are given in Table 1.

Culture on CHROMagar Candida plus yielded growth up to concentrations of approximately 80 CFU/mL. The specific *C. auris* colour was most distinguishable after 48 h of incubation, with some samples showing colony growth only between 24 and 48 h. No differences were observed between clade I and clade III strains.

The Altostar® *Candida auris* PCR yielded positive results up to a concentration of approximately 1000 CFU/mL (approximately 37 CFU/reaction) in four of the five tested strains. The AurisID showed positive results for two of the five approximate 1000 CFU/mL (approximately 22 CFU/reaction) solutions tested. PCR results in bold in Table 1 were considered positive when two out of three repeats were positive. ITS sequencing of low-concentration samples detected *C. auris* in 2 strains (Clade I & III) at approximately 100 CFU/mL and in one strain at approximately 80 CFU/mL (Clade I).

For both PCR assays, the addition of the internal control prior to extraction and its impact on PCR curves was investigated. For the AurisID assay, the internal control was positive in all samples, with a mean Ct-value of 28.16 (25.39 to 30.10). No negative influence on the detection of *Candida auris* was observed due to addition of the internal control. Similarly, for the Altostar® *Candida auris* PCR assay, the internal control was positive in all samples, with a mean Ct-value of 26.39 (25.34 to 27.51) and no negative influence was noted.

Table 1 Summary of results obtained by serial Dilution of five distinct *Candida auris* strains (NRC strain 1–5). Bold: samples were repeated in triplicate, and considered positive when 2/3 or 3/3 results were positive; NP: not performed

Approximate concentration (CFU/mL)	Altostar® Candida auris PCR (Altona)	AurisID (OLM)	CHROMagar Candida plus after 24 h/48 h (CHROMagar)	ITS sequencing
NRC strain 1 (Clade I)				
1 000 000	Positive	Positive	++++/++++	NP
10 000	Positive	Positive	+/++	NP
1 000	**Positive**	**Negative**	+/++	NP
400	**Negative**	**Negative**	Negative/+	NP
100	**Positive**	**Negative**	Negative/+	Negative
80	**Negative**	**Negative**	Negative/+ (one colony)	Negative
16	Negative	Negative	Negative/Negative	NP
NRC strain 2 (Clade III)				
1 000 000	Positive	Positive	++++/++++	NP
10 000	Positive	Positive	+/++	NP
1 000	**Positive**	**Positive**	++/++	NP
400	**Negative**	**Negative**	Negative/+	NP
100	**Positive**	**Negative**	Negative/+	Negative
80	**Negative**	**Negative**	Negative/+	Negative
16	Negative	Negative	Negative/Negative	NP
NRC strain 3 (Clade I)				
1 000 000	Positive	Positive	+++/++++	NP
10 000	Positive	Negative	++/++	NP
1 000	**Negative**	**Negative**	+/++	NP
400	**Negative**	**Negative**	+/+	Positive
100	**Negative**	**Negative**	Negative/+	Positive
80	**Negative**	**Negative**	Negative/+	NP
16	Negative	Negative	Negative/Negative	NP
NRC strain 4 (Clade I)				
1 000 000	Positive	Positive	++++/++++	NP
10 000	Positive	Negative	++/++	NP
1 000	**Positive**	**Negative**	+/++	NP
400	**Negative**	**Negative**	+/+	NP
100	**Negative**	**Negative**	Negative/+	Negative
80	**Negative**	**Negative**	Negative/+	Negative
16	Negative	Negative	Negative/+ (one colony)	NP
NRC strain 5 (Clade III)				
1 000 000	Positive	Positive	+++/++++	NP
10 000	Positive	Positive	++/++	NP
1 000	**Positive**	**Positive**	+/++	NP
400	**Negative**	**Negative**	+/+	NP
100	**Negative**	**Negative**	Negative/+	Positive
80	**Negative**	**Negative**	Negative/+ (two colonies)	Negative
16	Negative	Negative	Negative	NP

Feasibility of using TSB Salt enrichment broth for detection of *C. auris*

All samples showed growth of *C. auris* after 48 h of incubation on SAB agar (Thermo Fisher Scientific), CHROMagar Candida plus, and BBL CHROMagar Candida, except for two dilutions (100 and 50 CFU/mL) from strain five (Clade III) which exhibited no growth on SAB agar. Growth on SAB agar generally displayed fewer colonies and smaller colony morphology compared to the other media.

Clinical screening results

The patient characteristics of the 100 screened patients are summarized in Supplementary materials.

None of the 100 high-risk patients tested positive using the Altostar® *Candida auris* PCR assay. In contrast, the AurisID assay yielded three weakly positive results, each with a Ct-value of approximately 34.

Yeast growth was observed in fifty-five patients (55%) on the CHROMagar Candida plus. Information about the yeast species can be found in Table 2. Yeast recovery from nose,

Table 2 Species details on growth of non-Candida auris yeast in patient screening samples

	Species					
Specimen type	C. albicans (*n*)	*N.* glabrata (*n*)	C. tropicalis (*n*)	*P.* kudriavzevii (*n*)	C. parapsilosis (*n*)	Other (*n*)
Nose, throat, and perineum	33	14	5	4	7	8
Axillae and groints	10	5	3	0	2	0

throat and perineum samples occurred in 51% of cases, compared to 19% from groins and axillae in 19%. None of the patients screened showed growth of *C. auris* on CHROMagar Candida plus.

Discussion

C. auris is an emerging fungal pathogen that has caused outbreaks worldwide. Currently, in Belgium, only sporadic cases have appeared, but given the rise of *C. auris* in Europe and several outbreaks, this study aims to determine the optimal detection method in our hospital and to assess colonization rates among high-risk patients.

Both evaluated real-time PCR assays showed no cross-reactivity with DNA from 0.5 to 0.25 McF solutions of clinically important non-target yeasts, including *C. albicans*, *N. glabratus*, *C. tropicalis*, *C. parapsilosis*, *P. kudriavzevii*, *P. norvegensis* and *M. guillermondii*. These results align with those obtained by Sattler et al. (2021) in their evaluation of AurisID [24]. Evaluation of specificity continued during the clinical screening study as every yeast isolated on CHROMagar candida plus was identified using MALDI-TOF-MS. Fifty-five patients showed a positive yeast culture for non-*Candida auris* species on CHROMagar Candida Plus. Nose, throat and perineum culture yielded the highest positive rate (51%) compared to axillae and groins (19%). The previously mentioned yeast species, while the most common species in our laboratory, are not the closest genetically to *C. auris*. *C. haemulonii*, *C. duobushaemulonii* and *C. pseudohaemulonii* are more genetically alike and are known to cause false-positive results in the AurisID assay when present in sufficiently high concentrations [23]. During the current evaluation, two external control *C. haemulonii* and one external control *C. pseudohaemulonii* isolates were tested at two concentrations (0.25 and 0.5 McF), yielding no non-specific reactions for the Altostar® *Candida auris* PCR and the AurisID.

For the comparison of the analytical sensitivity, our findings suggest that culture on CHROMagar Candida plus after 48 h of incubation may detect *C. auris* at lower inoculum levels than real-time PCR assays under the conditions used in the current study. It should be emphasized that the purpose of this work was not to define an absolute analytical sensitivity threshold for any of the assays but rather to compare the three methods using the identical dilution series in a manner compatible with the current diagnostic workflow implemented at our centre. In the evaluation by Sattler et al. (2021) genomic DNA extract dilutions were used to determine the limit of detection of the PCR kit, whereas in the current study, serial dilutions of 0.5 McF solutions were used, all followed by extraction, with the extraction being the variable factor between both studies [23]. The extraction protocol applied in this evaluation corresponds to the routine method used for multiple yeast/fungi PCR in AZ Sint-Jan Hospital as well as sequencing of the ITS region.

As a limitation in this study, the internal control for extraction could not be added during the extraction as recommended by the manufacturer. In addition, extraction efficiency could not be evaluated, which may have influenced the observed PCR performance. The conversion of McFarland to estimated CFU/mL also introduces an inherent variability. Finally, only clade I and clade III isolates were included in the analytical evaluation, as these are currently the only isolated clades in Belgium, and the overall cohort size was relatively small.

To the best of our knowledge, this study is the first to investigate *C. auris* colonisation in a Belgian hospital. None of the 100 screened high-risk patients exhibited *C. auris* carriage determined by culture or Altostar® *Candida auris* PCR. However, three patients yielded a positive reaction using the AurisID real-time PCR for axillae and groins samples. In contrast, culture and Altostar® *Candida auris* PCR results were negative for these sites, as well as for the nose, throat and perineum screening samples. This discrepancy suggests a potential nonspecific reaction in the AurisID assay, with a cycle threshold value of approximately 34. No linkage could be found between both patients to suggest a true positive result. Repeatment in triplicate of two of these three extracts no longer yielded a positive result.

A similar study was conducted in Germany by Heindel et al.. (2023), involving 655 patients with a history of previous hospital stay or medical treatment abroad [13]. Another study from England screened 921 patients for *C. auris*, with the main risk factor being high rates of travel to risk countries [25]. Both studies, similar to our results, showed no detection of *C. auris*. They concluded that widespread screening for *C. auris* was not beneficial at that time and should be dependent on the local epidemiology of *C. auris*. When screening for *C. auris*, the optimal body sites for inclusion are not yet clearly defined. Screening of the

axillae is, however, included in each guideline, including the recommendations from the Center for Disease Control and Prevention (CDC) and the Belgian Superior Health Council [16, 26].

In a study conducted at Oxford University hospital's neuroscience intensive care unit, a substantial number of patients demonstrated initial colonisation in the axillae [11]. However, these findings possibly may not be generalized given the potential association between the outbreak with reusable axillary probes. Few studies have further investigated colonisation sites. Piatti et al. (2022) investigated the difference between skin and rectal colonisation for *C. auris* and found that intestinal carriers were fewer than cutaneous but were more continuously colonized, indicating that both skin and rectal swabs can be useful tools for surveillance [27]. More studies are needed to examine the number of screening sites and determine ideal screening sites.

Screening of the axillae and groins is not currently used for other multidrug-resistant organism screenings, meaning that caregivers need to take an extra sample. Given the considerable overlap between risk factors for *C. auris* colonisation and colonisation with other multidrug-resistant organisms, the aim was to streamline screening for *C. auris* and MRSA, which is performed as standard-of-care procedure, if possible. The usefulness of the TSB Salt enrichment broth for detecting *C. auris* was investigated, given the salt tolerance of *C. auris*. All examined dilutions showed growth on CHROMagar Candida plus and BBL CHROMagar Candida. Growth was not optimal on SAB agar, yielding less and smaller colonies and two dilutions showed no growth. These results suggest that screening of the naso-/oropharyngeal area and perineum can be done by inoculation of a CHROMagar Candida plus from the MRSA screening sample. However, a limitation of this evaluation is the use of pure *C. auris* suspensions; the potential impact of commensal flora on *C. auris* recovery requires further investigation.

Conclusion

This study demonstrates that culture on CHROMagar Candida Plus and molecular assays (Altostar® *Candida auris* PCR and AurisID) offer reliable detection of *Candida auris*. Screening strategies can be streamlined by integrating *C. auris* surveillance into existing MRSA workflows, using salt enrichment broth 2.5% sodiumchloride. No colonisation was detected among high-risk patients, but continuous surveillance remains essential.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10096-026-05409-4.

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Author contributions Conceptualization: KC, AM, MR, JH. Methodology, formal analysis and patient inclusion: KC, JH, TC, MV, MR, AM. Writing – original draft: KC. Writing - review and editing: AM, MR, JH, KF, TC, SS, AS, BD, MV. Supervision: AM, MR.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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