Original article

**Candida auris**: Diagnostic challenges and outbreak control in paediatric and neonatal intensive care unit in a tertiary care hospital - the first of many in Eastern India

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- Outbreak
- Surveillance
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**ABSTRACT**

**Problem considered:** An emerging global threat, Candida auris infections have poor prognosis, high transmission rate, and potential for outbreaks. In India, prevalence of Candida auris invasive infection has been calculated to be 5.3 %. Strict surveillance and preventive measures must be implemented in intensive care units because of its propensity for rapid adaptation and potential for antifungal resistance.

**Methods:** Outbreak investigational study was carried in paediatric and neonatal intensive care unit of a tertiary care hospital in eastern India. Clinical isolates from inpatients with candidemia were subjected to identification by microbiological tests. Deoxyribonucleic acid (DNA) sequencing done for molecular identification and determining clonal similarity of Candida auris isolates. Surveillance of intensive care units carried out to assess patient colonization, environmental contamination, and hand-carriage of yeast among healthcare workers following which strict infection control measures were implemented.

**Results:** Blood isolates from four candidemia patients identified microbiologically as Candida auris. Environmental surfaces found contaminated with Candida auris by surveillance included Ultrasound guided (USG) Accuprobe and Blood-pressure (BP) cuff used for all patients admitted, molecular identification of which showed homology with patient isolates. However, patients and hospital environment no longer harboured Candida auris owing to stringent Infection Prevention and Control (IPC) measures taken.

**Conclusion:** The article emphasises multidisciplinary approach towards investigation and containment of Candida auris outbreak and how prompt surveillance and simple preventive measures could eradicate Candida auris from patients and hospital setting.

1. Introduction

Since its initial discovery in the ear canal of a Japanese patient in 2009, multidrug-resistant Candida auris has become a global threat. In a short amount of time, numerous countries have documented a significant increase in mortality rates linked to this organism. 1 Because most research come from single centers and lack common denominators, the extent of Candida auris infection is yet unknown. Candida auris accounted for 74 out of 1400 (5.3 %) cases of candidemia in a study conducted in the year 2011–2012 in 27 intensive care units (ICUs) in India. 2 The mortality rate from candidemia due to Candida auris was as high as 50 % in India. 3 Despite the widespread belief that Candida auris is acquired nosocomially, and the fungus having been isolated from numerous locations in the patient environment, numerous studies into the ecological niche of this yeast within the hospital have been conducted without result. However, it has been shown that different Candida auris clonal lineages can be independently introduced into the same hospital, raising the prospect of community reservoirs. However, just 1 of 2246 (0.04 %) patients examined at the time of admission in an outbreak investigation at a cardiac hospital in the United Kingdom (UK) had Candida auris identified from the colonization site, indicating the hospital as the likely source of the organism. 4 Earlier reports of Candida auris infection have primarily been linked to nosocomial outbreaks, but

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sporadic instances have also been reported. Additionally, patients may become colonised with *Candida auris* without infection thus serving as a reservoir for the nosocomial spread of the infection. Understanding the dynamics of infected and colonised patients within the hospital is crucial for implementing control and eradication efforts. Furthermore, it is crucial that laboratories that offer diagnostic services to medical facilities are able to reliably and quickly identify *Candida auris* accurately in clinical samples.

This study summarises multidisciplinary approach towards the investigation and containment of *Candida auris* outbreak in a paediatric intensive care setting of a tertiary care hospital in Bihar and the comprehensive strategies in the outbreak response consisting of Infection Prevention and Control (IPC) measures, prospective surveillance efforts, healthcare staff training and teamwork employed to contain and prevent further *Candida auris* infections across the hospital.

2. Methods

The current outbreak investigational study was carried out prospectively in the paediatric and neonatal intensive care unit of a tertiary care multi-speciality hospital in eastern India that serves as the premier referral facility for complex surgical and medical cases, with a strong Antimicrobial Stewardship (AMS) program and a dedicated team for Antifungal Stewardship to ensure appropriate antifungal prescriptions. The institution also has an IPC team responsible for pathogen and location-based infection surveillance, which receives notifications from the clinical microbiology laboratory whenever *Candida auris* is isolated. Clinical isolates from paediatric inpatients admitted from August to October 2022 with laboratory confirmed *Candida auris* were included in this study.

In this study four patients of suspected sepsis in paediatric and neonatal intensive care unit in a tertiary level hospital in Bihar were diagnosed microbiologically as candidemia caused by a rare, highly pathogenic multidrug resistant strain of *Candida auris*. Table 6.A.1 highlights the definitions that were used to identify the outbreak that helped with the investigation and analysis. Table 6.A.2 contains patient specific information.

Blood culture samples from all four patients, incubated in BacT/ALERT 3D (bioMérieux), beeped within two days. Phenotypic identification was based on gram staining, colony color on CHROMagar, germ tube test, morphology on Corn-meal Agar with TWEEN 80, and growth at 37 °C, 42 °C, and 45 °C, with further identification using VITEK 2 ID (bioMérieux). Antifungal susceptibility testing (AST) of the isolates were performed using the VITEK 2 system (bioMérieux), with results interpreted based on CDC breakpoints. Patients were screened for *Candida auris* colonization at the axilla and groin using sterile cotton swabs (HI media PW005, India) moistened with sterile normal saline, which were then inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37 °C for 48 h, with growth subjected to VITEK ID and AST.

An investigation of the environment and healthcare workers hands were conducted in order to identify potential source in the ICU. The axilla and groin of the other patients admitted to the ICU at the same time were examined for *Candida auris* colonization. A thorough environmental survey was conducted, which included collecting samples from bed surfaces and bed railings, blankets, medicine trolleys, ventilator tubing, self-inflating bag, infusion pumps, pulse oximeter, Electrocardiogram (ECG) probes, USG Accu-probe, suction bottles and tips, fluids (injectable medications, disinfectants in use), syringes, door handles, glucometers, BP-cuff, stethoscopes, and mobile phones of doctors following which the samples were processed similarly. Growth detected was subjected to VITEK ID and AST. Following infection control procedures, repeat sampling was done from all environmental samples to ensure effectiveness of infection control measures.

For sequencing analysis, DNA was isolated from the blood culture isolate of patients and environmental isolate and its quality was evaluated on NanoDrop™ One (Thermo Fisher Scientific). Fragment of the Internal Transcribed Spacer (ITS) region was amplified using PCR, yielding a single amplicon of band of approximately 600 base pairs on an agarose gel. The amplicon was purified, and DNA sequencing was performed using ITS1 and ITS4 primers with the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. A consensus sequence was generated from the forward and reverse sequences. This sequence was analyzed with Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) GenBank database, aligned using Clustal W. Distance matrix and phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) 11.

3. Results

3.1. Microbiological identification and antifungal susceptibility testing

3.1.1. Microbiological identification

Gram staining of the blood culture samples received from all four patients which beeped within 2 days period showed Gram positive ovoid budding yeast cells [Figure 6.B.1(a)]. Culture on Sabouraud dextrose agar showed cream coloured, smooth colonies [Figure 6.B.1(b)] after overnight incubation at 37 °C. VITEK ID for the isolates identified them as *Candida auris* with a probability of 98–99 % by VITEK 2 Software version 8.01. CHROM-agar showed pink to purple-coloured colonies on prolonged incubation [Figure 6.B.1(c)]. Colonies of Dalmau Plating when seen under microscope showed ovoid or ellipsoidal budding yeast cells in singlets, doublets and clusters [Figure 6.B.1(d)] after incubation for 72 h at room temperature. The isolates grew well at 37 °C and 42 °C, whereas no growth was seen at 45 °C.

3.1.2. Antifungal susceptibility testing

The antifungal susceptibility result interpretation based on CDC breakpoints showed all four isolates were resistant to Fluconazole (Minimum inhibitory concentration (MIC) ≥ 32 μg/ml). Fluconazole susceptibility was considered as a surrogate for second generation triazole susceptibility assessment. However, isolates that are resistant to fluconazole may respond to other triazoles occasionally. The decision to treat with another triazole were made on case-by-case basis as per CDC guidelines. One of the three isolate was susceptible to Amphotericin B (MIC = 1 μg/ml) and other three were resistant (MIC ≥ 2 μg/ml). All four
isolates were however susceptible to Caspofungin (MIC ≤ 2 μg/ml) and Micafungin (MIC ≤ 4 μg/ml) making these the drug of choice against *Candida auris*. The antifungal given to the patients with duration are given in Figure 6.A.3.

Repeat blood cultures of first three patients after they had received the necessary antifungal therapy came negative whereas the fourth patient died due to cardiac arrest before repeat sampling could be done.

### 3.2. Colonisation study

Samples taken from axilla and groin of all the four patients who had fungemia due to *Candida auris* isolated the same organism with identical antifungal sensitivity pattern.

### 3.3. Environmental screening

Reusable patient monitoring equipments like USG Accu-probe and BP cuff, commonly used for all patients admitted in both paediatric and neonatal intensive care unit isolated the same organism.

### 3.4. Molecular sequencing

Isolates from patients and USG Accu-probe by molecular sequencing was found to be *Candida auris* and showed high similarity based on nucleotide homology and phylogenetic analysis. Isolate from first patient and that of USG Probe isolate was identified as *Candida auris* with nucleotide homology of 100 % with [Candida] auris isolate RICU4 (Accession number: MH118269.2) whereas the last patient isolate had 98.59 % homology with [Candida] auris isolate RICU4 (Accession number: MH118269.2). The clonal lineage of patients was identical to the clonal strain of *Candida auris* isolated from the USG-probe. However clonal lineage of the fourth patient slightly varied showing that a slightly mutated or different *Candida auris* clonal lineages can be independently introduced into the same hospital, raising the prospect of community reservoirs and need for strict surveillance to prevent outbreaks caused by the organism. The sequence obtained was submitted to the NCBI GenBank (Sequence number GenBank [Q0692814, Q0692815]). Figure 6.B.2 shows the phylogenetic tree of the identified isolates.

### 3.5. Infection-control response

Upon diagnosing candidemia due to *Candida auris*, the infection control team immediately notified the treating physician and recommended rigorous infection control measures. These included isolating the patient and using dedicated equipment such as stethoscopes, blood pressure cuffs, and pulse oximeters. Healthcare personnel were instructed to follow strict hand hygiene protocols using alcohol-based hand sanitizer and to wear gowns and gloves for all interactions that might involve contact with the patient or potentially contaminated areas in the patient’s environment. Additionally, surface disinfection with hydrogen peroxide or hypochlorite was advised every 6 h.

Following the identification of additional *Candida auris* candidemia cases in the same paediatric and neonatal intensive care unit, an intense surveillance effort was undertaken to pinpoint the outbreak’s source. This investigation identified the USG Accu-probe and BP-cuff as potential sources colonized by the pathogen. Consequently, thorough cleaning of the USG Accu-probe and other shared or reusable equipment was performed using a 70 % isopropyl alcohol solution.

An IPC team meeting with neonatal and paediatric ICU consultants, nursing, and paramedical staff was convened to review and enhance infection control protocols. Several measures were implemented as policy to contain the pathogen’s spread. These included alerting treating physicians whenever *Candida auris* is isolated, providing routine training on IPC practices for healthcare workers, and monitoring the implementation of IPC procedures in affected areas.

Specific infection control measures included standard precautions such as hand hygiene with alcohol-based sanitizer when hands are not visibly soiled, and washing with soap and water when they are. Enhanced barrier precautions involved using PPE for tasks like transferring patients and caring for devices such as central lines and ventilators. Transmission-based precautions required single-patient rooms for those with *Candida auris*, or cohorting patients if single rooms were unavailable. In multi-patient rooms, beds were spaced at least three feet apart to minimize pathogen transmission opportunities. Dedicated equipment for each patient and strict PPE protocols, including donning upon room entry and discarding before exit, were mandated.

Infection practices were intensified, with surfaces disinfected every 6 h using hypochlorite or hydrogen peroxide, and terminal cleaning of patient rooms was conducted. Shared or reusable equipment was cleaned and disinfected after each use with 70 % isopropyl alcohol solution. Environmental surfaces were cleaned frequently, and healthcare personnel were required to change PPE and perform hand hygiene before and after interacting with each patient. These precautions were maintained throughout all inpatient healthcare stays.

Following these enhanced infection control measures, repeat sampling was carried out, and all environmental samples tested negative for *Candida auris*, confirming the effectiveness of the implemented protocols in controlling the outbreak.

### 4. Discussion

Several studies have reported ability of *Candida auris* to survive on surfaces for several weeks and colonize human skin and persist for long periods. Here we report an outbreak of *Candida auris* infection in paediatric and neonatal intensive care unit. The survival of the organism on reusable patient equipments like USG Accu-probe and BP cuff, was the most convincing reason for the ongoing *Candida auris* transmission that we observed.

The management of *Candida auris* infections is very challenging due to the extremely high rates of fluconazole resistance and variable susceptibility to other azoles, amphotericin B, and echinocandins. A widespread fluconazole resistance and variable amphotericin B resistance has been documented, but echinocandin resistance is less common. In our study all isolates were found resistant to fluconazole and antifungal susceptibility pattern suggested Caspofungin and Micafungin as drug of choice.

Several studies evaluated effectiveness of chlorine-based disinfectants, which are most commonly used in the healthcare settings for disinfection of surfaces. Alcohol, peracetic acid, acetic acid, phenol, and glutaraldehyde have also been tested as disinfectants against *Candida auris*. According to one study, washing hands with soap and water following World Health Organization recommendations was just as effective in killing *Candida auris* as alcohol-based hand sanitizers with and without Chlorhexidine gluconate. 70 % isopropyl alcohol has also been seen to be effective against *Candida auris*. So, as a part of infection control, cohorting of patients, good hand hygiene, standard precautions, improved barrier precautions, transmission-based precautions, and appropriate disinfection of environmental surfaces with 70 % isopropyl alcohol disinfectant were implemented. When the specified disinfectant usage procedure (concentration and contact time) was strictly adhered to, the yeast was entirely eliminated from all contaminated surfaces. However, some patients may still be chronically colonized and there is always a chance for new outbreak by same or different clonal strain of the organism from other community reservoirs. Hence, to prevent candidemia caused by *Candida auris*, strict adherence to hand hygiene measures, adequate disinfection of medical equipment and ambient surfaces, and stringent surveillance to detect any new outbreaks are required.
5. Conclusion

This study highlighting an outbreak investigation in a tertiary care hospital to alert readers to the ominous presence of *Candida auris* in the Indian state of Bihar. For the diagnosis of fungemia due to *Candida auris* and for favourable clinical outcome, a high index of suspicion, prompt case discovery, constructive teamwork between the physician and the clinical microbiologist, adequate therapy, and strict surveillance and preventive measures are necessary.

Funding information

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Ethical statement

This current study was approved by Institutional Ethics Committee and an informed consent was waived off by ethics committee due to the audit nature of data collection in the event of an outbreak investigation.

Authors contribution

Dr. KP Anirima Investigation, Writing - Original Draft. Dr. Prathyusha Kokkayil Conceptualization, Writing - Review & Editing. Dr. Asim Sarfraz Methodology, Conceptualization, Writing - Review & Editing. Dr. Bhadesh Kant Chowdhy Visualization, Supervision. Dr. Bhaskar Thakuria Visualization, Supervision. Dr. Binod Kumar Pati Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix.

Table 6.A.1
Case definitions used to identify the outbreak of *Candida auris*

<table>
<thead>
<tr>
<th>Hospital acquired Candida auris infection</th>
<th>Isolation of Candida auris from any body fluids obtained from a specimen collected &gt;48h after hospital admission.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior antifungal exposure</td>
<td>Empirical or prophylactic therapy with antifungals within 30 days prior to the diagnosis of Candida auris infection.</td>
</tr>
<tr>
<td>Clinical cure</td>
<td>Complete resolution of all clinical signs and symptoms of focus of infections pertaining to Candida auris as evidenced by complete resolution of fever and attainment of hemodynamic stability, if normal before starting treatment.</td>
</tr>
<tr>
<td>Microbiological cure</td>
<td>Negative culture or absence of Candida auris in repeat cultures</td>
</tr>
</tbody>
</table>

Table 6.A.2
Patient specific information

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1 year</td>
<td>1 month</td>
<td>9 days</td>
<td>2 years</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Primary diagnosis</td>
<td>Purpura fulminans with septic shock with primary immunodeficiency</td>
<td>Extreme preterm 28weeks, perinatal asphyxia, early onset neonatal sepsis</td>
<td>Acyanotic heart disease, early onset neonatal sepsis, pneumonia</td>
<td>Cerebello-pontine angle tumour with cystic hygroma</td>
</tr>
<tr>
<td>Location at the time of isolation</td>
<td>Paediatric intensive care unit</td>
<td>Neonatal intensive care unit</td>
<td>Neonatal intensive care unit</td>
<td>Paediatric intensive care unit</td>
</tr>
<tr>
<td>Prior antifungal exposure</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Surgery in the last 30 days</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Duration of hospital stay prior to isolation of Candida auris (in days)</td>
<td>5 days</td>
<td>15 days</td>
<td>8 days</td>
<td>10 days</td>
</tr>
<tr>
<td>Specimen from which Candida auris was isolated</td>
<td>Blood</td>
<td>Blood</td>
<td>Blood</td>
<td>Blood</td>
</tr>
<tr>
<td>Clinical cure</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Microbiological cure</td>
<td>Yes</td>
<td>Yes</td>
<td>Not known (Patient left against medical advice)</td>
<td>Dead</td>
</tr>
<tr>
<td>Outcome</td>
<td>Alive</td>
<td>Dead</td>
<td>Dead</td>
<td>Dead</td>
</tr>
</tbody>
</table>

Table 6.A.3
Antifungal drugs given to patients and duration of antifungal therapy

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Antifungals given</th>
<th>Duration of antifungal treatment (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Fluconazole</td>
<td>23</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Fluconazole, Liposomal Amphotericin B, Caspofungin</td>
<td>5, 18 and 5</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Caspofungin</td>
<td>14</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Fluconazole, Caspofungin</td>
<td>5 and 3</td>
</tr>
</tbody>
</table>
Fig. 6.B.1. (a) Pink to purple-coloured colonies 2–3 mm on CHROMagar. (b) Ovoid or ellipsoidal budding yeast cells in singlets, doublets, and clusters, with some rudimentary pseudo-hyphae occasionally on Corn Meal Agar.

Fig. 6.B.2. Phylogenetic tree of the identified isolates from patient and environmental samples (where P1 = isolate from Patient 1, P2 = isolate from Patient 2, P3 = isolate from Patient 3, P4 = isolate from Patient 4, and UP = isolate from USG Accu-probe). The phylogenetic tree shows clonal lineage of P1, P2 and P3 were identical to the clonal strain of Candida auris isolated from the UP. However clonal lineage of P4 slightly varied thereby indicating a slightly mutated or different Candida auris clonal lineage.

References


