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ORIGINAL ARTICLE

Molecular identification and diagnostic challenges of *Kodamaea ohmeri* fungemia in a neonate - first report from Pakistan

Humaira Farooq[1*], Summiya Nizamuddin[2], Romena Qazi[2], Nasrullah Malik[2], Faisal Sultan[3]

[1]Department of Basic Sciences, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan
[2]Department of Pathology, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan
[3]Department of Internal Medicine and Infectious Diseases, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan

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ABSTRACT

Background and Objectives: *Kodamaea ohmeri* is an uncommon opportunistic yeast increasingly recognized as a cause of invasive infections, particularly in immunocompromised individuals and neonates. We report the first case of *Kodamaea ohmeri* fungemia in a neonate from Pakistan, highlighting the diagnostic challenges posed by its close resemblance to more common *Candida* species.

Materials and Methods: A one-month-old female neonate with suspected fungal sepsis yielded a yeast isolate. Initial identification was performed using API 20C AUX, followed by colony morphology assessment on Sabouraud Dextrose Agar. Molecular investigations included PCR amplification of the ITS1–5.8S rRNA–ITS2 region, restriction fragment length polymorphism (RFLP) with *MspI*, and definitive Sanger sequencing. Antifungal susceptibility testing was conducted using standard methods.

Results: The isolate was initially misidentified as *Candida guilliermondii* by API 20C AUX. Colony characteristics suggested an unusual yeast, prompting molecular analysis. PCR produced a ~400 bp amplicon, and RFLP yielded an undigested band, initially suggestive of *Candida auris* or *Candida haemulonii*. Sanger sequencing confirmed the organism as *K. ohmeri* with 99.8% identity. Antifungal testing showed low minimum inhibitory concentrations (MICs) for echinocandins and azoles, while fluconazole demonstrated a higher MIC.

Conclusion: This case emphasizes the limitations of conventional methods in identifying rare fungal pathogens, the critical role of molecular confirmation, and the importance of antifungal stewardship in guiding treatment. Reporting such cases contributes to global awareness, surveillance, and improved management of emerging yeast infections.

Keywords: *Kodamaea ohmeri*; Neonate; Fungemia; Molecular diagnostic techniques; Drug resistance; Pakistan

*Corresponding author: Humaira Farooq, Ph.D, Department of Basic Sciences, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan. Tel: +92-42-35905000 Fax: +92-42-35945208 Email: humairfarooq@skm.org.pk; humairafarooq12@gmail.com

INTRODUCTION

Kodamaea ohmeri is a rare, opportunistic yeast-like pathogen that has emerged from an environmental organism, originally isolated from food and environmental sources (1, 2), and has become a cause of serious infections, particularly in immunocompromised individuals such as those with malignancies, transplants, or on prolonged antimicrobial therapy (3, 4).

Clinically, *Kodamaea ohmeri* has been linked to bloodstream infections, fungemia, peritonitis, and wound infections (5), with reported mortality rates ranging from 40% to 50% (5, 6). Its phenotypic resemblance to more common yeasts such as *Candida tropicalis*, *C. albicans*, and *C. glabrata* often leads to misidentification using conventional methods, potentially resulting in underreporting and delayed treatment (5, 6).

Accurate identification is crucial in high-risk settings. However, its antifungal susceptibility remains inadequately defined, with variable resistance patterns reported, particularly to fluconazole, while some isolates are susceptible to amphotericin B, voriconazole, or echinocandins (7). This variability underscores the need for more data to guide therapy. Reporting rare cases helps expand understanding of their clinical profile and drug response (8, 9).

This report presents a literature review of *Kodamaea ohmeri* infections and details a case from our institution confirmed by molecular methods.

MATERIALS AND METHODS

A blood sample was obtained during routine diagnostics from a one-month-old female with suspected fungal sepsis. Initial identification used the API 20°C AUX system (bioMérieux, France). The isolate was sub-cultured on Sabouraud Dextrose Agar at 30°C for 48 hrs, producing small, dry, wrinkled white colonies.

Genomic DNA was extracted by boiling (10) and quantified with a NanoDrop spectrophotometer. The ITS1-5.8S rRNA-ITS2 region was amplified using ITS1 and ITS4 primers. PCR reactions were conducted in a 50 µL volume containing 25 µL of 2X PCR master mix (Green Taq Mix, Thermo Fisher Scientific), 1 µL of each primer (0.2 µM), 3 µL of template DNA, and 20 µL of nuclease-free water. PCR cycling conditions comprised an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 45 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. For species confirmation, PCR products were digested with *MspI* (Thermo Fisher Scientific), and fragments were resolved on 2% agarose gel (11). A clinical *Candida auris* strain served as a control.

Sanger sequencing was performed on purified products (QIAquick Kit) using BigDye v3.1 on an ABI 3500 Genetic Analyzer. Sequences were analyzed by BLASTn against GenBank (E-value cutoff 1e-5).

The nucleotide sequences of the ITS1-5.8S rRNA-ITS2 region were obtained directly from high-quality Sanger sequencing chromatograms and converted into FASTA format without gaps or ambiguous base calls. Quality was confirmed by manual inspection of chromatogram peaks, ensuring sharp, non-overlapping signals and minimal background noise. Each sequence was independently submitted to the NCBI GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for species identification. Both forward and reverse sequences demonstrated approximately 99.8% identity and 100% query coverage with *Kodamaea ohmeri* reference sequences, confirming the accurate species-level identification of the isolate.

Some positions in the ITS sequences are represented by standard IUPAC nucleotide ambiguity codes such as “K”, “M”, and “R”. These indicate sites where the Sanger sequencing chromatogram showed either two overlapping peaks or unclear signal, and therefore the exact nucleotide could not be determined with certainty. The codes follow IUPAC conventions: “K” = G or T, “M” = A or C, “R” = A or G. All ambiguous sites were manually inspected using their chromatograms to confirm true sequence heterogeneity or sequencing uncertainty. The presence of these codes does not affect species identification by BLAST.

Antifungal susceptibility was assessed using the YeastOne Sensititre YO10 system per CLSI M27-A3. MICs were visually read for fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, micafungin, anidulafungin, and amphotericin B after 24-hour incubation at 35°C.

RESULTS

Case summary. A one-month-old female neonate presented for evaluation with suspected fungal sepsis. The blood sample was obtained at Shaukat Khanum Memorial Cancer Hospital and Research Centre following referral from a local heart hospital. Unfortunately, detailed clinical history, including birth details, presenting symptoms, comorbidities, duration of treatment, and hospital course, was unavailable due to the outpatient nature of the referral and limitations in follow-up data collection. Despite this, the isolation of *Kodamaea ohmeri* from the blood culture indicates invasive fungal infection in a vulnerable patient population such as neonates. The clinical management and outcome could not be ascertained.

Microbiological identification and molecular analysis. The isolate was initially mistakenly recognized as *Candida guilliermondii* by API 20C AUX. However, distinctive colony morphology, dry, rough, white colonies with cerebriform areas on Sabouraud Dextrose Agar, and a lack of chromogenic color development indicated a species other than *Candida guilliermondii.*

PCR amplification yielded an approximately 400 bp product (Fig. 1), initially suggestive of *Candida auris* or *Candida haemulonii* (12). RFLP analysis showed a single undigested band, indicating no *MspI* restriction sites.

Sanger sequencing confirmed *Kodamaea ohmeri* with 100% query coverage and ~99.8% identity. The forward and reverse ITS1-5.8S rRNA-ITS2 sequences derived directly from high-quality chromatograms were independently submitted to the NCBI BLAST database. Both sequences demonstrated high similarity with *Kodamaea ohmeri*, showing approximately 99.8% identity and complete query coverage, confirming species-level identification. Manual inspection of chromatograms ensured the reliability of base calls without ambiguous nucleotides, supporting the accuracy of the molecular identification. The closest BLAST match is summarized in Table 1.

The representative sequences obtained are as follows:

>S_01_ITS1_F

TTTTTTTACAACAAAAMAAATCTATCTAAAAA-
CAATTCTTTACAAGAAATTCTTAAAACT
TTCAACAACGGATCTCTTGGTTCTCGCATCGAT-
GAAGAACGCAGCGAAATGCGATACGTA
ATACGAATCGCAGCTCTCGGAATCATCGAATCTTT-
GAACGCACATTGCACCATTGGGTAT
TCCCAATGGTATGCTTGTTTGAGCGAATACTTC-
CCTAATCCTCACGGATTGTATTGTGTT
TGCACGAAAATAATGACGACAGTACTCTA-
CAAAACGGTACCGTCAGTACACTCATTTTTT
TTCCTCAAATCAAGTAGGACTACCCGCTGAACTTA-
AGCATATCAATAAGCGGAG

>S_01_ITS4_R

TGAGTGTACTGACGGTACCGTTTTGTAGAGTACT-
GTCGTCATTATTTTCGTGCAAACACA
ATACAATCCGTGAGGATTAGGGAAGTATTCGCT-
CAAACAAGCATACCATTGGGAATACCC
AATGGTGCAATGTGCGTTCAAAGATTCGATGATTC-
CGAGAGCTGCGATTCGTATTACGTA
TCGCATTTCGCTGCGTTCTTCATCGATGCGAGAAC-

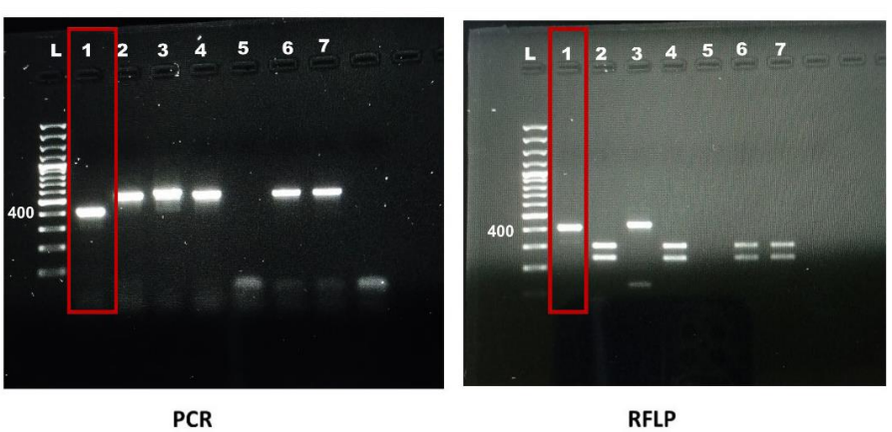

Fig. 1. Agarose gel electrophoresis images showing (left) PCR amplification of the ITS1-5.8S rRNA-ITS2 region and (right) restriction fragment length polymorphism (RFLP) analysis with *MspI*. For both gels, Lane L: 100 bp DNA ladder; Lane 1: clinical isolate from the neonate.

Table 1. NCBI BLAST analysis of *Kodamaea ohmeri* isolate DNA sequences

Sequence ID	Species Identified	Accession No.	E-value	Query Coverage (%)	Sequence Identity (%)	Band Size (bp)
S_01_ITS1_F	*Kodamaea ohmeri*	MT443964.1	1.00E-05	100	99.8	372
S_01_ITS4_R	*Kodamaea ohmeri*	OM287576.1	1.00E-05	100	99.8	411

CAAGAGATCCGTTGTTGAAAGTTTT
AAGAATTTCTTGTAAAGAATTGTTTTTAGA-
TAGATTTKTTTTGTTGTAAAAAAACAGTGT
GTAAGAATATTATGTTAAKGATCCTTCCGCAG-
GTTCACCTACGGARGG

Antifungal susceptibility testing showed that the *Kodamaea ohmeri* isolate was susceptible to all antifungal agents tested. Among the azoles, fluconazole had a relatively higher MIC of 1 µg/mL, whereas voriconazole, itraconazole, and posaconazole exhibited significantly lower MICs (≤ 0.03 µg/mL), indicating higher in-vitro activity. The isolate also showed strong susceptibility to echinocandins, with MICs of 0.03 µg/mL for caspofungin and anidulafungin, and 0.06 µg/mL for micafungin. Amphotericin B demonstrated good activity with an MIC of 0.25 µg/mL.

DISCUSSION

This report details the identification of *Kodamaea ohmeri* via PCR-RFLP and sequencing from a one-month-old infant's blood, indicating a true invasive fungal infection rather than contamination, given the patient's immature immune status. *Kodamaea ohmeri* is an emerging opportunistic pathogen, particularly in immunocompromised individuals and neonates, with 62 clinical isolates reported in a Chinese surveillance study over seven years (1, 13). Its rarity, phenotypic similarity to common *Candida* species, and variable antifungal susceptibility pose diagnostic and therapeutic challenges, emphasizing the need for accurate identification and tailored therapy (1). *Kodamaea ohmeri* is the only species within its genus with demonstrated medical significance (1, 13).

The isolated *Kodamaea ohmeri* exhibited distinctive dry, rough, white, non-mucoid, and cerebriform colonies on Sabouraud Dextrose Agar after 48 hours, differentiating it from the smooth, creamy colonies of *Candida albicans* (14, 15). Traditional biochemical methods are often insufficient for its identification, necessitating molecular methods like rRNA gene sequencing (6). This case, the first reported from Pakistan, highlights the global emergence of *Kodamaea ohmeri* and the critical role of molecular confirmation for unusual yeast isolates, similar to a prior case where initial PCR suggested *Candida auris* or *Candida haemulonii* before sequencing confirmed *Kodamaea ohmeri* (6).

Kodamaea ohmeri showed variable antifungal susceptibility. The isolate was susceptible to all tested agents, with lower MICs for echinocandins and newer azoles (≤0.03 µg/mL), while fluconazole had a higher MIC (1 µg/mL), raising concerns about its efficacy. Amphotericin B was active (MIC = 0.25 µg/mL) but limited by nephrotoxicity (16).

The isolate showed low MICs for echinocandins and amphotericin B, high activity of newer azoles (voriconazole, itraconazole, posaconazole ≤0.03 µg/mL), and a comparatively higher MIC for fluconazole (1 µg/mL). Similar patterns have been reported in previous studies, where *Kodamaea ohmeri* isolates often display variable fluconazole susceptibility but remain highly susceptible to echinocandins and amphotericin B (5, 8, 9, 13). Reports also suggest that voriconazole and amphotericin B are effective options, particularly in neonatal cases (7). Since formal CLSI/EUCAST breakpoints for *Kodamaea ohmeri* are not established, interpretation relies on MIC ranges and available epidemiological data. The susceptibility pattern observed indicates a likely wild-type phenotype, suggesting echinocandins or amphotericin B as preferred empiric options, while fluconazole should be used cautiously and guided by individual MIC values and patient response.

Taken together, these findings emphasize the importance of integrating molecular diagnostics and antifungal susceptibility testing in clinical settings along with conventional diagnostics, particularly when dealing with rare, opportunistic fungi (17).

CONCLUSION

This study documents the first case of *Kodamaea ohmeri* fungemia in a neonate from Pakistan, em-

phasizing significant diagnostic challenges due to phenotypic similarity to other *Candida* species. It highlights the critical role of molecular tools for accurate species identification for guiding antifungal treatment. The case further demonstrates the variable antifungal susceptibility of this rare pathogen, particularly reduced activity of fluconazole, reinforcing the importance of susceptibility testing to guide therapy. Increasing awareness, molecular surveillance, and prompt reporting of such infections are essential for improving recognition and management of emerging opportunistic yeasts.

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