Epidemiological study of a large cluster of fungaemia cases due to Kodamaea ohmeri in an Indian tertiary care centre

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Abstract

While performing molecular confirmation of phenotypically identified Candida tropicalis isolates, we re-identified a few isolates as Kodamaea ohmeri. This led us to the present epidemiological investigation of K. ohmeri fungaemia cases. All phenotypically identified C. tropicalis blood isolates during October 2008 through to December 2009 at our advanced paediatric centre were included for molecular identification by sequencing of the internal transcribed spacer and D1/D2 regions of rDNA. After identifying a large cluster K. ohmeri fungaemia cases, a case–control study was carried out retrospectively to analyse potential risk factors for K. ohmeri fungaemia. Molecular typing of the isolates was performed using a fluorescent amplified fragment length polymorphism (FAFLP) technique. The antifungal susceptibility testing was performed as per the M27-A3 protocol of CLSI. Thirty-eight (25.7%) of 148 phenotypically identified C. tropicalis isolates were confirmed as K. ohmeri by sequencing and FAFLP. By case–control analysis, piperacillin-tazobactam was significantly associated with the K. ohmeri fungaemia. The FAFLP analysis showed that all K. ohmeri isolates had >92% similarity. The azoles and echinocandins had good in vitro activity against K. ohmeri, though 86.8% of the isolates had MIC of 1 mg/L for amphotericin B. The response to antifungal therapy could be evaluated in 27 patients and 70.4% of patients recovered after antifungal therapy. The present study reports the largest cluster of K. ohmeri fungaemia from a single centre. The study also stresses the need for accurate identification of clinical yeast isolates.

Keywords: Antifungal susceptibility testing, Candida tropicalis, candidaemia, diagnosis, epidemiology, fungaemia, identification, Kodamaea ohmeri, molecular typing

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Introduction

Nosocomial fungaemia due to Candida spp. and related yeasts has become a persistent health problem in both developed and developing countries. The incidence of fungaemia varies at 8–10% of all nosocomial sepsis and 30–50% of these fungaemia cases occur in patients undergoing treatment in intensive care units [1–5]. The incidence and spectrum of causative agents of fungaemia varies in geographical regions. Though a higher rate of fungaemia due to non-albicans Candida species has been observed across the world, the proportion is almost 90% at certain hospitals in Asia [6–9]. While Candida glabrata and Candida parapsilosis are the leading non-albicans Candida spp. in the USA and European countries [1–5], Candida tropicalis is reported as the commonest agent in Asian countries [6–9].

While studying the molecular epidemiology of C. tropicalis candidaemia at our institute, we identified a few clinical isolates as Kodamaea ohmeri by sequencing. Those isolates had been identified earlier as C. tropicalis on the basis of phenotypic characters. Kodamaea ohmeri is a rare pathogen and the
majority of cases are reported from Asian countries. Previously known as *Pichia ohmeri* and *Yamadazyma ohmeri*, *K. ohmeri* is an ascosporogenous yeast and a teleomorph of *Candida guillermondii* var. *membranaeociens*, belongs to the class Ascomycetes and family Saccharomycetaceae. Of the five species reported under the genus *Kodakaea*, only *K. ohmeri* has the ability to grow at 37°C. The clinical importance of other *Kodakaea* species (*K. anthrophila*, *K. kakaduensis*, *K. laetipori* *K. nitidulidurum*) is not known [10]. *Kodakaea ohmeri* is reported to cause high mortality (50%) in paediatric populations [11–21]. From our tertiary care centre with 1740 beds we report a very high incidence of fungaemia (300–500 cases every year) and *C. tropicalis* is the commonest isolate [8,22–24]. However, *K. ohmeri* fungaemia had never been reported. We therefore planned a detailed epidemiological investigation of *K. ohmeri* fungaemia cases.

**Materials and Methods**

**Epidemiological investigation**

The Postgraduate Institute of Medical Education and Research is a 1740-bed multispeciality tertiary care centre in north India with an advanced paediatric centre with 243 beds.

**Isolates and identification.** *Candida tropicalis* isolates (n = 148; identified conventionally earlier by germ-tube test, urease production, morphology on corn-meal agar, and sugar fermentation and assimilation tests) from blood of patients admitted to the advanced paediatric centre from August 2008 to December 2009 were included in the study. Isolates from the hands of healthcare workers, which were collected by standard bag broth technique [9] twice (October 2008 and July 2009) from the neonatal surgical intensive care unit during the same period, were also included in the study. All 148 isolates used in the present study were re-identified by sequencing the internal transcribed spacer region and D1/D2 region of 26S ribosomal DNA [25,26].

**Patients.** Detailed clinical histories of patients with *K. ohmeri* fungaemia were retrieved from the archive and noted.

**Case–control study.** A retrospective case–control study was performed in the neonatal surgical intensive care unit (because the maximum number of cases of *K. ohmeri* fungaemia was identified from this area) to determine the potential risk factors of *K. ohmeri* infection. Consecutive patients with *K. ohmeri* fungaemia (30 cases), *C. tropicalis* fungaemia (25 cases) and patients without fungaemia who stayed in hospital for >7 days (22 cases) during October 2008 to December 2009 were studied. The last two groups served as controls.

**Fluorescent amplified fragment length polymorphism.** Molecular typing of the *K. ohmeri* isolates was performed using a fluorescent amplified fragment length polymorphism (FAFLP) technique [27]. The details have been provided in the Supplementary material (Supplementary text). In short, restriction enzymes *Mse* I and *HpyCH4IV* (New England Biolabs, Ipswich, MA, USA) and corresponding adapters were used. Amplification was performed using per-selective primers of *HpyCH4IV* (5′-GTA-GACTGCGTACCCGT-3′) and *Mse* I (5′-GATGAGTCCCTGA-CTAATG-3′). *HpyCH4IV* primer with one selective residue (5′-GTA-GACTGCGTACCCGT-3′), and *Mse* I primer with two selective residues were used (5′-GATGAGTCCCTGA-CTAACA-3′) and the primers were labelled with 6-FAM. Capillary electrophoresis of the amplified products (labelled with 6-carboxy fluorescein) and LIZ 500 (standard marker) was performed in an ABI automated DNA Sequencer 3130 (Applied Bioscience, Foster City, CA, USA). Typing data were imported to BioNumerics v 6.6 software (Applied Maths, Ghent, Belgium). The fingerprint curves were converted into bands and correct bands of each lane were assigned using the band position of the reference dye (LIZ500). The similarity coefficient was determined by Pearson correlation with negative similarities clip to zero. Cluster analysis was performed by Unweighted Pair Group Method with Arithmetic Mean using BioNumerics software.

**Antifungal susceptibility testing.** The MICs of the *K. ohmeri* isolates were determined by reference microbroth dilution antifungal susceptibility testing of yeasts as per document M27-A3 of CLSI [28]. The antifungal drugs included in the study were amphotericin B (Sigma Aldrich, Bangalore, India), fluconazole (Sigma Aldrich, India), itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer Central Research, Tadworth, UK), posaconazole (Merck Sharp and Dohme, Gurgaon, India) and caspofungin (Merck Sharp and Dohme, India).

**Statistical analysis.** The results of the study for the patient and the control groups were compared by multivariate analysis test. Normalcy of the three groups was determined by Kolmogorov–Smirnov test. Variables like age, duration of hospital stay and the time after which infection occurred were described in terms of means. The non-parametric Mann–Whitney *U*-test was applied to compare the differences between the groups. Qualitative variables like the sex of patient, risk factors, and antibiotics and antifungals used were compared using the Pearson chi-squared test. All the statistical tests were carried out using an a error of 5% and a β error of 0.2.
analyses used SPSS (version 19.0 for Windows) software.

Results

Isolates and identification
Of 398 culture-proven fungaemia cases that occurred during the study period of 17 months (August 2008 to December 2009) at the advanced paediatric centre, 148 isolates had been identified previously as C. tropicalis on the basis of biochemical and morphological tests. During the present study 38 (25.7%) of those 148 isolates were re-identified as K. ohmeri by genotypic characters. The K. ohmeri isolates had 98–100% identity with the sequence of standard strains (ATCC 46053, CBS 5367). The DNA sequences of the representative isolates have been deposited in the EMBL nucleotide sequence database with the Accession nos HG313954 to HG313978. Monthwise distribution of cases in pediatric ward is provided in Table S1. The details of each patient and isolate are presented in the Supplementary material (Table S2). During the same period, 47 yeasts were isolated from the hands of healthcare workers at the neonatal surgical intensive care unit and one isolate was identified as K. ohmeri (isolated during October 2008) was close to two blood isolates in one cluster, including the hand isolate (cluster H). The hand isolate (K5) and all blood isolates from 2008 were within a similarity coefficient of >92% [29].

Patients
The distribution of K. ohmeri cases during the study period is given in Fig. 1. The median age of the patients was 7 days, mean 87 days, range 1 day to 8 years with male preponderance (male : female ratio 2.4 : 1). The majority (87%) of the patients with K. ohmeri fungaemia were neonates and 79% (30/38) of them were from the neonatal surgical intensive care unit. The response to antifungal therapy could be evaluated in 27 patients, because eight patients died before the start of antifungal therapy and three patients left the hospital against medical advice. Twenty-three (85.1%) patients received fluconazole and four received caspofungin; 15 (65.2%) patients receiving fluconazole and all four patients receiving caspofungin recovered (the details of individual patients are available in the Supplementary material) (Table S2).

Case-control study
Risk factor analysis is presented in Table 1. In comparison to the C. tropicalis fungaemia, the only significant risk factor for acquiring K. ohmeri fungaemia in the hospital was piperacillin-tazobactam use (p 0.032). Whereas comparing the K. ohmeri fungaemia and non-fungaemia groups, prolonged hospital stay (p 0.037), piperacillin-tazobactam use (p 0.044), endotracheal intubation (p 0.002) and mechanical ventilation (p 0.033), were significant risk factors for the development of K. ohmeri fungaemia. Mortality was also significantly higher (50%) in K. ohmeri fungaemia patients compared with patients with C. tropicalis fungaemia (24%) or non-fungaemia (18.2%) groups. The attributable mortality in K. ohmeri fungaemia cases was 31.8%.

FAFLP
Thirty-eight isolates (37 from patients and one from the hands of a healthcare worker) were included for the FAFLP analysis. FAFLP profiles yielded fragments ranging from 40 to 500 bp but only fragments in the range of 50–250 bp were included for the analysis. A total of ~110 fragments were analysed. All K. ohmeri isolates had >92% similarity co-efficient. The FAFLP profile yielded eight clusters (Fig. 2). The similarity of ‘A’ ‘B’ and ‘C’ clusters was >96% with inter-cluster difference of >1%. The ‘D’, ‘E’, ‘F’ ‘G’ and ‘H’ clusters had 91–95% similarities. Except for one isolate (K5), all blood isolates from 2008 were in one cluster, including the hand isolate (cluster H). The hand isolate (isolated during October 2008) was close to two blood
isolates of 2008 (K2 and K6) with variation of <4%. The *C. tropicalis* isolate had only 40% similarity with *K. ohmeri* isolates.

### Antifungal susceptibility testing

The *in vitro* activities of each antifungal agent against the *K. ohmeri* isolates including the range of MIC, geometric mean, MIC$_{50}$ and MIC$_{90}$ and cumulative percentage of the isolates inhibited at the MIC at various concentrations of antifungal agents are presented in Table 2 and Table S3. MIC$_{90}$ of azoles and echinocandins were ≤0.50 mg/L. However, 33 (86.8%) isolates had MIC of 1 mg/L against amphotericin B. Only one isolate had high MICs against azoles (fluconazole MIC >64 mg/L, voriconazole 8 mg/L, itraconazole 8 mg/L, and posaconazole 4 mg/L). The response to antifungal therapy in the patient harbouring the *K. ohmeri* isolate with high MIC against azoles could not be evaluated because the patient left against medical advice.

### Discussion

The present series of *K. ohmeri* fungaemia is the largest cluster (38 cases) of such cases from a single centre reported and the majority (78.9%) of those were reported from a neonatal surgical intensive care unit. The infection was often serious and fatal (attributable mortality 31.8%). *Kodamaea ohmeri* is rarely reported to cause human infection [11–21]. Before the present report, only 39 cases of *K. ohmeri* infection had been reported. Among them, 30 (76.9%) cases presented as bloodstream infections [11,12,16,19], and the remaining occurring as peritonitis (two cases), endocarditis (three cases), urinary tract infection (one case), polymicrobial wound infection (one case) and oral ulcer (three cases) [13,17,18,20]. The majority (75%) of those patients were immunosuppressed when they acquired the infection and had 38% mortality. *Kodamaea ohmeri* fungaemia may not be rare in India, as the fungus was isolated from ~1% of fungaemia cases from nine of 27 intensive care units in a recently conducted study across India (unpublished observation).

A high incidence of fungaemia due to non-*albicans* *Candida* species has been reported from Asian countries including India [6–22–24], and *C. tropicalis* is reported as the commonest non-*albicans* *Candida* species in contrast to *C. glabrata* and *C. parapsilosis* in the USA and Europe[1–5]. The emergence of *C. glabrata* is linked to widespread use of azole antifungal agents and *C. parapsilosis* is linked with catheter use [1–3]. However, such an explanation could not be provided for the emergence of our *C. tropicalis* isolates, as the resistance to azoles in those isolates was not beyond 10% [8,22,23]. This could be a result of suboptimal infection control practices in hospitals in developing countries [30]. Similarly, the majority (97.3%) of *K. ohmeri* isolates in the present study had low MIC against azoles and echinocandins.

To investigate the reasons for emergence we conducted a case–control analysis. Prolonged hospital stay, endotracheal intubation and mechanical ventilation were significantly associated with *K. ohmeri* fungaemia. These risk factors are already
known as risk factors for fungaemia [1–3]. The other common risk factors for fungaemia, such as central line and surgery, were not found to be significant in this risk factor analysis. However, while comparing commonly occurring C. tropicalis candidaemia cases, piperacillin-tazobactam use was found to be a significant risk factor for K. ohmeri fungaemia. This single risk factor could not explain the emergence of K. ohmeri fungaemia. Moreover, we did not find any change of clinical and laboratory procedures and hospital care practices during the study period. K. ohmeri is commonly used in the food industry, especially in the preparation of pickles because of its fermentation capability [10]. Though Indians are fond of pickles, they are not usually supplied in the diet of hospitalized children. Therefore, it is difficult to ascertain the actual reason for the emergence of K. ohmeri fungaemia. The patients with K. ohmeri fungaemia had significantly higher (50%) mortality than those with C. tropicalis fungaemia and than patients without fungaemia. The high mortality due to K. ohmeri infection may be related to the virulence factors of the organism, though no study has yet been conducted to confirm this.

We performed molecular typing of the K. ohmeri isolates both from patients and the hand isolate. In an earlier study, pulsed-field gel electrophoresis was used to type K. ohmeri clinical isolates, which differentiated 13 isolates into six different types [11]. As amplified fragment length polymorphism is a more robust technique, we employed the fluorescence-based technique to differentiate the isolates. The resulting FAFLP pattern indicates that the majority (63.2%) of the isolates (Group A and B) had a possible clonal origin with >96% similarity. Interpretation of the

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<th>TABLE 2. In-vitro antifungal susceptibility data (MIC, mg/L) of 38 Kodamaea ohmeri isolates against six antifungal agents</th>
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<td>Antifungal agent</td>
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<td>Amphotericin B</td>
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<td>Itraconazole</td>
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<td>GM, geometric mean.</td>
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FAFLP data has no clear guidelines. Savelkolk et al. mentioned that patterns with 90–100% homology are considered to be derived from identical strains whereas patterns with 60–90% homology indicate different strain from the same species [29]. However, these percentages are arbitrary and may be influenced by many technical variations and imperfections. The minimal change among the isolates of 2008 and 2009 may be due to chromosomal instability or rearrangement. We could not ascertain the method of spread of the organism in our hospital because it was a retrospective study and we had only one hand isolate. Reports in the literature reveal that K. ohmeri isolates have low MIC to all antifungal agents except for a high range of MIC against fluconazole in a few isolates [11–13,21]. Similarly, all isolates in the present study, except one, had low MIC to azoles and caspofungin. However, 86.8% of our isolates had moderately high MIC (MIC 1 mg/L) to amphotericin B, in contrast to earlier reported cases [13,21]. This observation is relevant because amphotericin B deoxycholate is a commonly used systemic antifungal agent in India despite its toxicity due to low cost. One isolate (K6) with high MIC to three commonly used azoles was isolated in 2008. Fortunately that strain either did not spread or underwent loss of fitness. This high MIC isolate was closely related (99% similarity) to another isolate (K2) that had a low MIC to all the azoles tested, and was isolated from the same ward during the same period.

Though echinocandins are recommended for management of candidaemia in intensive care units [31] and most (84.2%) of the patients were from ICUs in the present study, 85.1% of patients were treated with fluconazole and 65.2% of patients responded to fluconazole therapy. It is possible that clinicians prescribed fluconazole because of its low cost and that the isolates had low MICs against fluconazole.

The difference in phenotypic characteristics of K. ohmeri and C. tropicalis is minimal. Inclusion of raffinose and D-xylose in the routine panel of sugars that is used in the assimilation test for yeast identification may differentiate the two species, as K. ohmeri can assimilate raffinose but not D-xylose whereas C. tropicalis can assimilate D-xylose but not raffinose (Table S4) [10]. However, the majority of laboratories in India do not perform a full battery of sugar assimilation tests when identifying Candida species. The commercial systems Vitek-2 (bioMérieux, Inc., Hazelwood, MO, USA) and API 20 (bioMérieux SA, Marcy-l’Etoile, France) may differentiate the two species. Sequencing of the internal transcribed spacer region and D1/D2 region of ribosomal DNA accurately identifies most yeast species. However, commercial systems and molecular identification are rarely employed in public sector hospitals in India because of their high cost and laboratories mostly rely on restricted biochemical tests and morphological identification of yeasts. As K. ohmeri has the potential to cause outbreaks in hospitals [11], it is important to identify this fungus.

In conclusion, the present study highlights the epidemiology of the largest cluster of K. ohmeri fungaemia from a tertiary care centre in Asia. The study also emphasizes the need for accurate identification of clinical yeast isolates.

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Transparency Declaration

The authors have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Monthwise distribution of Kodamaea ohmeri isolates at different wards in the Advanced Paediatric Centre of the Institute.

Table S2. The clinical details of 38 patients with Kodamaea ohmeri fungaemia.

Table S3. Minimum inhibitory concentrations (in µg/mL) of Kodamaea ohmeri isolates against different antifungal agents.

Table S4. Phenotypic characters of Kodamaea ohmeri and Candida tropicalis.

References


