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]. The reason for the emergence of candidaemia due to *C. tropicalis* in Asian countries is not clear. Furthermore, large outbreaks due to unusual species like *Pichia anomala*, have also been reported from India [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 guilliermondii* var. *membranaefaciens*, belongs to the class Ascomycetes and family Saccharomycetaceae. Of the five species reported under the genus *Kodamaea*, only *K. ohmeri* has the ability to grow at 37°C. The clinical importance of other *Kodamaea* species (*K. anthrophila*, *K. kakaduensis*, *K. laetipori K. nitidulidarum*) is not known [10]. *Kodamaea 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 multispecialty 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 *HpyCH4*IV (New England Biolabs, Ipswich, MA, USA) and corresponding adapters were used. Amplification was performed using per-selective primers of *HpyCH4*IV (5′-GTAGACTGCGTACCCGT-3′) and *Mse*I (5′-GATGAGTCCTGACTAA-3′). *HpyCH4*IV primer with one selective residue (5′-GTAGACTGCGTACCCGTC-3′), and *Mse*I primer with two selective residues were used (5′-GATGAGTCCTGACTAACA-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 α error of 5% and a β error of