Current Research in Microbial Sciences 8 (2025) 100359

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Kodamaea ohmeri: An emergent yeast from a One Health perspective

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ARTICLE INFO

Keywords:
Agrochemicals
Hospital disinfectants
Yeasts
Cross-resistance

ABSTRACT

Kodamaea ohmeri is an emerging and opportunistic yeast associated with a high mortality rate in humans. As it is commonly found in the environment, it is possible that environmental conditions and agricultural practices contribute to the adaptation of this yeast and the selection of antifungal resistance. During a multicentric study in Brazil, conducted under a One Health perspective, 14 isolates of *K. ohmeri* were identified from different sources: three from blood cultures, three from animals (swine and poultry), and eight from animal environments (swine and poultry). Yeasts were isolated using CHROmagar® *Candida* medium and identified by MALDI-TOF MS and ITS rDNA barcoding. Minimum inhibitory concentration (MIC) was determined using the broth microdilution method for clinical (azoles, echinocandins, pyrimidine analogs, and polyenes), and environmental antifungals (tebuconazole, pyraclostrobin, carbendazim, and mancozeb), and hospital disinfectants (quaternary ammonium compounds). Of note, color variations of *K. ohmeri* were noted on CHROmagar® depending on the incubation time, which is likely to complicate its identification. Following polyphasic identification and taxonomic confirmation, all isolates demonstrated low MIC values for clinical antifungals, disinfectants, and tebuconazole. However, all isolates were able to grow in the presence of carbendazim, mancozeb, and pyraclostrobin. Together, these findings highlight the risks associated with the use of environmental azoles, such as tebuconazole, as they may impact non-target fungi of medical importance, but other fungicides do not present the same risk. This is the first study to demonstrate that *K. ohmeri*, an important emerging yeast in human medicine, can be isolated from various sources, including patients. Although the isolates exhibited low MIC values for clinical antifungals, it is

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https://doi.org/10.1016/j.crmicr.2025.100359

Available online 11 February 2025
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crucial to monitor changes in sensitivity patterns over time in emerging microorganisms to prevent the development of multidrug resistance, which may originate in the environment.

1. Introduction

Kodamaea ohmeri is an ascomycetous yeast belonging to the family Saccharomycetaceae, which also includes other medically important yeasts such as those of the *Candida* genus (Diallo et al., 2019; Zhou et al., 2021). Previously, it was classified under the genera *Yamadazyma* and *Pichia*, where it was known as *Yamadazyma ohmeri* and *Pichia ohmeri*, respectively (Kurtzman et al., 2011).

K. ohmeri was first isolated in 1984 and initially regarded as an environmental contaminant. Since then, it has been isolated from various environmental sources, including sand beach, fruits, plants, seawater, swimming pools, and is also commonly associated with insects and other organisms (Kurtzman et al., 2011; Maciel et al., 2019; Santos et al., 2020). Ten years after its initial description, *K. ohmeri* was recognized as a human pathogen, being responsible for a wide spectrum of infections including fungemia (Jin and Jin, 1994).

Nowadays, *K. ohmeri* is considered an emerging and opportunistic yeast in humans. Among the reported infections, fungemia is the most common disease, along with endocarditis and peritonitis. Less frequent infections include keratitis, skin infections, disseminated infections, urinary and lower respiratory tract infections, oral mucositis, subcutaneous infections, cellulitis, and onychomycosis (Zhou et al., 2021; Ioannou and Papakitsou, 2020). *K. ohmeri* has recently caused outbreaks and has been associated with high mortality rates, reaching up to 50 % (Otag et al., 2005; Liu et al., 2013; Chakrabarti et al., 2014).

The most used treatment for *K. ohmeri* infections has been amphotericin B (AMB). However, other antifungal agents, such as fluconazole (FCZ), voriconazole (VRC), itraconazole (ITZ), caspofungin (CSP), micafungin (MCF), and 5-flucytosine (5-FC), have also been employed. Less frequently used treatments include anidulafungin (AND), ketoconazole (KCZ), and isavuconazole (ISV). Drug combination therapies, such as AMB and FCZ, have also been reported (Zhou et al., 2021; Ioannou and Papakitsou, 2020).

Despite the high mortality rate associated with *K. ohmeri* infections, no evidence for acquired resistance have been yet described. As an environmentally prevalent yeast, it is possible that *K. ohmeri* develops antifungal resistance due to exposure to antifungals in its natural habitat (McEwen and Collignon, 2018). Environmental resistance can arise from selective pressures such as temperature fluctuations or exposure to antimicrobials, agrochemicals, and quaternary ammonium-based disinfectants released into the environment by human activities (Williams et al., 2024).

Environmental azoles, used as agrochemicals, have been shown to select for resistance to clinical azoles in fungal species such as *Aspergillus fumigatus, Cryptococcus* spp., and *Candida* spp. (Bastos et al., 2021). Among the most commonly used agrochemicals are tebuconazole (TEB), an azole with a mechanism similar to clinical azoles that inhibits lanosterol alpha-demethylase; pyraclostrobin (PYR), a strobilurin that blocks ATP production by disrupting mitochondrial electron transport; carbendazim (CBZ), which inhibits fungal growth by preventing tubulin polymerization; and mancozeb (MCZ), an agrochemical with a poorly understood mechanism believed to target multiple sites (Bastos et al., 2021; Fungicide Resistance Action Committee).

Similarly, exposure to quaternary ammonium compounds (QACs) in hospital environments may select for species with reduced susceptibility to disinfectants (Eagan et al., 2022). These disinfectants act as cationic surfactants that directly interact with the microbial cell membranes. This interaction increases membrane permeability, leading to the loss of proteins and intracellular components, resulting in cell lysis and death (Bureš, 2019; Dewey et al., 2022; Nguyen et al., 2017).

In this context, the One Health approach emerges as a comprehensive strategy to address antifungal resistance. This perspective emphasizes the interconnectedness of human, animal, and environmental health, recognizing that many diseases affecting humans also impact animals and that the microorganisms responsible are widespread in the environment (McEwen and Collignon, 2018; Williams et al., 2024; Naddeo, 2021).

As part of a One Health multicentric study conducted across four Brazilian states, 14 *Kodamaea ohmeri* isolates were collected from diverse sources. Yeasts isolates were identified using CHROmagar® *Candida*, MALDI-TOF MS and ITS rDNA barcoding, and underwent antifungal testing with clinical, environmental, and disinfectant drugs to assess their possible resistance patterns.

2. Material and methods

2.1. Ethical statement and sample collection

The samples analyzed in this study are part of the collection from a multicenter study conducted in four Brazilian states: Minas Gerais (southeast region), Mato Grosso do Sul (central-west region), and Ceará, and Rio Grande do Norte (both in the northeast region).

Yeasts were collected and isolated from two different contexts: a farming environment, where samples were taken from production animals (swines and poultries) and their living environment; and a human clinical environment, where samples were collected from patients and the hospital environment. This study was approved by the Animal Use Ethics Committees at the following institutions: Federal University of Ceará (UFC) (protocol number 4897120623), Federal University of Minas Gerais (UFMG) (CEUA 7/2023), Federal University of Grande Dourados (UFGD) (protocol number 23004), and Federal University of Rio Grande do Norte (UFRN) (protocol number 042/2023, certificate code 356.042/2023). Furthermore, the study was approved by the Research Ethics Committees of UFC (CAAE: 70,154,823.5.1001.5054), UFMG (CAAE 00883118.0.0000.5149) and Eduardo de Menezes Hospital, Minas Gerais State Hospital Foundation (FHEMIG - CAAE 00883118.0.3001.5124), UFGD (CAAE: 76,088,323.2.0000.5160), and UFRN (CAAE: 67,951,123.2.0000.5537).

Participating hospitals in each state isolated yeasts following the standard laboratory procedures of their respective institutions. Samples were collected from various clinical specimens, such as blood, cerebrospinal fluid, urine, respiratory samples (tracheal aspirate, bronchoalveolar lavage, and sputum), biopsies, secretions, and bone marrow aspirates, with samples from skin, hair, nails, or mucous membranes excluded. After identification all yeasts were sent to research laboratories at the universities involved in the project.

Hospital samples were collected from medical devices, corridors, patient beds, doors, doorknobs, and surfaces in medical and nursing areas (including computers and the staff cafeteria), as well as from various wards and intensive care units using sterile swabs moistened with saline solution (0.85 % (w/v)). Samples were then transferred to 2 mL microtubes containing peptone water with chloramphenicol (0.5 g/L) and sent to the laboratory for further analysis.

Farm animals (poultry (specifically, chicken) and swines) were sample using sterile swabs moistened with saline solution (0.85 % (w/v)) were inserted into the rectum of swines, and into the cloaca and oral cavity of poultry. Additional samples were collected from the animals' environment, including soil surfaces, walls, door handles, taps, feed containers, drinking fountains, and cages. Fecal samples from both species were also collected. Swabs were transferred to 2 mL microtubes containing peptone water with chloramphenicol (0.5 g/L) and transported to the laboratory for further analysis.

2.2. Isolation and species identification

Upon reception, all samples were homogenized and incubated for two hours at 30 °C, before 0.1 mL of each sample was inoculated onto CHROmagar® *Candida* (BD® difco) plates, which were incubated at 30 °C for seven days. Yeast growth was monitored daily.

Growing yeasts were first examined based on their color and morphology on chromogenic media. Each individual phenotype was the subsequently identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) with the Bruker Biotyper 3.1 library (version 4613; Bruker Daltonik), following the protocol described by Pinto et al. (2022). In summary, approximately 10^6 yeast cells (c.a. 1g) were transferred from the culture plate to a 500 µL tube containing 20 µL of 70 % formic acid (v/v). From each sample, 1 µL of the supernatant was transferred to a paraffin film, mixed with 10 µL of acetonitrile, and 1 µL of this mixture was then spotted onto a stainless MALDI-TOF MS plate (Bruker Daltonics, Germany). A matrix solution (1 µL) containing α-cyano-4-hydroxycinnamic acid (CHCA, Fluka, Buchs, Switzerland) was applied to cover the sample. Each analysis was performed in triplicate.

All identified isolates were cryopreserved at -80°C in YPD (Yeast Peptone Dextrose) liquid medium supplemented with 10 % glycerol.

2.3. Colony PCR and ITS rDNA barcoding

Initially, yeasts grown on SDA plates at 30 °C for 48 h were used for colony PCR, according to Corrêa-Moreira et al. (2024). Briefly, a small portion of an isolated colony was picked using a micropipette tip and added to the PCR tubes as the DNA template. Cells were then heated in a microwave for 90 s and immediately placed on ice to prevent DNA degradation. Amplification was performed in a 50-µl reaction volume, with 10 pmol of universal fungal primers ITS1 (CGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC), according to Lindsley et al. (2001), on a 96-well thermocycler (Applied Biosystems).

PCR products were purified using the QIAquick® PCR Purification Kit (QIAGEN®) according to the manufacturer's protocol, and sequencing was performed at Fundação Oswaldo Cruz - PDTIS/FIOCRUZ, Brazil. Nucleotide sequences were edited using the CodonCode Aligner software and compared by BLAST (Basic Local Alignment Search Tool) with sequences available from NCBI / GenBank. The phylogenetic analysis was inferred using the Maximum Parsimony (MP) method of Eck and Dayhoff (1966), with 1000 replicate bootstraps (Felsenstein, 1985) (Fig. 1). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei and Kumar, 2000) with search level 3 (Felsenstein, 1985; Nei and Kumar, 2000) in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree is drawn to scale, with branch lengths calculated using the average pathway method (Nei and Kumar, 2000) and are in the units of the number of changes over the whole sequence. The codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There was a total of 221 positions in the final dataset, out of which 35 were parsimony informative. Phylogenetic analyses were conducted in MEGA4 (Nei and Kumar, 2000; Tamura et al., 2007).

2.4. Culture on CHROmagar® Candida

After identification by MALDI-TOF, *K. ohmeri* isolates were inoculated onto CHROmagar® *Candida* plates to observe their growth over the time. Plates were incubated at 30°C and photographed at the following time points: 24, 48, 72, and 120 h.

2.5. Antifungal susceptibility testing

The *in vitro* antifungal susceptibility assays were performed using the broth microdilution method, as described by the Clinical and Laboratory Standards Institute. RPMI-1640 medium and an inoculum of 0.5–2.5 ×

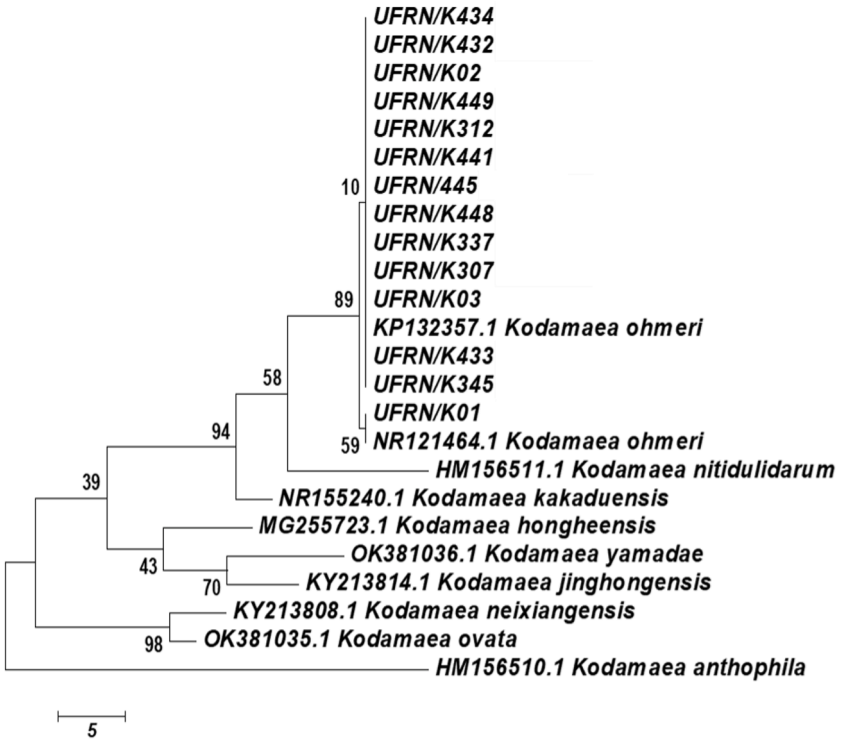

Fig. 1. Evolutionary relationships of 24 taxa. ITS rDNA phylogenetic tree of the 14 *Kodamaea ohmeri* isolates using Maximum Parsimony method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed.

10^3 cells/ml were used in 96-well plates. *In vitro* susceptibility was determined for nine clinical antifungals (fluconazole (FCZ), itraconazole (ITZ), voriconazole (VRC), posaconazole (PSC), isavuconazole (ISV), micafungin (MCF), caspofungin (CSP), amphotericin B (AMB), and 5-flucytosine (5-FC), four environmental fungicides (tebuconazole (TEB), pyraclostrobin (PYR), carbendazim (CBZ), and mancozeb (MZB)), and two hospital disinfectants (benzalkonium chloride (BZK) e didecyldimethylammonium chloride (DDA)).

Except for FCZ, which was diluted in water, all tested antimicrobials were diluted in dimethyl sulfoxide (DMSO). The compounds were tested at the following concentrations: FCZ, 5-FC and TBZ (0.125–64.0 μg/mL); PYR, CBZ, MZB, BZK, and DDA (0.062–32.0 μg/mL); AMB and ITZ (0.031–16.0 μg/mL); VRC, PSC, ISV, MCF, and CSP (0.007–4.0 μg/mL). The minimum inhibitory concentrations (MIC) were defined as the lowest concentration capable of inhibiting 50 % of the fungal growth for FCZ, ITZ, VRC, PSC, ISV, MCF, CSP, 5-FC, and TEB. For AMB, PIR, CBZ, MZB, BZK, and DDA, the MICs were considered as the lowest concentration capable of inhibiting 100 % of fungal growth.

As neither breakpoints nor epidemiological cut-offs have been yet defined by the CLSI (2017) for *K. ohmeri*, MIC values were compared to those obtained for *C. albicans* ATCC 90029, which was used as a control in this study.

3. Results

3.1. Isolation of Kodamaea ohmeri from different sources

During a multicentric surveillance study aimed at isolating, identifying, and assessing the susceptibility profile of yeasts from various sources to multiple drugs, 14 *Kodamaea ohmeri* isolates were recovered. All isolates were identified by MALDI-TOF MS as *K. ohmeri* with scores ≥1.7 (Table 1). The species identification was further confirmed by ITS rDNA barcoding (Table 1, Fig. 1). All sequences have been deposited in GenBank under the following accession numbers: PQ754840 to PQ754853.

Interestingly, although four states participated in this surveillance study, all *K. ohmeri* isolates originated from the same state, Rio Grande do Norte, Brazil (Table 1). *K. ohmeri* was found in production animals, specifically poultry and swine, with isolates recovered from the cloaca of poultry, rectal of swine, and their surrounding environment (Table 1). Furthermore, *K. ohmeri* was identified as an infectious agent in two hospitalized patients, with isolates obtained from their blood samples (Table 1).

Despite some isolates were obtained from the same animal, such as UFRN/K337 and UFRN/K433 (Table 1), both recovered from the cloaca of animal 5, as well as the environmental isolates UFRN/K441 and UFRN/K449, and UFRN/K445 and UFRN/K448 (Table 1), they were

Table 1
Informations about the different isolates of *Kodamaea ohmeri* ($n = 14$).

Isolate	Source	Sample Source	Polyphasic Taxonomy			
			Phenotypic identification	MALDI-TOF MS/Score	ITS sequencing partial	GenBank accession number
UFRN/K01	Patient 1	Blood	Pink colonies at 24 and 48 h of incubation. Blue/green colonies after 72 h.	*Kodamaea ohmeri/1.79*	*Kodamaea ohmeri*	PQ754853
UFRN/K02	Patient 2	Blood	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.75*	*Kodamaea ohmeri*	PQ754846
UFRN/K03	Patient 2	Blood	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.75*	*Kodamaea ohmeri*	PQ754847
UFRN/K307	Environment (Swine)	Nursery 12 - Drinking Fountain of Pen 3	Pink colonies with a blue background appearing within the first 24 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.76*	*Kodamaea ohmeri*	PQ754848
UFRN/K312	Environment (Swine)	Nursery 11 - Drinking Fountain of Pen 1	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.78*	*Kodamaea ohmeri*	PQ754850
UFRN/K337	Poultry	Cloaca (Poultry 5)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.75*	*Kodamaea ohmeri*	PQ754844
UFRN/K345	Swine	Rectum (Swine 18)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.87*	*Kodamaea ohmeri*	PQ754841
UFRN/K432	Environment (Poultry)	Feeder (Henhouse 5)	Pink colonies with a blue background appearing within the first 24 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.74*	*Kodamaea ohmeri*	PQ754851
UFRN/K433	Poultry	Cloaca (Poultry 5)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.74*	*Kodamaea ohmeri*	PQ754840
UFRN/K434	Environment (Poultry)	Feeder (Henhouse 5)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.74*	*Kodamaea ohmeri*	PQ754843
UFRN/K441	Environment (Poultry)	Drinking Fountain (Henhouse 4)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.77*	*Kodamaea ohmeri*	PQ754852
UFRN/K445	Environment (Poultry)	Drinking Fountain (Henhouse 3)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.71*	*Kodamaea ohmeri*	PQ754842
UFRN/K448	Environment (Poultry)	Drinking Fountain (Henhouse 3)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.71*	*Kodamaea ohmeri*	PQ754845
UFRN/K449	Environment (Poultry)	Drinking Fountain (Henhouse 4)	Pink colonies with a blue background appearing after 48 h of incubation. Blue/green colonies after 72 h, with the presence of lilac tones.	*Kodamaea ohmeri/1.84*	*Kodamaea ohmeri*	PQ754849

considered distinct. This distinction was based on the observation that the colonies of these isolates exhibited different morphotypes (Data not showed).

3.2. Color variation of K. ohmeri in CHROmagar® Candida

K. ohmeri was isolated from environmental and animal samples using the spread plate technique on CHROmagar® *Candida* medium. During the initial presumptive identification, the yeasts were screened based on the observed color pattern on this media. Of note, we observed variations in color, including purple, blue, green, pink/lilac, and white across *K. ohmeri* from different isolation sources and expected these variations could be attributed to the prolonged incubation time, exceeding 120 h, which was employed to maximize yeast recovery.

This observation prompted us to cultured again all 14 yeasts on CHROmagar® *Candida* and track any color change of the colony over time. After 24 h of incubation, most isolates exhibited a pink color similar to that of *Candida glabrata (Nakaseomyces glabratus)* and *Candida krusei* (*Pichia kudriavzevii*) (Odds and Bernaerts, 1994) (Fig. 2). In isolates UFRN/K307 and UFRN/K432, a blue background was also observed (Fig. 2).

After 48 h of incubation, a transition from pink to blue was observed, as documented by Zhou et al. (2019) (Fig. 2). On CHROmagar® *Candida,* a blue color is attributed to *C. tropicalis* (Odds and Bernaerts, 1994). Of note, one isolate (UFRN/K01), retained a pink color with purple tones and developed a drier colony texture compared to other isolates, which remained creamy (Fig. 2). The color transition continued after 72 h with multiple color patterns, although green predominated and remained after 120 h of incubation (Fig. 2). Except for UFRN/K01, all isolates maintained a creamy appearance.

3.3. In vitro susceptibility profile of K. ohmeri to clinical antifungals

After identifying *K. ohmeri* as an agent of human infections, alongside its isolation from the environment, we aimed to determined its MIC to the main clinical antifungals available. As *K. ohmeri* does not have defined breakpoints for clinical antifungals, we used the *C. albicans* ATCC 90029 strain, the principal representative of the Ascomycota phylum (to which *K. ohmeri* also belongs), as a comparative control. The MICs obtained for clinical antifungals are shown in Table 2.

The highest MIC observed was for FCZ, the most used azole to threat invasive infection, with MIC ranging from 2.0 to 4.0 μg/mL and MIC_{90} of 2.0 μg/mL. In contrast, VRC was the azole that presented the lowest MIC_{90} (0.03 μg/mL), compared with ITR (0.25 μg/mL), PSC (0.12 μg/mL) and ISV (0.12 μg/mL). Notably, 43.75 % (n = 7) of the isolates showed low MIC for ISV (0.007 μg/mL), including samples from patients, animals, and the animal environment. For echinocandins, MCF and CSP had MIC_{90} values of 0.5 μg/mL and 0.25 μg/mL, respectively. On the other hand, AMB showed no MIC variation, with clinical, animal, and environmental samples showing susceptibility at a concentration of 0.5 μg/mL. Finally, 5-FC MIC_{90} was 0.12 μg/mL. Overall, no difference in azole susceptibility profiles was observed across the different isolation sources.

3.4. In vitro susceptibility of K. ohmeri to agrochemicals

To investigate the susceptibility of *K. ohmeri* in a One Health context, we then assessed the susceptibility of our isolates to four major agricultural fungicides, namely TEB, PYR, CBZ, and MZB.

All *K. ohmeri* isolates had a MIC of 1.0 μg/mL (Table 3), which is lower than the MIC obtained for *C. albicans* ATCC 90029 (4.0 μg/mL).

On the contrary, as for *C. albicans*, PYR and CBZ failed to inhibit fungal growth, as all *K. ohmeri* isolates exhibited MIC values exceeding the highest concentration tested for these agrochemicals (MIC >32 μg/mL) (Table 3).

Eventually, while MCZ demonstrated a MIC of 4 μg/mL for *C. albicans* ATCC 90029, we observed higher MICs for all isolates of *K. ohmeri* (MIC_{90}=16 μg/mL) ranging from 8.0 to 32.0 μg/mL (Table 3). No association between the isolation sources and MIC was noted.

3.5. In vitro susceptibility of K. ohmeri to hospital disinfectants

Considering that *K. ohmeri* is an important emerging human pathogen and has been responsible for outbreaks in ICUs, we evaluated the susceptibility of the isolates to the most commonly used hospital disinfectants in Brazil, namely BZK and DDA. As shown in Table 4, MIC of BZK for *K. ohmeri* despite great variations (range 0.5–4.0 μg/mL, MIC_{90}=4.0 μg/mL) were similar to *C. albicans* ATCC 90029 (2.0 μg/mL).

Again, for DDA, the MIC against *C. albicans* ATCC 90029 was 1.0 μg/mL, whereas for *K. ohmeri*, it ranged from 0.06 to 2.0 μg/mL, with an MIC_{90} of 1.0 μg/mL (Table 4).

4. Discussion

Fungal diseases, though often overlooked, contribute to approximately 3.75 million deaths annually (Denning, 2024). This figure is particularly concerning in light of the growing resistance to available antifungal treatments, a phenomenon that has been widely documented

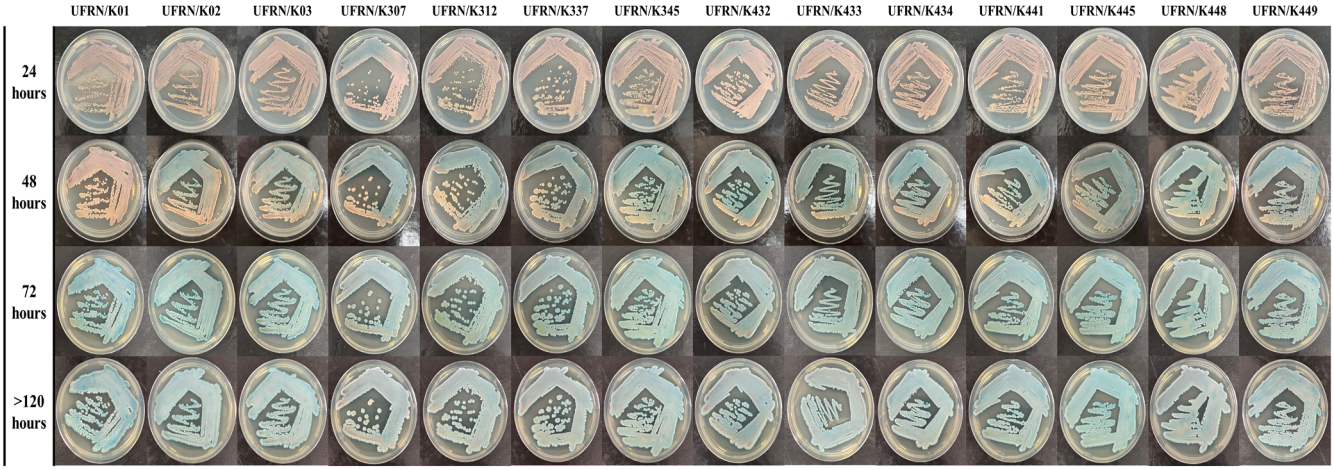

Fig. 2. Variation of color and aspect of 14 *Kodamaea ohmeri* isolates on CHROmagar® *Candida* over time. Overall, after 24 h of incubation, the colonies display a pink or lilac color, with a blue background that may or may not be visible. After 48 h, the color gradually shifts to blue, and after 72 h or more of incubation, the colonies of *K. ohmeri* become green.

Table 2
Minimum inhibitory concentrations (MIC) (μg/mL) of 14 *K. ohmeri* isolates determined by the CLSI for clinical antifungals.

Specie	Isolates	FCZ	ITR	VRC	PSC	ISV	MCF	CSP	AMB	5FC
Candida albicans	ATCC 90029	**2.0**	**0.12**	**0.12**	**0.12**	**0.12**	**0.03**	**0.25**	**0.5**	**0.05**
Kodamaea ohmeri	UFRN/K01	2.0	0.25	0.03	0.12	0.12	0.25	0.25	0.5	0.12
	UFRN/K02	2.0	0.12	0.01	0.03	0.007	0.25	0.25	0.5	0.12
	UFRN/K03	2.0	0.12	0.01	0.03	0.007	0.25	0.25	0.5	0.12
	UFRN/K307	2.0	0.25	0.03	0.12	0.12	0.25	0.25	0.5	0.12
	UFRN/K312	2.0	0.12	0.03	0.12	0.12	0.25	0.25	0.5	0.12
	UFRN/K337	2.0	0.12	0.03	0.12	0.06	0.25	0.25	0.5	0.12
	UFRN/K345	4.0	0.25	0.03	0.12	0.06	0.5	0.03	0.5	0.12
	UFRN/K432	2.0	0.12	0.01	0.03	0.007	0.25	0.25	0.5	0.12
	UFRN/K433	2.0	0.12	0.01	0.03	0.007	0.5	0.03	0.5	0.12
	UFRN/K434	2.0	0.5	0.06	0.25	0.25	0.5	0.03	0.5	0.25
	UFRN/K441	2.0	0.12	0.01	0.03	0.007	0.25	0.03	0.5	0.12
	UFRN/K445	4,0	0.12	0.01	0.03	0.007	0.25	0.03	0.5	0.12
	UFRN/K448	2.0	0.12	0.01	0.12	0.12	0.25	0.03	0.5	0.12
	UFRN/K449	2.0	0.5	0.06	0.25	0.25	0.25	0.03	0.5	0.25
	MIC range	**2.0 - 4.0**	**0.12 - 0.5**	**0.01 - 0.06**	**0.03 - 0.25**	**0.007 - 0.25**	**0.25 - 0.5**	**0.03 - 0.25**	**0.5**	**0.12 - 0.25**
	MIC_{90}	**2.0**	**0.25**	**0.03**	**0.12**	**0.12**	**0.5**	**0.25**	**0.5**	**0.12**

Fluconazole (FCZ), itraconazole (ITZ), voriconazole (VRC), posaconazole (PSC), isavuconazole (ISV), micafungin (MCF), caspofungin (CSP), amphotericin B (AMB),5-flucytosine (5FC).

Table 3
Minimum inhibitory concentrations (MIC) (μg/mL) of 14 *K. ohmeri* isolates for four agrochemicals, determined by the CLSI.

Species	Isolates	TEB	PYR	CBZ	MZB
Candida albicans	ATCC 90029	**4.0**	**>32.0**	**>32.0**	**4.0**
Kodamaea ohmeri	UFRN/K01	1.0	>32.0	>32.0	16.0
	UFRN/K02	1.0	>32.0	>32.0	8.0
	UFRN/K03	1.0	>32.0	>32.0	8.0
	UFRN/K307	1.0	>32.0	>32.0	16.0
	UFRN/K312	1.0	>32.0	>32.0	16.0
	UFRN/K337	1.0	>32.0	>32.0	16.0
	UFRN/K345	1.0	>32.0	>32.0	16.0
	UFRN/K432	1.0	>32.0	>32.0	8.0
	UFRN/K433	1.0	>32.0	>32.0	8.0
	UFRN/K434	1.0	>32.0	>32.0	32.0
	UFRN/K441	1.0	>32.0	>32.0	8.0
	UFRN/K445	1.0	>32.0	>32.0	8.0
	UFRN/K448	1.0	>32.0	>32.0	16.0
	UFRN/K449	1.0	>32.0	>32.0	32.0
	MIC range	**1.0**	**>32.0**	**>32.0**	**8.0 - 32.0**
	MIC_{90}	**1.0**	**-**	**-**	**16.0**

Tebuconazole (TEB), pyraclostrobin (PYR), carbendazim (CBZ), and mancozeb (MZB)

Table 4
Minimum inhibitory concentration (MIC) (μg/mL) of 14 K. ohmeri isolates for two hospital disinfectants, determined by the CLSI.

Species	Isolates	BZK	DDA
Candida albicans	ATCC 90029	**2.0**	**1.0**
Kodamaea ohmeri	UFRN/K01	1.0	0.12
	UFRN/K02	4.0	2.0
	UFRN/K03	4.0	2.0
	UFRN/K307	0.5	0.12
	UFRN/K312	0.5	0.12
	UFRN/K337	0.5	0.12
	UFRN/K345	0.5	0.06
	UFRN/K432	4.0	1.0
	UFRN/K433	4.0	1.0
	UFRN/K434	0.5	0.25
	UFRN/K441	4.0	0.5
	UFRN/K445	4.0	1.0
	UFRN/K448	0.5	0.12
	UFRN/K449	1.0	0.5
	MIC range	**0.5 - 4.0**	**0.06 - 2.0**
	MIC_{90}	**4.0**	**1.0**

Benzalkonium chloride (BZK), didecyldimethylammonium chloride (DDA)

and is linked to the emergence of previously unknown species now responsible for outbreaks and an ever-increasing number of people at risk for IFIs (Vitiello et al., 2023; Fisher et al., 2022).

From a One Health perspective, human practices and environmental factors play a critical role in facilitating the adaptation of microorganisms, enabling the emergence of human pathogens, and promoting the selection of multidrug-resistant strains (McEwen and Collignon, 2018; Williams et al., 2024; Naddeo, 2021).

Kodamaea ohmeri is a yeast belonging to the phylum Ascomycota that has emerged as a human pathogen since its first report in 1994 (Jin and Jin, 1994). It is an opportunistic pathogen, frequently associated with invasive infections in patients hospitalized in ICUs. Furthermore, in most reported cases, infections caused by *K. ohmeri* were associated with patients having underlying conditions such as pneumonia, bacterial sepsis, leukemia, cancer, diabetes, and renal and hepatic insufficiencies. Despite its increasing relevance in human infections, there is limited epidemiological data characterizing this infection. However, it is estimated that its mortality rate, when associated with invasive infections, can reach up to 50 % (Zhou et al., 2021).

In this study, we report the presence of *K. ohmeri* as a human pathogen, isolated from the blood of two patients during hospitalization in Brazil. Simultaneously with its isolation in infections, *K. ohmeri* was isolated in a study investigating the distribution of pathogenic yeasts in the environment and in animals. In Rio Grande do Norte state (Brazil), *K. ohmeri* was first reported in 2006 as the causative agent fungemia and we highlight here two additional cases of this infection in Brazil (De Barros et al., 2009). More recently, it was isolated in the same state from milk in the city of Ceará Mirim (da Silva Campos et al., 2023). Therefore, the presence of *K. ohmeri* in both infections and animal samples suggests that this yeast may possibly be a relevant pathogen for the state of Rio Grande do Norte. Intriguingly, the other three states that participated in our research program did not report any K. *ohmeri* in patients nor animals in the same period of time.

On chromogenic medium, *K. ohmeri* displayed a multiple color pattern (pink, blue, and green) which may explain why *K. ohmeri* can be confused with other yeasts on CHROmagar® *Candida*, and possibly lead to its underdiagnosis in the clinical setting as other yeasts, such as *C. glabrata, C. krusei*, and *C. tropicalis*, shares similar colors (Odds and Bernaerts, 1994), depending on the incubation time. Although the color change of *K. ohmeri* occurs mainly after 48 h, the manufacturer of the CHROmagar® *Candida* medium recommends reading between 24 and 48 h, emphasizing that full color development occurs after 42 h of incubation. It is important to emphasize that in some hospitals in developing countries, the only available means for isolation and presumptive or confirmatory identification of fungi is CHROmagar® *Candida*.

Consistently with our data, a previous study conducted in China evaluated yeasts isolated from hospitals over a seven-year period. A total of 62 *K. ohmeri* strains were isolated using various diagnostic methods, including CHROmagar® *Candida*. However, when these strains were reidentified by 26S rDNA sequencing, only 29 (46.8 %) belong to *K. ohmeri*. The highest rate of misidentification occurred when using CHROmagar® *Candida*, resulting in false positives for *C. albicans, C. glabrata*, and *C. tropicalis* (Zhou et al., 2019).

To minimize the risk of misidentification, methods such as MALDI-TOF MS, combined with molecular and computational approaches, may provide greater accuracy in distinguishing closely related yeast species (Leaw et al., 2006; Zhao et al., 2018; Li et al., 2024; Chew et al., 2022).

In our study, all 14 *K. ohmeri* isolates were identified using MALDI-TOF MS, showing 100 % concordance with ITS rDNA barcoding. This result highlights the capability of this technique to accurately identify fungal species within the *Kodamaea* genus.

There are no defined breakpoints to interpret MIC determined for *K. ohmeri*. Therefore, we used MICs obtained against *C. albicans* ATCC 90029 as a comparator.

The antifungals FCZ (Diallo et al., 2019; Paul and Kannan, 2019; Santino et al., 2013; Sathi et al., 2023; Yang et al., 2009), CSP (Diallo et al., 2019; Janvier et al., 2012), ITZ (Menon et al., 2010) and AMB (Sathi et al., 2023) have shown high MIC values against strains of *K. ohmeri*. Furthermore, a study comparing the MICs of clinical antifungal agents against clinical and reference strains showed that the MIC range for FCZ against clinical strains varied from 4 to 64 μg/mL, while against reference strains it did not exceed 4 μg/mL. For the other azoles, no significant differences in MICs were observed between clinical and reference strains (Desnos-Ollivier et al., 2012).

A comprehensive review by Zhou et al. (2021) compiled epidemiological data on *K. ohmeri* up until March 31, 2021. This review highlighted that *K. ohmeri* strains exhibit varying antifungal susceptibility. The most significant MIC variations were observed for FCZ (MIC up to >128 μg/mL) and CSP (range 0.125–≥ 16 μg/mL).

In our findings, for FCZ, CSP, and 5-FC, the isolates presented MIC_{90} values of 2, 0.25, and 0.12 μg/mL, respectively, using the broth microdilution method. Importantly, no differences were found in the MIC of clinical or environmental samples. Therefore, although these are the drugs with the greatest MIC variation among strains from different locations, our findings are consistent with what has been reported.

In comparison with MIC values reported for other antifungals against *C. albicans* ATCC 90029, we found *K. ohmeri* quite susceptible to clinical antifungals. Some studies (Zhou et al., 2021; Ortiz et al., 2024) have shown low AMB, MCF, ITR, VRC, ISV and PSC MICs against *K. ohmeri*. These observations were consistent with our findings.

Considering the agricultural environment, the use of agrochemicals has increased over the decades. In Brazil, there was a 190 % increase in the use of these substances between 2000 and 2012 (Rigotto et al., 2014). This reality underscores the importance of evaluating the influence these agents may have on the selection of antifungal resistance in the environment. Although agrochemicals play a key role in protecting crops from environmental fungi (Bastos et al., 2021), their excessive use can cause ecological imbalances and may possibly contribute to increased resistance or modify the biology of non-target fungi (Castelo-Branco et al., 2022). As there multiple agrochemicals, each with a distinct mode of action and purposes, we tested the *in vitro* susceptibility of *K. ohmeri* to four main agrochemicals classes known for their antifungal effects (TEB, PYR, CBZ, and MZB), and used in Brazil and worldwide.

Overall, Both PYR and CBZ do not appear to be effective against *K. ohmeri* (nor it was on the *C. albicans* control strain), as it was able to grow at the highest concentration tested (32 μg/mL). This may indicate their ineffectiveness against these yeasts, unlike with *Cryptococcus* spp., which show MIC values below 2 μg/mL for PYR (Bastos et al., 2019).

On the contrary, TEB, which has the same mechanism of action that of clinical azoles, seems effective against *K. omheri* which presented lower MIC than *C. albicans* ATCC 90029.

Bastos et al. (2021) reported that exposure to environmental azoles can select for resistance to clinical azoles in *Aspergillus fumigatus, Cryptococcus* spp., and *Candida* spp., representing the phenomenon known as cross-resistance. However, this was not observed here, as MICs for both clinical azoles and tebuconazole were low, sometimes even lower than those observed against *C. albicans* control strain. Furthermore, although high MICs were observed for MZB, PYR, and CBZ, it did not impact MIC determined for clinical antifungals which remained in the expected ranges.

During the COVID-19 pandemic, there was a significant increase in the use of quaternary ammonium-based disinfectants in households and hospitals, driven by the recommendation of these compounds, which have proven effective in inactivating enveloped viruses, including SARS-CoV-2 (Dewey et al., 2022). Like agrochemicals, quaternary ammonium compounds (QAC) have also been described as having the potential to promote cross-resistance. In *Pseudomonas aeruginosa*, adaptation to different concentrations of benzalkonium chloride has been associated with decreased porin expression and reduced negative membrane charge, mechanisms also observed in antibiotic-resistant strains (Kim et al., 2018; Machado et al., 2013).

In yeasts, Eagan et al. (2022) describe how exposure to QACs can select for species that are less susceptible to disinfection in a hospital setting. Specifically, the study reports that hospital disinfectants based on quaternary ammonium and hydrogen peroxide were able to select clones of *Candida glabrata* with cross-resistance to azoles.

In this study, the MIC_{90} of disinfectants against *K. ohmeri* were 4.0 μg/mL for BZK and 1.0 μg/mL for DDA. Although the BZK MIC was one concentration higher than that of the *C. albicans* control strain, which showed inhibition by BZK at 2.0 μg/mL and DDA at 1.0 μg/mL, both values are still considered low. Therefore, no cross-resistance was evidence in *K. ohmeri*.

Although *K. ohmeri* is found in various environments, the results of this study do not indicate the emergence of antifungal resistance or cross-resistance. This finding may be related to the limited representativity of the isolates analyzed, particularly those of human origin. However, considering the emerging status of this yeast and its widespread environmental prevalence, continuous monitoring of its spread and resistance profile is crucial, especially from a One Health perspective.

5. Conclusion

This multicentric study demonstrated the occurrence of invasive infections caused by *K. ohmeri*, as well as its isolation in production animals, such as swine and poultry. Nevertheless, for the first time, we reported the importance of use of One Health strategies for screening this emergent yeast.

On the CHROmagar® *Candida* medium, *K. ohmeri* showed a variable color pattern over time, starting from pink, transitioning to a dual color (pink and blue), and finally turning green. This pattern may be confused with contamination or with other species of the *Candida* genus, which may potentially lead to underreport this yeast in clinical labs without access to MALDI-TOF mass spectrometry or phenotypic identification tests.

Although the fungus is present in the environment, the study was unable to establish a relationship of cross-resistance to antifungals, which may be due to the limited number of clinical samples compared to environmental ones. Nevertheless, our findings highlight the remarkable adaptability of *K. ohmeri*, which appears capable of evolving in diverse ecological niches, from the environment to humans and animals. Importantly, this highlights the need for vigilant surveillance, not only for multidrug-resistant bacteria in the environment and colonized hosts but also for fungal pathogens. Monitoring shifts in sensitivity patterns over time among emerging microorganisms remains essential to inform

effective control strategies.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT-OpenAI to improve the language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Funding

This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq and Brazilian Ministry of Health (408540/2022-2 and 444501/2023-1), and the National Institute of Science and Technology (INCT) Funvir from Brazil (Grant No. 405934/2022). This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001. D.A.S. (303762/2020-9) is a research fellow of the CNPq.

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Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

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