



First Case of Identification of *Candida kefyr* and *Pichia kluyveri* in Lebanese Water

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Abstract *Candida kefyr* (*Khuyveromyces marxianus*) is a clinically important infectious yeast. It is found in nature, and is known to cause diseases in humans. No previous studies known have shown the detection of *Candida kefyr* in water sources of any country, and no studies have detected it anywhere in Lebanon. This present study is carried out to assess the quality of Lebanese water in terms of yeast pollution. The occurrence of *Candida* spp. (species) was inspected in 84 water samples that tested positive for containing *Candida*. Out of all isolates, 12 isolates were of *C. kefyr* and two were of *P. kluyveri*. The identification of *Candida* sp. was carried out by using HiCrome™ M1297A and M1297AR chromogenic media. Isolates identified were confirmed by MALDI-TOF MS and 26S rRNA sequencing. The isolates varied from sensitive to resistant to common antifungal agents, including Itraconazole, clotrimazole, fluconazole, miconazole, and nystatin, via disk diffusion assay. These results are the first to confirm the presence of *C. kefyr* in

Lebanese water supplies and suggest that water consumed in Lebanon may be a potential transmission route for serious *Candida*-based infections.

Keywords *Candida* · *Khuyveromyces marxianus* · *Pichia kluyveri* · *Candida eremophila* · *Candida Kefyr* · Lebanon · Water · Yeast · HiCrome · MALDI-TOF · 26s rRNA

1 Introduction

The genus *Candida* is made up of a heterogeneous group of fungi, with more than 17 different species known to be the most common pathogenic fungi to humans (Sardi et al. 2013). Its pathogenicity is not only restricted to mucosal or cutaneous infections but may also spread as systemic infections (Turner and Butler 2014). Candidiasis is named after *Candida*; it is an opportunistic fungal disease, which has varied types. It usually affects individuals suffering from immunosuppression, such as those with AIDS (acquired immune deficient syndrome) (Calderone and Clancy 2011).

The most famous and most studied species of *Candida* is *C. albicans*; all other species are enlisted as non-*albicans Candida* (NAC). The most common NAC species are *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*; other less frequently studied NAC species include *C. guilliermondii*, *C. lusitaniae*, *C. kefyr*, *C. famata*, *C. inconspicua*, *C. rugosa*, *C. dubliniensis*, and *C. norvegensis* (Sanguinetti et al. 2015). Certain *Candida* species are considered rare; these include *C. lambica* and *C. eremophila* (Johnson 2009).

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As known, there is no vaccine against *Candida* (Whibley and Gaffen 2015), to fight the multitude of diseases it causes. The diseases caused by *Candida* include Mucosal candidiasis, which comprise oral candidiasis (Patil et al. 2015), candida vulvovaginitis (Kumari et al. 2013), candida balanitis (Nyirjesy and Sobel 2013), esophageal candidiasis (Ally et al. 2001), gastrointestinal candidiasis (Vazquez and Sobel 2002), and respiratory candidiasis (El-Ebiary et al. 1997).

Candida is also known to cause cutaneous candidiasis (Masters and Mandell 2015) which includes candidial folliculitis (Kapdağlı et al. 1997), candidal intertrigo (Kalra et al. 2014), candidal paronychia (Rockwell 2001), perianal candidiasis (Pirone et al. 1992), candidid (James et al. 2006), chronic muco-cutaneous candidiasis (Puel et al. 2011), congenital cutaneous candidiasis (Darmstadt et al. 2000), diaper candidiasis (James et al. 2006), erosio interdigitalis blastomycetica (Schlager et al. 2018), and candidal onychomycosis (Bedaiwy et al. 2017). *Candida* is also known to cause other forms of global and fatal infections when entering the bloodstream; these infections are referred to as systemic candidiasis (Clancy and Nguyen 2013).

Candida kefir, also named as *Kluyveromyces marxianus*, is known as industrial yeast, it is found in milk and kefir, and hence its name, but it is also found in other non-dairy product such as chocolate and tequila (Varela et al. 2018). It has other synonymous subspecies and asexual forms such as *Kluyveromyces fragilis*, *K. bulgaricus*, *C. pseudotropicalis*, and *K. lactis*, which highlights its use as a neutral lactase producer in food industries (Dekker and Daamen 2011). *C. kefir* is acknowledged to play a significant role in well-documented neutropenic patients' infections (Sendid et al. 2006). It has been isolated from the oral cavity of cancer patients, and linked to oral lesions (Aslani et al. 2018). However, the method by which this species adapted to human pathogenicity, and the precise mechanisms of virulence and persistence is to be determined (Sendid et al. 2006).

The increase in *Candida kefir*-based frequencies of infection raises the doubts about the efficacy of antifungal agents as protective or curative agents on the epidemiology of this yeast (Dufresne et al. 2014). Hence, the pursuit of *Candida* spp. in water supplies has been continuous and elaborate. To date, these efforts to enhance the understanding of water pollution by *Candida* never halted, driving several researchers to achieve the point of successfully detecting multiple species of *Candida* viable in various types of water (Ashbolt 2015; Babič et al. 2016; Curutiu et al. 2019; Bakhiet et al. 2016). However, *C. kefir*, has not been isolated or considered a water pollutant to date.

Pichia kluyveri, also known as *Candida eremophila*, is considered a member of a major genus involved in cocoa fermentations. Its use is intended for increasing the quality of fermentation of cocoa. *Pichia* genera, including *P. kluyveri*, have been described as possessing killer toxins. It is able to produce toxins that kill other yeast genera (Batista et al. 2015).

In Lebanon, a recent and sole study realized by our research team (Halawi et al. 2019) has revealed the presence of several *Candida* species in Lebanese water supplies, including pathogenic *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. glabrata* which were resistant to common antifungal agents. The search for other *Candida* species to be isolated from water is a continuous quest that targets the full understanding of the presence, the source, and the impact of these yeasts on the public health. Recently, as more research articles reported the misidentification of certain *Candida* species (Desnos-Ollivier et al. 2008; Castanheira et al. 2013; Kathuria et al. 2015), and as our previous study relied only on species identification based on SDA and HiCrome™ M1297A media observation, which, albeit its usefulness, remain insufficient for definitive *Candida* sp. identification. Thus, we have decided to retest our previously acquired *Candida* sp. isolates (Halawi et al. 2019) and to investigate a possible misidentification using newly developed chromogenic agar (HiCrome™ M1297AR), matrix-assisted laser desorption ionization–time of flight mass spectrometry and 26S rRNA sequencing.

2 Materials and Methods

2.1 Study Area

Lebanon (35°0'N 35°0'E) is a mostly mountainous Mediterranean country of 10,452 km² total area. Lebanon constitutes of seven geographic provinces which are Akkar and North-Lebanon in the northwest, Baalback-El Hermel in the Northeast, Mount Lebanon and Bekaa in the Middle West and East respectively and South Lebanon and Nabatieh in the South. The Lebanese climate is determined by its geography and physiography, which is known to be a typical Mediterranean climate along the coastal and the middle mountain range. One the other hand, it also has a sub-alpine or mountain climate on the highest slopes, covered by snow during most days of the year. Furthermore, the climate changes to arid in some of

the northern plains (Dabaja et al. 2019). The area studied in this paper covers all seven provinces of Lebanon.

2.2 Sample Collections

A total of 84 *Candida* positive water samples, previously and randomly collected across Lebanon, were tested. The 500 ml/site samples included community water, potable water, and spring water, which all are delivered to the final consumer. The samples were collected in sterile glass bottles with sodium thiosulfate (10% w/v) and transported to Beirut Arab University-Debbieh laboratories in ice for culturing within 24 h of collection.

2.3 Culture and Isolation of *Candida* Spp. from Water Samples

Sabouraud Dextrose Agar supplemented with chloramphenicol was used for isolation of yeast colonies; the agar was prepared according to the manufacturer's instructions (Sigma-Aldrich®). The medium was then sterilized by autoclaving at 121 °C at a pressure of 15 pounds for 15 min. Water samples were processed using the membrane filter method. The plates were incubated at 37 °C for 48 h. The discrete colonies that form on the surface of the membrane were then sub-cultured to confirmation media (Pontara et al. 2011). *Candida* isolates were then stocked in culture media with 15% glycerol (v/v) and stored at – 80 °C for further use.

2.4 Primary Identification of *Candida* Spp.

Isolated *Candida* spp. from agar plates were then preliminary identified by their colony appearance, color and growth patterns on chromogenic agar M1297A-HiCrome™ (Himedia, Mumbai, India) according to manufacturer's guidelines. Colony growth and its specific color development were monitored over a 48-h period. This was sufficient to attain a stable chromogenic identification of the different *Candida* sp. (Bandh et al. 2016) In cases of inconclusive results, M1297AR HiCrome™ agar (Himedia, Mumbai, India) was used mainly to discriminate *C. kefyr* isolates, according to manufacturer's guidelines.

2.5 Verification of *Candida* Spp. Using MALDI-TOF Mass Spectrophotometry

Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) Vitek-MS (bioMérieux, Marcy

l'Etoile, France) was used to confirm the results obtained by the chromogenic agar following the manufacturer's standard instructions. Briefly, each *Candida* isolate was selected as a single colony, then suspended in 1 ml of 70% ethanol and homogenized. Samples were subsequently cleared by centrifugation at 15,000×g for 2 min, and the supernatant was removed. The remaining pellet was then dried at room temperature. After drying, 70% formic acid was added to suspend the pellet. An equal amount of pure acetonitrile was later added and left incubated at room temperature for 3 min. Samples were then centrifuged at 15,000×g for 3 min, and 1 µl of the supernatant was spotted onto an analysis plate. The spot was afterwards overlaid with 1 µl of α-cyano-4-hydroxycinnamic acid (CHCA) matrix, dissolved in 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid. The matrix was left to dry at room temperature prior to analysis (Lacroix et al. 2014). Isolates were processed using the Vitek® MS V3.2-clinical use–preparation software and analyzed by Myla software.

2.6 Molecular Identification of *Candida* Spp. Using 26s rRNA Sequencing

Selected *Candida* isolates (all *C. kefyr*, and some other species) were sent to Microgen Inc. (Seoul, South Korea) for DNA extraction and 26s rRNA (D1, D2 regions) sequencing; all DNA targets were sequenced in both directions (Lacroix et al. 2014). Blastn-NCBI program (<http://www.ncbi.nih.gov/blast>) was used to identify the generated sequences.

2.7 Antifungal Disk Diffusion Susceptibility Test

Antifungal susceptibility testing was performed as described by Hazen et al. (2003). Mueller Hinton-methylene Blue agar plates 4.0 mm thick and 90 mm diameter were used. The agar surface was streaked by a sterile swab soaked in a *Candida* spp. suspension adjusted to a 0.5 McFarland standard by spectrophotometry. The plates were incubated at 37 °C for 24 h. Five antifungal discs were used, which included fluconazole 10 µg (item no. SD114), Miconazole 50 µg (item no. SD272), Clotrimazole 10 µg (item no. SD115), Nystatin 100 u (item no. SD025), and Itraconazole 10 µg (item no. SD221), which were all purchased from Himedia, Mumbai, India. MIC interpretive criteria were in accordance with those of Morris et al. 2017.

3 Results

3.1 Chromogenic Identification of *Candida* Species

Samples were plated on HiCrome™ M1297A medium for primary identification. Green isolates were assumed as *C. albicans*, cream to white colonies were assumed as *C. glabrata*, and purple fuzzy colonies were assumed as *C. krusei* according to manufacturer's manual. As the M1297A medium was not able to generate a distinctive color for *C. kefyr* colonies (Data sheet 1/2011), and due to the variation of appearance of colonies on SDA medium, we used the M1297AR chromogenic agar for this aim. *Candida kefyr* isolates appeared as white to cream colonies (Fig. 1), whereas the color of the other *Candida* species appeared as obtained with the M1297A medium.

3.2 Identification of *C. kefyr* Species Using MALDI-TOF MS and 26s rRNA Sequencing

As the manufacturer's guidelines stated, HiCrome™ M1297A cannot identify *C. kefyr*, and as shown in Fig. 1, both chromogenic agar media were not able to discriminate between *C. kefyr* and *C. glabrata* isolates, yielding a similar white to cream color (Table 1). Thus, the test of choice was MALDI-TOF MS for this aim. All isolates were then tested using this technique. MALDI-TOF MS yielded a total of 14 positive *C. kefyr* isolates which were previously identified as *C. krusei* using the HiCrome™ M1297A medium. Considering a number of articles that reported MALDI-TOF MS misidentification of *Candida* species (Kathuria et al. 2015; Haas et al. 2016), and to further confirm our findings, we sequenced the 26s rRNA (region D1, D2) of the 14 *C. kefyr* isolates identified by The MALDI-TOF MS technique (Fig. 2). The identity of each generated sequence was obtained using the NCBI-Blastn program. Out of these 14 isolates, the 26s rRNA sequences revealed 12 *C. kefyr*, and two *Pichia Kluyveri* isolates, which were misidentified by MALDI-TOF MS.

3.3 Antifungal Susceptibility Test

Disc diffusion test was used to measure the amount of resistance for the 14 isolates to 5 common antifungal agents: itraconazole, clotrimazole, fluconazole, miconazole, and nystatin. The area of inhibition caused by each disc was recorded and concluded in Table 2.

Resistant isolates are noted as R, susceptible isolates as S, and susceptible-dose-dependent as SDD.

The isolates of *C. kefyr* and *P. kluyveri* had variable susceptibilities to various antifungal drugs. One isolate of *P. kluyveri* (no. 2) was susceptible to itraconazole, fluconazole, and nystatin. On the other hand, it was susceptible but dose dependent to clotrimazole and resistant to miconazole. The other isolate (no. 4), was susceptible to itraconazole only and resistant to all the remaining antifungal agents tested (Fig. 3). *C. kefyr* isolates were all highly susceptible to at least one antifungal agent, except isolate no. 8, which was only susceptible dose-dependent to itraconazole and nystatin, while being resistant to all the remaining antifungal agents used (Fig. 4).

In total, itraconazole was the most potent antifungal agent against *C. kefyr*, with only 41.6% of isolates being non-susceptible to it, compared to 50% of the isolates unsusceptible to fluconazole and nystatin, 66.6% unsusceptible to miconazole and 75.3% unsusceptible to clotrimazole. Reversely, 58.3% of the 12 isolates were susceptible to itaconazole, 33.3% susceptible to miconazole, 50% susceptible to fluconazole and nystatin, and only 25% susceptible to clotrimazole (Table 3).

4 Discussion

This study presents the first of its kind to successfully isolate *Candida kefyr* and *Pichia Kluyveri* from water sources reaching end users in Lebanon. The isolates' identities were confirmed by using two types of chromogenic agars, MALDI-TOF MS and 26s rRNA sequencing. Most of the isolates showed significant resistance to antifungal drugs tests, which raises concerns about the water quality consumed. It also sets an alarm to warn against a new form of water pollution in Lebanon, pollution by pathogenic yeasts.

Microbiological drinking water safety has been traditionally considered by bacterial parameters, which are indicative of fecal contamination. Meanwhile, the presence of opportunistic fungi in drinking water poses health risks to consumers. Though the presence of fungi in water distribution system and the associated health risks are well documented in the scientific literature, inclusion of fungi in the drinking water regulations is scarce. Most national and international guideline documents (including the World Health Organization) list

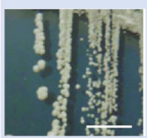
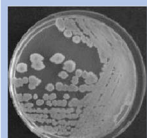
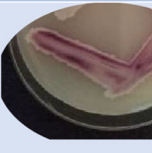
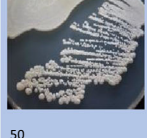
<i>Candida</i> Species	SDA	M1297A	M1297AR	Typical appearance on agar with reference
<i>C. albicans</i>				 44
<i>C. glabrata</i>				 45
<i>C. lambica</i>		 *	 *	 46
<i>C. parapsilosis</i>		N.A.		 47
<i>C. kefyr</i>		 *		 48
<i>C. Krusei</i>				 49
<i>Pichia kluyveri</i>		N.A.	 *	 50

Fig. 1 Different *Candida* spp. colony-morphology on various agar media; N.A.: photograph is unavailable due to the media being inappropriate. *The resulting colors are not to be used for

reference, studies cannot be based upon them and further biochemical testing for confirmation is required

fungi among the “nuisance organisms” causing odor problems and do not deem dedicated monitoring

necessary. The US EPA considered the inclusion of microsporidia in drinking water regulations earlier, but

Table 1 Identification of *Candida* isolates by different techniques

New sample number	HiCrome M1297A	Conclusion	HiCrome M1297AR	Conclusion	MALDI-TOF result	26S rRNA
1	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
2	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>Pichia Kluyveri</i>
3	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
4	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>Pichia Kluyveri</i>
5	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
6	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
7	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
8	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
9	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
10	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
11	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
12	Purple fuzzy	<i>C. krusei</i>	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
13	Pink to violet	Unknown	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>
14	Pink to violet	Unknown	Cream to white	<i>C. kefyr</i>	<i>C. kefyr</i>	<i>C. kefyr</i>

VITEK® MS Review Detail

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Isolate information

Accession ID: DrZaayer114-1

Specimen Type: -

Confidence Level:



Number of identifications: 1

List of identifications

Position	Analysis Date	Organism Name	Pathogenicity	Confidence Value	Confidence Level	Acquisition/Computation message(s)
C2	3/27/19 11:19 AM	<i>Candida kefyr</i>		99.9		

Key: [Validate selection](#) [Add comment](#)

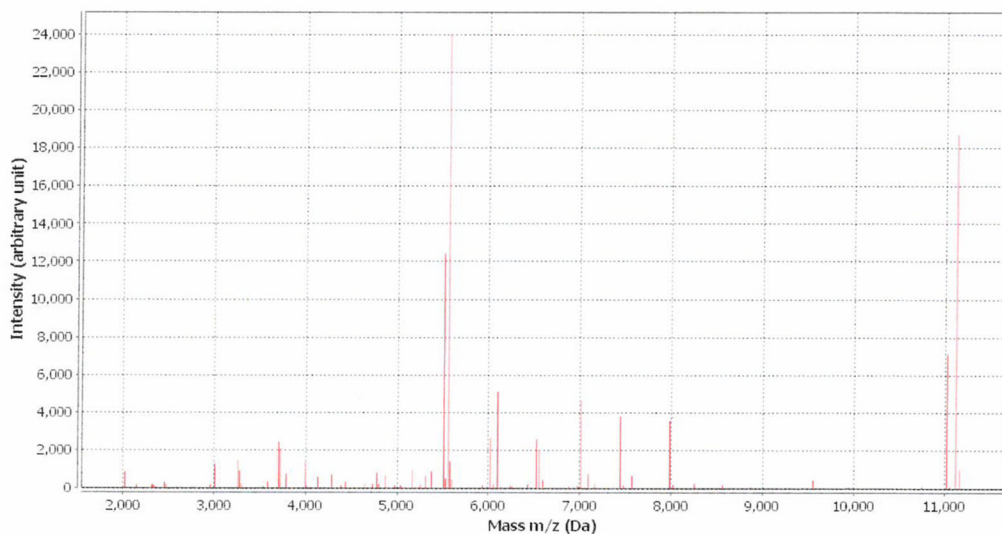


Fig. 2 *C. kefyr* spectrum-MALDI-TOF MS identification

Table 2 Disc diffusion zone of inhibition (mm) to respective isolates

Isolate number	Candida species	IT	CC	FLC	MIC	NS
1	<i>C. kefyr</i>	S	R	S	S	SDD
2	<i>Pichia Kluyveri</i>	S	SDD	S	R	S
3	<i>C. kefyr</i>	R	S	S	SDD	S
4	<i>Pichia Kluyveri</i>	S	R	R	R	R
5	<i>C. kefyr</i>	R	SDD	S	R	S
6	<i>C. kefyr</i>	S	S	SDD	SDD	S
7	<i>C. kefyr</i>	S	SDD	S	R	S
8	<i>C. kefyr</i>	SDD	R	R	R	SDD
9	<i>C. kefyr</i>	S	R	R	R	R
10	<i>C. kefyr</i>	S	R	R	R	R
11	<i>C. kefyr</i>	S	SDD	S	S	S
12	<i>C. kefyr</i>	R	S	S	S	SDD
13	<i>C. kefyr</i>	S	SDD	R	R	SDD
14	<i>C. kefyr</i>	R	SDD	R	S	S

IT itraconazole, CC clotrimazole, FLC fluconazole, MIC miconazole, NS nystatin

it was withdrawn from the list of “Contaminant Candidate List” in a later phase. The European Union drinking water directive does not address fungi explicitly either. However, the directive states that wholesome drinking water should be “free from any micro-organisms and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health.” This definition implies that the presence of pathogenic or allergenic fungi in the drinking water is not acceptable either. The obligatory microbial drinking water parameters (*E. coli*, Enterococci, coliforms or clostridia) have no indicative value of fungal contamination. The indicator parameter heterotrophic plate count (HPC), however, may include fungi as well. HPC is widely used to indicate changes in microbial concentration. Regulatory value is generally not

rendered to HPC. The EU directive does not give a parametric value; compliance is defined as “no abnormal change” (Babič et al. 2017). On the other hand, fungi occur widely in drinking water sources (Pereira et al. 2009), and several studies worldwide have showed the possibility of the occurrence of viable *Candida* cells in water (Hageskal et al. 2009; Siqueira et al. 2011; Yamaguchi et al. 2007). *Candida* species are frequently isolated and has been found in the USA, in almost 50% of samples in France, and in 50% chlorinated water samples in Finland. In Greece, *Candida* species were isolated from hospital water supplies and even from hemodialysis units (Arvanitidou et al. 2000).

Recreational waters may also pose a threat to human health from candidiasis. *Candida* species are frequently isolated from human-impacted surface water and

Fig. 3 Two samples of *Pichia kluyveri* isolates with variable susceptibility to five antifungal discs. **a** Showing sample no. 2. **b** Showing sample no. 4

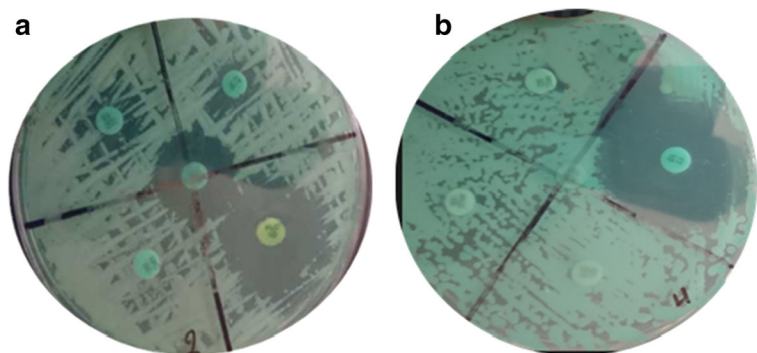
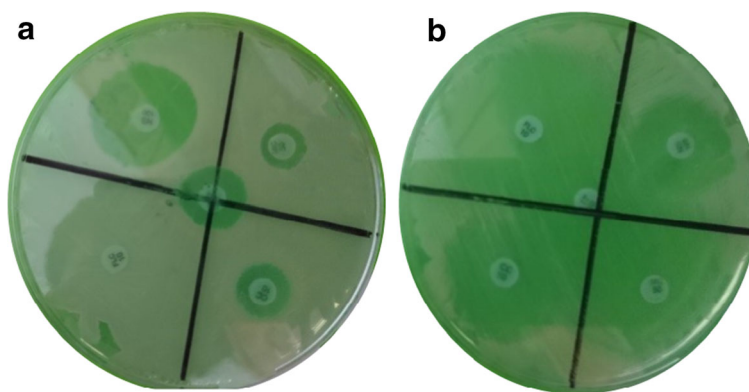


Fig. 4 Two samples of *Candida kefyr* isolates with variable susceptibility to five antifungal discs. **a**, Showing sample no. 8. **b** Showing sample no. 11



beaches. A study of the river water entering Lake Michigan just west of Mount Baldy recreational beach showed that *C. krusei* was the most numerous *Candida* species (Brinkman et al. 2003).

Kluyveromyces marxianus (*C. kefyr*) has been well studied, and its strains have been isolated from a wide array of habitats. It is known to possess a high degree of metabolic diversity and polymorphism (Fonseca et al. 2008). This yeast is known for its industrial and biotechnological capacities and is often used in dairy and ethanol production (Lane and Morrissey 2010). However, *K. marxianus* is also known to be a human pathogen (Papon et al. 2013). It has been implicated in infections affecting neutropenic patients (Sendid et al. 2006; Reuter et al. 2005) and hematological malignancies (Dufresne et al. 2014).

Pichia Kluyveri is a known fermenter yeast; it is usually isolated from fruits. *P. kluyveri* occurs naturally on crops and coexists with *Kluyveromyces marxianus*. It is one of the microbes responsible for food spoilage (Crafack et al. 2013). It is known to produce killer toxins in the presence of other yeasts or even yeast extracts (Middelbeek et al. 1979). It is also known to kill other *Candida* species especially *C. albicans* (Middelbeek

et al. 1980). Consequently, the absence of other *Candida* species in certain water samples holding killer toxins can be questioned.

The hygienic standards of water in Lebanon are low, and it is widely known to be highly polluted with various pollutants, bacteria, yeast, and heavy metals (Halawi et al. 2019). Nevertheless, Lebanon is a dairy-producing counter, with more than 500,000 tons produced annually. The regulations regarding dumping industrial wastes, especially dairy factory production wastes, are hardly met, as cleaner technologies are still considered new concepts in Lebanon and the industry still has not even adopted a pre-treatment system for wastewater resulting from the production processes before dumping (Regional Activity Centre for Cleaner Production (RAC/CP), 2002).

In the absence of any kind of water treatment before dumping wastewater into rivers and sea, devastating amounts of pollutants are continuously found across Lebanese water supplies (Azar and Nasser 2018). There is not a study yet that has isolated *C. kefyr*, from tap or drinking water, but apparently, this yeast is slowly finding its way to end users, and even worse, it is showing resistance to antifungal drugs.

Table 3 Number and percentage of isolates of *C. kefyr* and their susceptibility to various antifungal agents

Species name	Number and percentage	CC	FLC	IT	MIC	NS
<i>Candida kefyr</i> (N = 12)						
Non-susceptible ^a	n	9	6	5	8	6
	%S	75.3	50	41.6	66.6	50
Susceptible	n	3	6	7	4	6
	%S	25	50	58.3	33.3	50

^a Includes susceptible-dose dependent, intermediate and resistant isolates

5 Conclusions

The existence of pathogenic resistant strains of *Candida* poses a gigantic risk to health. From this current study, it was clear that both HiCrome™ media used were not conclusive when it comes to identification of certain *Candida* species, although HiCrome™ M1297AR manual stated it was designed to identify *C. kefyr*, the color produced by the chromogenic agar was misleading. *P. Kluyveri*, a species not known to be pathogenic so far, has been isolated from water sources, which raises questions about water supplies pollution from industrial and agricultural malpractices. It is necessary and highly recommended to any researcher who intends to adopt the chromogenic agar approach for identification, to accompany it by observation of colony morphology on SDA, along with other more accurate tests (i.e., 26S rRNA sequencing) if possible. Our findings suggest the need for broader tests for water quality, not to only include famous *Candida* species (e.g., *C. albicans*) but all known pathogenic *Candida* species such as *C. kefyr*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, and *C. krusei*.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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