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Concerning the presumptive identification of *Candida kefyr* on Uriselect™ 4 agar

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ABSTRACT

Introduction. Non-albicans *Candida* species, such as *Candida kefyr*, are emerging pathogens. Chromogenic media are highly useful for the diagnosis of urinary tract infections (UTIs). The aim was to describe the behavior of this specie on a non-specific chromogenic medium.

Material and methods. A retrospective study of cases of candiduria detected in the Microbiology laboratory of the Virgen de las Nieves Hospital in Granada (Spain) between 2016 and 2021 (N=2,130). Urine samples were quantitatively seeded on non-selective UriSelect™ 4 chromogenic agar.

Results. Between 2016 and 2021, *C. kefyr* was the seventh most frequent *Candida* species responsible for candiduria in our setting (n=15). The macroscopic appearance of *C. kefyr* colonies, punctiform and bluish, allowed the direct identification of these microorganisms.

Conclusions. This study provides the first description of the specific behavior of *C. kefyr* on UriSelect™ 4 agar, which differentiates it from other *Candida* species based on its enzymatic characteristics.

Keywords: *Candida kefyr*, urinary tract infection, chromogenic agar.

Sobre la identificación presuntiva de *Candida kefyr* en agar Uriselect™ 4

RESUMEN

Introducción. Las especies de *Candida* no-albicans, como *Candida kefyr*, son patógenos emergentes. Los medios cromogénicos son muy útiles para el diagnóstico de infec-

ciones del tracto urinario (ITU). El objetivo era describir el comportamiento de esta especie en un medio cromogénico no específico.

Material y métodos. Estudio retrospectivo de casos de candiduria detectados en el laboratorio de Microbiología del Hospital Virgen de las Nieves de Granada (España) entre 2016 y 2021 (N=2.130). Las muestras de orina se sembraron cuantitativamente en agar cromogénico no selectivo Uri Select™ 4.

Resultados. *C. kefyr* fue la séptima especie de *Candida* responsables de la candiduria en nuestro medio (n = 15). El aspecto macroscópico de las colonias de *C. kefyr*, puntiformes y azuladas, permitió su identificación presuntiva directamente.

Conclusiones. Este estudio proporciona la primera descripción del comportamiento específico de *C. kefyr* en agar Uri Select™ 4, que lo diferencia de otras especies de *Candida* en función de sus características enzimáticas.

Palabras clave: *Candida kefyr*, infección urinaria, agar cromogénico.

INTRODUCTION

Chromogenic media have been advantageously employed for years in clinical microbiology laboratories for presumptive identification of the main microorganisms involved in different diseases. Cultures in non-selective media such as blood agar permit the growth of numerous microorganisms, whose initial identification is frequently based on the appearance of colonies. Chromogenic media differentiate among microorganisms according to their enzymatic capacity to metabolize the chromogenic substrates, obtaining colonies with characteristic colors. These media include UriSelect™ 4 agar (Bio-Rad, USA) and CHROMID® CPS® agar (bioMérieux, USA), which are especially useful for the diagnosis of UTIs, permitting the ready differentiation of the main microorganisms involved.

Candida species are yeasts that form part of skin, genito-

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urinary, and gastrointestinal microbiota. *Candida* species, most frequently *Candida albicans*, are responsible for the majority of fungal infections [1,2]. However, non-*albicans* *Candida* species are emerging pathogens that may be related to the increased prevalence of fluconazole-resistant species [3]. Reported multi-resistant species include *Candida krusei* and, less frequently, *Candida glabrata*. More rarely, resistance has also been described in *Candida kefyr* (previously *Candida pseudotropicalis*) [4]. By contrast, the usefulness of fluconazole in the prolonged treatment of urinary infections has been confirmed [5].

The currently recognized teleomorph of *Candida kefyr* is *Kluyveromyces marxianus* [6,7]. *C. kefyr* continues to be a rare cause of disease; however, it has recently been described as an emerging pathogen in patients with hematological neoplasms, among others, and should therefore be considered [8, 9], although its isolation in urine is less common [10]. No published data are available on the behavior of *C. kefyr* on either generic or specific chromogenic media. The aim of this paper was therefore to describe the behavior of this species on a non-specific chromogenic medium.

MATERIAL AND METHODS

We undertook a retrospective study of cases of candiduria detected in the Microbiology laboratory of the Virgen de las Nieves Hospital in Granada (Spain) between 2016 and 2021. Urine samples were quantitatively seeded on non-selective UriSelect™4 (Bio-Rad) chromogenic agar plates and incubated at 35 ± 2 °C for 18–24 h, following a previously described protocol [11]. This study only assessed samples with a significant count, evaluating the growth of *Candida* spp. and the macroscopic appearance of colonies. A definitive diagnosis was

obtained by MALDI-TOF MS (Bruker Biotyper, USA), which frequently required the use of formic acid for the identification.

Ethical statement. The study protocol was carried out in accordance with the Declaration of Helsinki. This was a non-interventional study based solely on routine procedures using biological material only for standard urinary tract infection diagnostics as prescribed by attending physicians. There was no additional sampling or modification of the routine sampling protocol, and data analyses were carried out using an anonymous database. Therefore, ethical approval was considered unnecessary according to national guidelines. The Clinical Management Unit of Infectious Diseases and Clinical Microbiology of the University Hospital Virgen de las Nieves, Spain granted permission to access and use the data.

RESULTS

Table 1 exhibits the significant clinical isolates detected (N=2,130). Between 2016 and 2021, *C. kefyr* was the seventh most frequent *Candida* species responsible for candiduria in our setting. The colonies of all isolated *Candida* species, which represent the most abundant in episodes of candiduria, had a similar morphology to each other; they had a creamy appearance and a white color. However, the colonies of *C. kefyr*, on the other hand, acquired a characteristic blue-violet hue, which allows it to differentiate itself from the most frequent *Candida* species. The macroscopic appearance of *C. kefyr* colonies (Figure 1), punctiform and bluish, allowed the direct identification of these microorganisms, because colonies of all other species were white with variable size and surface creaminess. These differences became evident after 24 h of incubation and were increased but not modified at 48 h.

Table 1	Prevalence of <i>Candida</i> species from 2016 to 2021 isolated from urine.						
	2016 n	2017 n	2018 n	2019 n	2020 n	2021 n	TOTAL
<i>C. albicans</i>	219	274	174	177	185	213	1,242
<i>C. glabrata</i>	69	75	72	74	85	100	475
<i>C. tropicalis</i>	35	60	40	34	33	38	240
<i>C. parapsilosis</i>	7	9	6	17	8	17	64
<i>C. krusei</i>	5	10	10	7	11	10	53
<i>C. lusitaniae</i>	7	5	4	4	6	3	29
<i>C. kefyr</i>	0	1	6	4	1	3	15
<i>C. inconspicua</i>	0	0	1	0	2	0	3
<i>C. famata</i>	2	1	0	0	0	0	3
<i>C. dubliniensis</i>	0	0	1	0	1	0	2
<i>C. guilliermondii</i>	2	0	0	0	0	0	2
<i>C. orthopsilosis</i>	0	0	0	0	1	0	1
<i>C. metapsilosis</i>	0	0	0	1	0	0	1

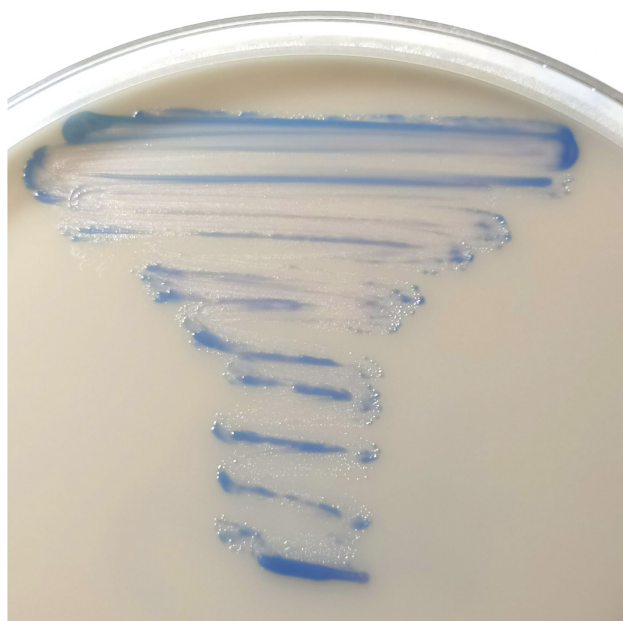


Figure 1 | Appearance of *Candida kefyr* colonies on UriSelect™4 agar (Bio-Rad).

DISCUSSION

In this study, we describe the enzymatic characteristics of *C. kefyr* in a non-selective chromogenic medium, UriSelect™4 agar. Chromogenic media that permit the direct differentiation and identification of microorganisms are highly useful for the diagnosis of UTI, among other diseases. Besides different peptones and tryptophan, UriSelect™4 agar contains a chromogenic mixture that permits the differentiation of microorganisms according to the color resulting from the action of their specific enzymes. This medium allows the action of β -galactosidase, β -glucosidase, and tryptophan deaminase to be evaluated, generating a pink color for colonies with β -galactosidase activity, turquoise blue for those with β -glucosidase action, blue-violet for those with β -galactosidase and β -glucosidase action, and a brownish halo around orange-brown colonies for those with tryptophan deaminase action. The chromogenic medium used in the study is also used to identify bacteria present in the urinary tract. All of them grow differently from *C. kefyr*, based on the size, consistency, and color of the colonies, although these results have not been exposed.

C. kefyr colonies are soft, creamy, smooth, and blue-violet on UriSelect™4 (Figure 1), being differentiated from other *Candida* species on this medium. *C. kefyr* produces β -galactosidase and β -glucosidase [12,13].

The enzymatic activity of yeasts varies depending on the substrate used and the environmental conditions of growth. Fonseca et al. describe that the highest beta-galactosidase activity occurs at 37°C, and that it decreases rapidly at temperatures above 40°C, while in some strains of *C. kefyr*, the

enzyme is very active at 50°C. Likewise, when different substrates for the production of β -galactosidase by *Kluyveromyces marxianus* were studied, lactose maintained the highest enzymatic activities [14]. The beta-galactosidase activity is used in some biochemical galleries for the identification of yeast species (*Candida* and non-*Candida*), such as API *Candida* system (bioMérieux, France), in which, of all the species tested, the only one with beta-galactosidase activity is *C. kefyr*. This enzymatic activity is more studied in species of interest to the food industry. Different biotechnological applications have been investigated with this yeast, but the vast majority of studies published on *C. kefyr* have not aimed to investigate its biochemistry or metabolism, but investigate its application for food or beverage production, without focusing on what really happens at the intracellular level [14]. In the case of *Candida* species of clinical interest, their metabolic activity is barely described.

Finally, Iranian authors describe the beta-glucosidase activity of some *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicales* and *C. kefyr*, among others) in which *C. albicans* has a positive reaction, while the rest negative [15]. This differs from what we and other authors have described, being justified by the existence of different *Candida* strains, isolating in different places, and demonstrating some metabolic diversity and intraspecific polymorphism. So, it seems clear that beta-galactosidase activity is the main differentiating factor of *C. kefyr* compared to other *Candida* species that are usually isolated in clinical samples in our environment.

In conclusion, this study provides the first description of the specific behavior of *C. kefyr* on UriSelect™4 agar, which differentiates it from other *Candida* species based on its enzymatic characteristics. Although not one of the most prevalent *Candida* species, *C. kefyr* is of increasing importance as an emerging pathogen. The creamy blue-violet appearance of the colonies allows its direct identification, facilitating the workflow and reducing costs by avoiding the need for confirmatory tests.

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None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest

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