Speciation of *Candida* isolates obtained from diarrheal stool

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Introduction
Candida is the most common fungi causing diarrhea. As pathogenesis and susceptibility to antifungals might vary in different species of Candida, their speciation is essential.

Objective
The aim of this study was to identify different species of Candida and investigate their antifungal susceptibility pattern.

Materials and methods
Of 2036 diarrheal stool samples, 212 samples were microscopically positive for budding yeast cells. We selected 50 of these samples and cultured on blood agar and MacConkey agar and incubated at 37°C for 18–24 h. Tiny colonies confirmed by means of Gram staining as budding yeast cells were subcultured on Sabouraud's dextrose agar. Speciation of Candida was carried out using the germ tube test and inoculation into chrome agar and corn meal agar. Antifungal susceptibility testing was carried out as per CLSI guidelines using voriconazole, fluconazole, itraconazole, and amphotericin B.

Results
Candida was mostly isolated from children younger than 12 years (50%) and from male patients. A total of 64 isolates were obtained. *Candida krusei* (52%) was the most common isolate, followed by *Candida tropicalis* (16%), *Candida albicans* (0.02%), and *Candida parapsilosis* (0.01%). The isolates were mostly susceptible to voriconazole (97.4%), itraconazole (79.4%), amphotericin B (66.6%), and fluconazole (18%).

Conclusion
Candida diarrhea was mostly seen in individuals younger than 12 years, most commonly caused by *C. krusei*. Resistance to fluconazole was high. A rising resistance to amphotericin B is alarming. Speciation of *Candida* is important to see the difference in antifungal susceptibility in different species.

Keywords:
Candida, *Candida krusei*, diarrhea, speciation

Introduction

Diarrhea is an important health problem and is responsible for a significant degree of morbidity among individuals in developing and developed countries. Fungi that cause diarrhea are species of *Candida*, *Trichosporon*, and *Geotrichum* [1]. *Candida*, a universal genus of yeast existing in the environment, is a part of the normal flora in the gastrointestinal tract and on the skin and mucous membranes [2]. *Candida* spp. are commonly found in the hospital environment, in air and various surfaces. They could also be detected on the hands of healthcare personnel that could lead to nosocomial spread among patients [3]. *Candida albicans* is the most commonly isolated yeast from human stool, and it is identified in 65% of stool samples of healthy adults. However, several studies have reported that it might cause diarrhea [4]. A variety of *Candida* spp. can cause various opportunistic infections and nosocomial diarrhea.

The pathogenesis and susceptibility to antifungal agents might vary in different species of *Candida*, making it essential to carry out speciation of the *Candida* isolate obtained from the stool of diarrheal patients.

Materials and methods

The present study was conducted in the Diarrhea Laboratory of the Department of Microbiology of a tertiary level hospital in North India. Of a total of 2036 diarrheal stool received in our laboratory, 212 samples were positive for budding yeast cells microscopically. A total of 50 of these samples had

abundant budding yeast cells on microscopic examination and were selected for the study. They were cultured on blood agar and MacConkey agar plates and incubated at 37°C for 18–24 h. Tiny white dry colonies of budding yeast cells confirmed by means of gram staining were subcultured on Sabouraud's dextrose agar. Identification of different *Candida* spp. was carried out using the germ tube test, and inoculation into chrome agar (Fig. 1) and corn meal agar (Figs 2 and 3) using standard mycological procedures. The antifungal susceptibility testing was carried out using the disc diffusion test using voriconazole (1 μg), fluconazole (25 μg), itraconazole (10 μg), and amphotericin B (100 U). The results were interpreted according to CLSI criteria M44A. *C. albicans* ATCC 90028 was used as control [5].

Results

Of the 50 samples of diarrheal stool with abundant budding yeast cells on microscopic examination, *Candida* spp. was isolated marginally more from male patients (54%) and less in female patients (46%), with a male-to-female female ratio of 1.17 : 1. Most of the cases with *Candida* diarrhea were children younger than 12 years (50%). followed by those between 12 and 60 years of age (42%). However, in individuals older than 60 years it was less common (Table 1).

From a total of 50 samples, 64 *Candida* of different species were isolated. Of these, 36/64 (56.3%) were obtained as single isolated growth and 28/64 (43.7%) were obtained as mixture. *C. krusei* (52%) was the more commonly isolated one, followed by *Candida tropicalis* (16%), *C. albicans* (0.02%), and *Candida parapsilosis* (0.01%). A mixture of *C. albicans* and *C. tropicalis* (12%) and *C. krusei* and *C. tropicalis* (16%) were also isolated (Fig. 4).

The antifungal susceptibility pattern of the isolates showed that they were mostly susceptible to voriconazole (90.6%), followed by itraconazole (81.2%), amphotericin B (75%), and fluconazole (26.6%). All *C. albicans* were susceptible to voriconazole, whereas fluconazole sensitivity was seen in 57.2% of the isolates. The *C. krusei* were mostly sensitive to voriconazole (94.1%), followed by itraconazole (88.2%) and amphotericin B (76.4%). The *C. tropicalis* were mostly susceptible to voriconazole (81.8%) and amphotericin B (72.7%). A single *C. parapsilosis* was isolated and it was sensitive to all four drugs used (Table 2).

Figure 1
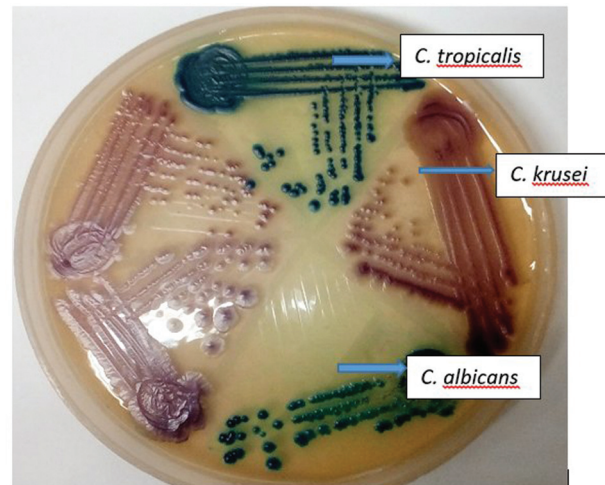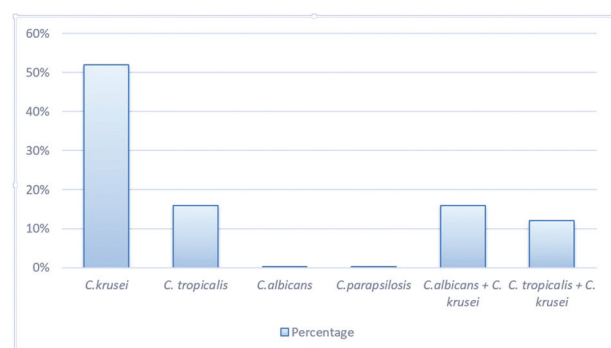

Different *Candida* spp. on Hi-chrome agar.

Figure 2
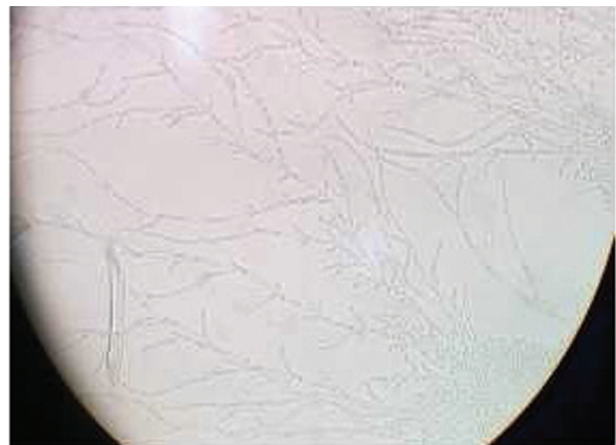
Candida krusei on corn meal agar.

Figure 3

Candida tropicalis on Corn meal agar.

Discussion

The infection of fungal origin is rising significantly. The approaches for the prevention and control of candidiasis

Table 1 Demography of the diarrheal cases (N=50)

Age groups (years)	Male	Female	Total [n (%)]
<12	15	10	25 (50)
12–60	10	11	21 (42)
>60	2	2	4 (8)
Total [n (%)]	27 (54)	23 (46)	50

Table 2 Antifungal susceptibility pattern using the disc diffusion method (N=64)

Candida isolates	N (%)	Sensitive [N (%)]			
		Amphotericin B	Fluconazole	Itraconazole	Voriconazole
Candida albicans	7 (10.9)	5 (71.4)	4 (57.2)	6 (85.7)	7 (100)
Candida krusei	34 (53.1)	26 (76.4)	–	30 (88.2)	32 (94.1)
Candida tropicalis	22 (34.3)	16 (72.7)	3 (13.6)	15 (68.1)	18 (81.8)
Candida parapsilosis	1 (0.01)	1 (100)	1 (100)	1 (100)	1 (100)
Total	64 (100)	48 (75)	8 (26.6)	52 (81.2)	58 (90.6)

Figure 4

Distribution of different Candida spp. isolated (N=64).

depend on whether the fungus is transmitted from exogenous or endogenous source. The gastrointestinal tract is the major endogenous source of Candida, whereas cross-transmission from the environment appears to be the exogenous source [6]. There is a high occurrence of bacterial diarrhea in north India, but the trend of fungal diarrhea, particularly by Candida, is rising and it has motivated us to look for the occurrence of different Candida spp. in both symptomatic and asymptomatic individuals, as most of the time it is ignored as a normal commensal.

In this study, Candida was isolated marginally more from male patients (54%). Most of the cases with Candida diarrhea were children younger than 12 years (50%). The predominance among male patients in our study might be due to the higher incidence of migration of men for work, and hence greater exposure to infection or harboring of the resistant flora. Moreover, there was less awareness among women about seeking medical support, and hence lesser reporting of cases.

All patients in our study were under antibiotic therapy with one or more antibiotics, including cephalosporins, aminoglycosides, and macrolides. However, some were administered vancomycin and imipenem. Payne et al. [7] in their study had shown that normal gut flora can exert 'natural' resistance to C. albicans, but this resistance is lost with antibiotic intake. According to Helstrom and Balish [8], the endogenous intestinal flora is reduced with antibiotic intake, and thus leads to uninhibited multiplication of the Candida spp. The authors suggested that antibiotic intake may also damage the anatomical integrity of the intestinal mucosa and modify the intestinal immune response, making it more vulnerable for infection by Candida spp. [8]. Extremes of age and administration of antibiotics and steroids may act as predisposing factors for Candida diarrhea [9]. This is in accordance with the findings in the present study.

In our study, 36/64 (56.3%) Candida isolates were obtained as single isolates and 28/64 (43.7%) Candida isolates were obtained as mixture. C. krusei (52%) was the more commonly isolated one, followed by C. tropicalis (16%), C. albicans (0.02%), and C. parapsilosis (0.01%). A mixture of C. albicans and C. tropicalis (12%) and C. krusei and C. tropicalis (16%) were also isolated. Enweani et al. [1] in their study conducted in Bangladesh on fungal diarrhea found C. albicans (59.4%) to be the most common species, followed by C. tropicalis (30.9%), Candida pseudotropicalis (5.0%) from diarrheal cases, and Candida glabrata (3.0%) and C. parapsilosis (1.8%)

from nondiarrhea cases. Krause *et al.* [10], in their study conducted in Germany found that, in patients with antibiotic associated diarrhea, *C. albicans* was the most frequent isolate (55%), followed by *C. glabrata* (26%), *C. tropicalis* (5%), *C. krusei* (3%), and other *Candida* spp. (25%) out of 395 patients. Mixed *Candida* cultures were found in 122 (31%). In addition, in a previous study in our hospital in 2003, the most common *Candida* spp. isolated from diarrheal stool was *C. albicans* (40%). Other species isolated were *C. krusei* (20%), *C. tropicalis* (15%), *Candida kefyr* (10%), *Candida guilliermondii* (97.5%), and *C. parapsilosis* (7.5%) [9]. However, in a recent study from the same hospital in 2013 by Banerjee *et al.* [11], the most common *Candida* spp. obtained from chronic diarrhea cases was *C. tropicalis* (43.8%), followed by *C. albicans* (15.6%), *C. krusei* (15.6%), *Candida famata* (6.3%), *C. parapsilosis* (3.1%), *Candida lusitaniae* (3.1%), and *C. guilliermondii* (3.1%). Moreover, in the current study, the most common species of *Candida* isolated was a non-albicans *Candida* (i.e. *C. krusei*). Both the above and the current study from our hospital reflects that there is an increasing occurrence of non-albicans *Candida* isolates causing diarrhea in our hospital.

The antifungal susceptibility pattern of the isolates in this study showed that they were mostly susceptible to voriconazole (90.6%), followed by itraconazole (81.2%) and amphotericin B (75%). However, sensitivity to fluconazole (26.6%) was low. The sensitivity to fluconazole was 57.2% in *C. albicans* and was more than that in the non-albicans species – that is, *C. tropicalis* (13.6%). In a previous study in the same hospital in 2013, the isolates were mostly resistant to fluconazole followed by flucytosine [11]. However, there was no resistance to voriconazole at all in that study. However, in our study, 9.4% of the isolates showed resistance to voriconazole, showing a rising resistance to voriconazole in our hospital. Most of the isolates were sensitive to amphotericin B in our study (75%). In a study by Babin *et al.* [12] in Kerala in 2013 on vulvovaginal candidiasis, the *C. albicans* were resistance to fluconazole in 16.27% followed by itraconazole (13.95%) and voriconazole (9.30%). In the azole groups, the highest resistance was observed against fluconazole (53.84%), followed by itraconazole (32%), voriconazole (21.79%), flucytosine (26.92%), and amphotericin (12.8%) among the non-albicans isolates. The result of this study was similar to ours. However, the resistance to voriconazole was less and that of amphotericin B was high. In our study, 25% of the isolates were resistant to amphotericin B, which is a higher percentage and alarming in the current scenario.

In another study by Roopa and Biradar [13] in Karnataka in 2015, all species of *Candida* isolates (136) were susceptible to amphotericin B and nystatin followed by the next effective drug ketoconazole (84.6%). Fluconazole was least effective (55.8%) of all antifungals. In their study, Patel *et al.* [14] have reported that the sensitivity to azole group was 25.5% among *C. albicans* and 18.7% among *C. tropicalis* to fluconazole, whereas the sensitivity to amphotericin B varied from 75.6 to 100% among all isolated species of *Candida*. Kashid *et al.* [15] in their study found that 30.6% of the isolates were resistance to fluconazole, 28.5% were resistance to clotrimazole, 11.56% were resistance to nystatin, and all isolates (100%) were sensitive to amphotericin B. We have not looked for the sensitivity profile of echinocandins even though it is available for long time, because it is not routinely used in our hospital and the sensitivity of amphotericin B is fairly high and it is the recommended drug for resistant isolates in our hospital.

There is also a variability in the occurrence of different *Candida* spp. of different individuals from different geographical area, which might be due to age factor, immunity, endocrinopathies, use of antimicrobials and steroids, dietary factors, disruption of the mucosal integrity, duration of stay in the hospital, etc. [16]. This is true for our study as there were different *Candida* spp. isolated from the diarrheal stool in our patients who were on different antibiotic therapy.

Conclusion

Candida in diarrheal stool was mostly seen in individuals younger than 12 years, *C. krusei* being the most common isolate, showing a rising trend of infection with non-albicans *Candida*. Resistance to fluconazole was high. A rising resistance to amphotericin B was seen alarming. Speciation of *Candida* is essential as the different species vary in their susceptibility to different antifungals. Moreover, the identification of a particular species is important in epidemiological point of view to trace the point source.

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