RESEARCH ARTICLE

Internal transcribed spacer for identification of yeast species isolated from cancer patients at the Isotope and Radiation Center, Khartoum, Sudan: A cross-sectional, case-control study [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: Cancer patients have a high risk of fungal infections, especially by *Candida species*. Non-*C. albicans* *Candida* infections and less common yeast infections have been increasing in recent years. Identification by conventional methods can be difficult and sometimes inconclusive. This study aimed to detect the prevalence of oral yeast species isolated from cancer patients, from oral swab, sputum and urine, using Internal Transcribed Spacer (ITS) sequence analysis, since little is known about this problem in Sudan.

Methods: The study involved 333 cancer patients (168 patients under treatment [study group] and 165 patients before treatment [control group]). Oral swabs were collected from all patients. Urine or sputum specimens were collected from patients under treatment showing clinical features of UTI or lower respiratory tract infection, respectively. ITS1 and ITS2 region of isolated yeast were amplified by PCR and sequenced. The obtained sequences were compared to reference sequence available in the GenBank database using BLAST.

Results: Culture results showed oral yeast species were isolated from 69/168 (41.1%) and 74/165 (44.8%) of patients among study and control groups, respectively (P value > 0.05). 2/9 (22.2%) patients were urine growth positive and 8/14 (57.1%) patients were sputum culture positive. Sequence analysis showed, *C. albicans* was the most

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prevalent organism (93; 52.5%) followed by *C. tropicalis* (29; 16.4%), and *C. glabrata* (24; 13.6%). Non-*C. albicans* *Candida* and uncommon rare yeast were found to be associated with oral infections and colonization among the study and control groups, whereas *C. albicans* was the most common species (66.7%) associated with oral candidiasis among the treated patients.

Conclusion: Cancer patients were highly colonized with different oral yeast species, which indicates that ITS sequence analysis is an accurate method for identification. This will aid effective management to prevent dissemination of disease especially among those who are under chemo and/or radiotherapy treatment.

Keywords

Yeast species, ITS sequencing, Cancer patients.

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Introduction

As a result of the immunocompromised state and the effect of chemotherapy, cancer patients are more susceptible to fungal infections, especially by *Candida* species (Al-Dwairi *et al.*, 2014). *Candida* species are commensal in human bodies, and become opportunistic pathogens in immunodeficient patients. Sometimes they can cause systemic infections and colonize different organs due to their dissemination from the mucosal infected regions (Miceli *et al.*, 2011). Pfaller *et al.* (2006) and Wilson *et al.*, (2002) reported that the systemic infections have considerable morbidity among those with severely paralyzed immune system.

The most common fungal infection is oropharyngeal candidiasis, which is more prevalent among cancer patients (Akapan & Morgan, 2002). Recently infections with non-*Candida albicans* and rare common yeast genera, such as *Pichia*, *Rhodotorula*, and *Saccharomyces*, have been implicated; however, *C. albicans* remains the most prevalent species (Han *et al.*, 2004 and Walsh *et al.*, 2004).

Yeast identification is of great importance for targeting proper treatments (Ramani *et al.*, 1998), since different species yield different antifungal response (Pfaller *et al.*, 2003). Conventional methods for yeast identification may be difficult and inconclusive (Reiss *et al.*, 1998) especially for less common yeast. Thus more rapid and accurate molecular methods have been developed, among which ITS sequence analysis is found to be more accurate for species delineation (Chen *et al.*, 2001 and Iwen *et al.*, 2002).

The ITS region is located between the highly conserved genes coding for 18S and 28S rRNA. The ITS region includes two non-coding regions ITS1 and ITS2, which are separated by the highly conserved 5.8S rRNA gene (White *et al.*, 1990). The more genetic variability of ITS1 and ITS2 regions enables better identification of closely related species other than the adjacent rRNA gene (Ciardo *et al.*, 2006).

This study aimed to detect the prevalence of oral yeast species isolated from cancer patients by oral swab, in addition to other specimens (sputum and urine), using ITS sequence analysis, since little is known about this problem in Sudan.

Methods

Study design and participants

This was a cross-sectional, case-control study conducted in a period between April 2013 and December 2017.

The study involved 333 cancer patients referred to Isotope and Radiation Center, Khartoum, who were seeking anticancer treatment and during a routine check-up were enrolled in this study. The participants were classified into 168 patients under chemo and/or radiotherapy treatment (study group) and 165 cancer patients prior to starting anticancer treatment (control group). Study participants included 185 females and 148 males (mean age, 48 years old).

Written informed consent and structural questionnaire (see [Supplementary File 1](#)), including demographical data, site of cancer and cancer treatments, was obtained from each patient. The participants were ensured of anonymity and that only group findings will be reported.

To reduce any possible bias matching criteria was done, which included age, sex and type of cancer. *Inclusion criteria*: Any patient diagnosed with any type of cancer during or before starting cancer treatment, having age equal or above 18 year old, attending Isotope and Radiation Center, Khartoum was included in this study. *Exclusion criteria*: Patients on antifungal therapy for past two weeks were excluded from the study.

Sample collection

Oral swabs were collected from each patient (333 patients) to detect the prevalence of yeast infections and colonization among the treated and non-treated patients. Urine (n=9) and sputum (n=14) samples were collected from patients under treatment (study group), who exhibited the clinical features of urinary tract infection (UTI) and/or lower respiratory tract infection (LRT), respectively. These samples were taken in order to detect whether there is any dissemination from a patient's own oral yeast due to action of cancer treatments.

All specimens (n=356) were cultured without delay in Sabouraud's dextrose agar plates (SDA) to which chloramphenicol (0.05g/l) was added. Then the plates were incubated at 37°C for 24–48 hours. Phenotypic identification was made using Gram's stain and germ tube test. Purified colonies were preserved on glycerol stock solution for molecular identification (Sherman *et al.*, 1986).

DNA extraction

The isolated strains were subbed from the stock solution on Sabouraud's dextrose agar medium and DNA extraction was performed from colonies that had been incubated for 48 hrs using Guanidine Chloride method as described by Gassoum *et al.* (2014). Three to five colonies were washed with 5 ml phosphate buffer saline (PBS) (Sigma Aldrich) for three times. 2 ml white cell lysis buffer and 20 µl of proteinase K (10 mg/ml; iNtRON Inc, Korea) were added, vortexed and incubated at 37°C for overnight. Then 1 ml from Guanidine chloride (7M; iNtRON Inc, Korea) and 350 µl of ammonium acetate (7M; Loba Chemie, India) were added. The tubes were vortexed and incubated at 65°C in an oven for 2 hours. Then the supernatant was mixed with 2ml pre chilled chloroform (sd Fine-Chem limited, India) at 6000 RPM for 20 minutes and this was transferred into a new Falcon tube and completed to 10 ml volume with pre chilled absolute ethanol (Carlo Erba, France) and incubated overnight at -20°C for completion of DNA precipitation.

After incubation the tubes were centrifuged at 6000 RPM for 20 minutes, then the ethanol was poured off and the same step was repeated with 70% ethanol. After that the tubes were left to air dry. Finally DNA was suspended in 80 µl TE buffer (iNtRON Inc, Korea) and incubated at 4 °C until used. Nanodrop ND 1000

Spectrophotometer (NanoDrop Technologies, Inc.) was used to measure quality and quantity of DNA.

PCR amplification procedure

The universal fungal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Macrogen Inc. Korea) were used to amplify the entire ITS rDNA region (Zimbeck *et al.*, 2010). PCR mixture contained 5 µl pre mix (iNtRON Inc., Korea), 22 µl deionized sterile water, 1 µl from each forward and reverse primer, and 1 µl of genomic DNA, which served as the DNA template in a final volume of 25 µl.

PCR cycling conditions were as follows: an initial denaturation step of 5 min at 95°C followed by 35 cycles of 45 s at 94°C, 45 s at 55°C, and 45 s at 72°C, with a final extension of 5 min at 72°C. The reactions were carried out in an ESCO thermocycler (AERIS-BG096, China).

The PCR products were analyzed on 2% agarose gels (iNtRON Inc., Korea) stained with ethidium bromide (10 ng/100 ml; Fisher Scientific, USA) and visualized under a UV transilluminator apparatus (Saratoga, CA.95070, USA Gel Documentation System) and Biodoct BDA system (Biometra, Germany).

DNA sequencing

PCR products of 90 isolates from the cases and 87 isolates from the control group were purified and commercially sequenced using forward primer ITS1 and backward primer ITS4 by Macrogen Company (Seoul, Korea).

The sequences obtained in this study were identified by searching databases using BLAST sequence analysis tool (<http://www.ncbi.nlm.nih.gov/BLAST/>). The sequences were compared using nucleotide-nucleotide BLAST (blastn) with default setting except that sequences were not filtered for low complexity. Species were identified based on the highest similarity score (100%) with reference database sequence.

Statistical analysis

Data was analyzed using SPSS 21. Frequencies and percent were obtained for frequency tables, Chi-squared test was used for goodness of fit. The relationships between variables tested were obtained using cross tables and Chi-squared (Fisher exact) test for independence. P-value ≤0.05 was considered as significant.

Ethical statement

Scientific approval was obtained from Faculty and Department of Medical Microbiology Management, Al-Neelain University (No AU/FMLS/7/1). Ethical clearance was obtained from the Directorate of Research Department, Ministry of Health, Khartoum (dated on 21-04-2013).

Results

The collected oral swabs (333 specimens), from 168 cancer patients with chemo and/or radiotherapy treatment (study group)

and from 165 cancer patients (control group) without treatment were examined by cultural technique. Oral *Candida* species were isolated from 69/168 (41.1%) and 74/165 (44.8%) of patients among study and control groups, respectively.

Of the 168 study group, 23 patients with clinical symptoms of UTI and/or LRT infection had urine (n=9) and sputum (n=14) samples collected for detection of *Candida* species. The results showed that 2 out of 9 patients and 8 out of 14 patients were culture positive for *Candida* spp. (Table 1).

Among the culture positive patients (n=69 study group; n= 74 control group), the prevalence of oral candidiasis were 33.3% and 10.8% ,while the prevalence of oral colonization were 66.7% and 89.2% among the study and control patients, respectively (Table 2).

Among the 69 positive cases in the study group, 13 patients exhibited mixed growth of oral yeast, while among the control group 25 patients exhibited mixed growth out of 74. As a result, the numbers of total isolates collected from different specimens from the study group were 92 and 99 isolates from the control group.

PCR products of ITS polymerized region revealed different band size for different species. *C. albicans* exhibited 500 bp (Figure 1). In contrast, non-*C. albicans Candida* and less common yeast represented different band sizes ranging from 400–750 bp (Figure 2).

Table 1. Culture results of urine and sputum specimens collected from the study group.

Growth	Specimens		
	Sputum N (%)	Urine N (%)	Total N (%)
Positive	8 (57.1)	2 (22.2)	10 (43.5)
Negative	6 (42.9)	7 (77.8)	13 (56.5)
Total	14 (100)	9 (100)	23 (100)

Table 2. Prevalence of oral candidiasis and colonization among the study and control groups.

Patients	Symptoms		Total N (%)	P- value
	Yes (infection) N (%)	No (colonization) N (%)		
Study	23 (33.3)	46 (66.7)	69 (100)	< 0.05
Control	8 (10.8)	66 (89.2)	74 (100)	< 0.05

Figure 1. Agarose gel showing the results obtained for conventional PCR of isolated yeast genomic DNA. From left to right lanes: L, 100bp DNA ladder, L1 negative control, L2,4,5,6 *C. albicans* (500bp).

Figure 2. Agarose gel showing the results obtained for conventional PCR of isolated yeast genomic DNA. From right to left lanes: L, 100bp DNA ladder, L1,2,3,6 *C. glabrata* (750bp), L9,11 *C. glabrata* (600bp), L4,8 *P. kudriavzevii* (450bp), L10,12 *P. kudriavzevii* (400bp), L5,7 *C. tropicalis* (450bp), L13 *L. fermentati* (500bp).

DNA sequencing was performed for isolated yeast species (only 90 isolates from 67 out of 69 positive patients in study group and 87 isolates from 66 out of 74 positive patients in the control group). The result of the BLAST sequence analysis for species identification is shown in [Table 3](#).

The distribution of oral mixed species among the 67 study and 66 control groups is shown in [Table 4](#).

[Table 4](#) shows the distribution of oral mixed species among the (67) study and (66) control groups. It was found that although some patients represented different colonial morphology of mixed oral isolated yeast, they exhibited the same species on sequence identification.

The identified species were classified according to presence or absence of symptoms of oral candidiasis among the

Table 3. Species distribution among the study and control groups.

Species	Study N (%)	Control N (%)	Total N (%)
<i>Clavispora lusitaniae</i>	0 (0.00)	4 (4.60)	4 (2.30)
<i>C. albicans</i>	59 (65.6)	34 (39.1)	93 (52.50)
<i>C. glabrata</i>	7 (7.80)	17 (19.50)	24 (13.60)
<i>C. tropicalis</i>	13 (14.40)	16 (18.40)	29 (16.40)
<i>Pichia.sporocuriosa</i>	1(1.1)	0 (0.00)	1(0.60)
<i>Saccharomyces cervisiae</i>	0 (0.00)	2 (2.30)	2 (1.10)
<i>Magnusiomyces capitatus</i>	2 (2.20)	2 (2.30)	4 (2.30)
<i>Pichia kudriavzevii</i>	7 (7.80)	11 (12.60)	18 (10.20)
<i>C. doblensis</i>	0 (0.00)	1 (1.10)	1 (0.60)
<i>Lachancea fermentati</i>	1 (1.10)	0 (0.00)	1 (0.60)
Total	90 (100)	87 (100)	177 (100)

Table 4. Frequency of mixed oral yeast species among Sudanese cancer patients.

Species	Study N (%)	Control N (%)	Total N (%)
<i>Clavispora lusitaniae</i>	0 (0.0)	4 (6.1)	4 (3.0)
<i>C. albicans</i>	42 (62.7)	23 (34.8)	65(48.9)
<i>C. glabrata</i>	3 (4.5)	6 (9.1)	9(6.8)
<i>C. tropicalis</i>	3 (4.5)	4 (6.1)	7 (5.3)
<i>Saccharo cervisiae</i>	0 (0.0)	2 (3.0)	2 (1.5)
<i>Magnusiomyces capitatus</i>	2 (3.0)	2 (3.0)	4 (3.0)
<i>Pichia kudriavzevii</i>	3 (4.5)	4(6.1)	7 (5.3)
<i>C.dobliensis</i>	0 (0.0)	1 (1.5)	1 (0.8)
<i>Lachane fermentati</i>	1 (1.5)	0 (0.0)	1 (0.8)
<i>C. tropicalis+C. glabrata</i>	3 (4.5)	3 (4.5)	6 (4.5)
<i>C. albicans+C. albicans</i>	4 (6.0)	2 (3.0)	6 (4.5)
<i>C. tropicalis+C. tropicalis</i>	3 (4.5)	2 (3.0)	5 (3.8)
<i>Pichi kudriavzevii+Pichi kudriavzevii</i>	1 (1.5)	0 (0.0)	1 (0.8)
<i>C. glabrata+C. glabrata</i>	0 (0.0)	4 (6.1)	4 (3.0)
<i>C. albicans+C. tropicalis</i>	0 (0.0)	2 (3.0)	2 (1.5)
<i>Pichi kudriavzevii+C. tropicalis</i>	0 (0.0)	2 (3.0)	2 (1.5)
<i>C.albicans+pichia sporocuriosa</i>	1(1.5%)	0(0.0%)	1(0.8%)
<i>C.albicans+Pichi. kudriavzevii</i>	1(1.5%)	5(7.6%)	6(4.5%)
Total	67 (100)	66 (100)	133 (100)

study and control group to detect their association with oral infection and colonization as in Figure 3. This showed that *C. albicans* was the most common organism associated with oral infection (14 out of 21) and colonization (28 out of 46) among the study group. In the control group it had the same percentage (2/8; 25%) as *C. tropicalis* and mixed *C. glabrata* among oral symptomatic patients, whereas it was found to be the most common cause of colonization (21/58; 36.2%).

Identification of sputum isolates showed that *C. albicans* was isolated from both oral and sputum specimens from four patients, while three patients exhibited *C. albicans* from sputum specimens only (oral swabs were negative), and *C. tropicalis* was isolated from sputum specimen only from one patient. Regarding urine specimens, *C. albicans* was isolated from both oral and urine samples from one patient in contrast to mixed infections (*Pichia kudriavzevii* and *Pichia kudriavzevii*) that were isolated from oral swabs. *C. glabrata* was also isolated from the urine of another patient.

Dataset 1. Demographical data of cancer patients

<http://dx.doi.org/10.5256/f1000research.14019.d199685>

Sheet 1: For case group (168 cancer patients under treatments); Sheet 2: For control group (165 cancer patients); Sheet 3: Pictures demonstrated the colonial morphology for different isolated yeast species.

Discussion

In the present study it was found that oral *Candida* isolates were obtained from 69 (41.1%) patients who were under treatment with chemo and/or radiotherapy treatments and from 74 (44.8%) patients without cancer treatment. Xu *et al.* (2013) found that oral infection was prevalent in 46% (391/850) of all cancer patients, while another study reported the incidence of oral candidiasis ranging from 7 to 52% in cancer patients on chemotherapy and/or radiotherapy (Lone *et al.*, 2014).

The present study revealed that 8 out of 14 patients were positive for sputum culture among the study group (Table 1). Mohammed *et al.* (2016) and Ungureanu *et al.* (2016) reported slightly lower percentages of 30.50% and 33.75%, respectively, in comparison to this study (57.15%). This may be due to differences in study population, since cytotoxic and immuno-suppressive therapies promote dissemination of *Candida* spp. through induction of cytopenias and/or immune cell dysfunction (Blot *et al.*, 2008; Cruciani & Serpelloni, 2008). Also this was expressed in the isolation of 22.2% of *Candida* species from urine culture of the treated patients among this study, which is relatively similar to Nigar *et al.* (2016), who found out of 64 culture positive clinical specimens *Candida* species were identified from 18.75% urine specimens.

Among the study and control groups, the present study found that oral colonization was significantly ($p < 0.05$) more common than oral *Candida* infection (66.7% vs 33.3% and 89.2% vs 10.8%, in both groups, respectively). Similar results have

Figure 3. Frequency of yeast species associated with oral *Candida* infection (symptoms) and colonization (no symptoms) among the (A) study group and (B) control group.

been reported by Lone *et al.* (2014), who found the total colonization to be prevalent in 50% and oral candidiasis in 30% of all cancer patients. In contrast, oral *Candida* infection was more common ($p < 0.05$) among the study group compared with the control group (33.3% vs 10.8%). A systematic review carried out by Lalla *et al.* (2010) reported that for all cancer treatments, the weighed prevalence of clinical oral fungal infection was found to be 7.5% pretreatment and 39.1% during treatment. This may be due to chemotherapeutic agents and therapeutic radiation that disrupts the mucosal barrier of the mouth, leading to severe oral mucositis, gingivitis, and oral candidiasis. Blasting results of ITS sequence analysis in the present study revealed *C. albicans* was the most prevalent organism in the two groups (65.6% and 39.1%, study and control groups, respectively). These results are in agreement with previous reports (Ismet *et al.*, 2016 and Aldossary *et al.*, 2016) that studied the prevalence of oral *Candida* spp and demonstrated that *C. albicans* was the most prevalent organism.

The majority of oral infections are due to *C. albicans* but non-*albicans* strains, such as *C. glabrata* and *C. tropicalis*, have increasingly been implicated in causing disease (Bagg *et al.*, 2003). Similar findings were observed in this study *C. tropicalis* was found to be the second most common isolate among the study group (14.4%) followed equally (7.8%) by *C. glabrata* and *P. kudriavzevii*, whereas among the control group *C. glabrata* was the second most common isolate (19.5%) followed by *C. tropicalis* (18.4%).

It was found that *P. kudriavzevii* (anamorph of *Candida krusei*) represented 12.6% among the control group, and *P. sporocuriosa* was isolated once from the study group only. In addition, *C. lusitanae* (teleomorph of *Candida lusitanae*) represented 4.6%, and *C. dubliniensis* represented 1.1% among the control group only (Table 3). These findings are in agreement with Pfaller *et al.* (2010), who performed a recent ten-year analysis of the worldwide distribution of non-*albicans* *Candida* species, and indicated that *C. glabrata* remains the most common non-*albicans* species and that *C. parapsilosis*, *C. tropicalis*, and *C. krusei* are also frequently isolated.

Less common yeast species were also detected in small numbers. *L. fermentati* appeared only once and *M. capitatus* twice among the study group, while among the control group the later along with *S. cerevisiae* were detected among two patients (2.2%). These findings were in agreement with a study done by Han *et al.*, 2004 and Walsh *et al.*, 2004, who reported that, recently, infections caused by less common yeast species such as *Pichia*, *Rhodotorula*, *Trichosporon*, and *Saccharomyces* spp. and other rarely encountered species have been reported.

Table 4 shows the presence of identified mixed oral yeast species among the study and control groups. Similarly de Sousa *et al.* (2016) found the presence of more than one yeast among orogastric cancer patients.

Regarding distribution of species according to presence or absence of symptoms of oral candidiasis, it was found that *C. albicans* was the most common isolate associated with infection 14/21 (66.7%) and colonization 28/46 (60.9%) among the study group. While among non-*albicans* *Candida*, the prevalence of *C. tropicalis* was 9.5% vs. 2.2% and *C. glabrata* was 4.8% vs. 4.3% among the oral symptomatic and non-symptomatic study patients, respectively (Figure 3A). Similarly Lone *et al.*, (2014) observed *C. albicans* to be the most common species (74.39% vs. 65.4%) causing colonization and candidiasis in cancer patients, respectively. Whereas they found *C. glabrata* was the second most common species followed by *C. tropicalis* and *C. parapsilosis* to cause colonization as well as candidiasis in cancer patients. This may be due to geographical region variation.

In contrast, among the control group *C. albicans* was detected as the same percentage (2/8; 25%) as *C. tropicalis* and the mixed *C. glabrata* among oral symptomatic patients, whereas it represents the most common cause of colonization (21/58; 36.2%) followed by *C. glabrata* (Figure 3B). This may be due to small number of symptomatic control patients due to the absence of any cancer treatment since it is a predisposing factor for initiation of oral infection by the colonized organisms, mainly by *C. albicans*, which is the most virulent organism.

Among the isolated *Candida* species from sputum and urine specimens, *C. albicans* was isolated from both oral and sputum specimens from four patients and from both oral and urine from one patient. These patients may have gained the infection or colonization in these organs from their own oral colonized *Candida* species. Other studies reported that colonized *Candida* can invade the underlying mucosa and enter the blood stream leading onto disseminated disease with considerable morbidity and mortality if not treated promptly (Lalla *et al.*, 2010 and Shokohi *et al.*, 2011).

Conclusion

The present study demonstrates that cancer patients were highly colonized with oral yeast species. *C. albicans* was the most common isolate associated with oral infection and colonization among the treated cancer patients. In contrast with control group it occupied a higher percent among the colonized species only. As the control group were not under cancer treatment, this lead to oral infection and disseminations to other organs. So early detection and identification of colo-

nized yeast is of great value especially among patients undergoing cancer treatments.

Although *C. albicans* was the most prevalent species, other non-*albicans* *Candida* and rarely encountered yeast were also isolated. This indicates that use of proper and accurate molecular methods for yeast identification, especially for unusual yeast species, and prior antifungal treatment as required in cancer patients.

Data availability

Dataset 1: Demographical data of cancer patients. *Sheet 1:* For case group (168 cancer patients under treatments); *Sheet 2:* For control group (165 cancer patients); *Sheet 3:* Pictures demonstrated the colonial morphology for different isolated

yeast species. DOI, [10.5256/f1000research.14019.d199685](https://doi.org/10.5256/f1000research.14019.d199685) (Nagla *et al.*, 2018).

Sequences from the patients are available on GenBank under accession numbers: MH037201 to MH037237, MH019244 to MH019255, MH016295-MH016371, MH061321-MH061334, MH084778-MH084790, MH016252 to MH016274 and MH104613.

Competing interests

No competing interests were disclosed.

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Supplementary material

Supplementary File 1: Demographic questionnaire.

[Click here to access the data.](#)

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Lucia Bulacio

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The subject is very interesting since the early study of the epidemiology of infectious diseases in immunocompromised patients contributes to reduced frequency of complications due to invasion and dissemination, which cause increased morbidity and mortality of patients.

- *Saccharo* genus does not exist (*Saccaromyces*?)
- *C. dobliensis* does not exist (*C. dubliniensis*?)
- *Pichi kudriavzevii* does not exist (*Picchia*?)
- *P. sporocuriosa*? *L. fermentati*?

The first time they are mentioned in the text, all isolates must be named using the complete nomenclature; this is the name of the genus followed by the specific epithet, e.g. *Candida albicans*, *Saccharomyces cerevisiae*.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mycology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 02 November 2018

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Zoe Weiss

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This is an interesting study, but it is rather poorly analyzed. Also, some of the definitions need to be readdressed.

1. First of all, even in cancer patients, growth of *Candida* from sputum or urine does not mean dissemination; rather colonization. Even if the patients have symptoms, *Candida* UTIs and even less pneumonia are extremely rare. The authors need to provide solid data such as histopathology, define that there were no other potential pathogens, and symptoms resolved only after antifungal.
2. I am not sure how appropriate it is to use chi-square to document that one condition (colonization) is more frequent than another (infection) within the same group. Besides, so what? If infection is properly defined, the authors should investigate if there was a statistically significant difference in % between their "case" and "control" groups.
3. Speaking of infection, any cases of candidemia? This is the main clinical syndrome that can result from heavy *Candida* colonization, mucosal breach, foreign bodies, especially lines, immunosuppression, elimination of other flora with antibacterials etc.
4. More clinical information is required and needs to be properly analyzed (e.g. risk factors for non-*albicans* species, different clinical syndromes etc.).

5. It might be good to analyze the data on different species presented in the article tables for statistically significant differences in the frequencies of different *Candida* species.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 02 Nov 2018

nagla masaad, Al-Neelain University, Khartoum, Sudan

1. The first point of referee comment. Answer: I didn't confirm that it was dissemination. I suggested it may be dissemination due to the presence of the same strain in the oral cavity and urine or sputum in symptomatic patients so I recommend for further investigation so as to confirm it.
2. Point number 2: The authors stated that there was a statistical significant difference between infection and colonization among the case and control group.
3. Point number 4: It would be good to investigate that but, it wasn't one of my objectives.

Thanks for your good comment.

Nagla

Competing Interests: No competing interests were disclosed.

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