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Microbiology Section

Risk Factors,Diagnosis and Outcome of Proven and Probable Invasive Trichosporonosis in a Tertiary Care Hospital: A Cross-sectional Analytical Study

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ABSTRACT

Introduction: *Trichosporon* species are basidiomycetous yeast like fungus that is ubiquitous in the environment. They form part of the normal flora in humans. In recent years, they have been causing invasive infections, especially in the immunocompromised hosts. Disseminated cases including trichosporonemia can rapidly progress, leading to increased morbidity and mortality.

Aim: To diagnose and identify risk factors and outcomes of proven and probable invasive *Trichosporon* species infections.

Materials and Methods: This cross-sectional analytical study was done at Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India, over a period of one year (March 2023 till February 2024). All the clinical samples sent for routine diagnostics to the laboratory during the study period and archived samples were included (Total isolates, n=60). European Organisation for Research and Treatment of Cancer/Invasive Fungal Infection Cooperative Group (EORTC/IFICG) and the National Institute of Allergy and Infectious Disease/Mycoses Study Group (NIAID/MSG) definitions were used to categorise samples as proven invasive and probable invasive trichosporonosis. Phenotypic characterisation was done by colony morphology on Sabouraud Dextrose Agar (SDA), Gram stain, Dalmau technique and urea hydrolysis. Genotypic characterisation was done by *Trichosporon* genus specific and *Trichosporon asahii* specific Polymerase Chain Reaction (PCR). The reference strains used as quality control were *Trichosporon asahii* Microbial Type Culture Collection (MTCC) 6179, *Trichosporon jirovecii* MTCC 9036 and *Candida albicans* American Type Culture Collection (ATCC) 90028. All statistical analysis was performed using Microsoft Excel (2016) and descriptive statistics were presented as numbers and percentages.

Results: All the 60 (100%) isolates were characterised as *Trichosporon asahii*. Amongst them 4 (6.7%) were categorised as proven invasive trichosporonosis and 56 (93.3%) were probable invasive trichosporonosis. Some important risk factors for invasive disease were Intensive Care Unit (ICU) admission, antibiotic usage, diabetes and hypertension. All 4 (6.3%) proven cases were isolated from blood and 2 (50%) amongst them succumbed to the disease. Amongst the probable cases, 16 (28.6%) succumbed to the disease.

Conclusion: Invasive infections caused by *Trichosporon* species have high mortality especially amongst immunocompromised hosts. Hence, identification at an early stage by appropriate diagnostic methods and initiating appropriate antifungal agents can result in better outcome of the patient.

Keywords: Fungaemia, Invasive fungal infection, *Trichosporon* species

INTRODUCTION

Trichosporon species are yeast like anamorphic organisms that are widely distributed in nature and are more commonly found in the tropical and temperate regions. In humans, they are a part of the normal microbiome and can be seen in the gastrointestinal tract, oral cavity and transiently colonising the respiratory tract and skin [1]. Most of the time *Trichosporon* species are isolated from the laboratories as part of a superficial infection or as colonisers. In recent times this opportunistic pathogen has gained more importance due to the increase in the incidence of invasive infections worldwide. Invasive infections are most commonly seen in patients with immunosuppression especially haematological malignancies [1]. The EORTC/IFICG and the NIAID/MSG have put forth certain guidelines for invasive fungal infections which categorises patients with the same into “Proven Invasive” and “Probable Invasive” infection [2]. These definitions don’t include the guidelines for clinical interpretation of *Trichosporon* species recovered from respiratory samples [1]. In general, such guidelines can help in identification of reasonably homogenous group of patients that can better help in the management strategies as well as clinical and epidemiological research. For proven invasive fungal infections, detection of the fungus by culture of normally sterile samples or histopathological findings of invasive fungal infection is enough for diagnosis. In the case of probable invasive fungal infections, there are more elements like mycological evidence, host as well as clinical factors [2]. The presentation of infections caused by *Trichosporon* species is similar to other fungal infections and because of this it gets misdiagnosed frequently and is also difficult to treat [3]. Members of this genus can be provisionally identified by phenotypic characteristics like growth of dry colonies, microscopic features like arthroconidia, blastoconidia, pseudohyphae and ability to hydrolyse urea. Once a provisional diagnosis is made, further confirmation can be done by molecular methods like PCR or sequencing of the IGS1 (Intergenic spacer 1) region [1]. *Trichsoporon* species are difficult to identify and invasive infections have a poor outcome. Very few studies have been done in India analysing invasive trichosporonosis. Considering the increasing incidence of invasive trichosporonosis especially amongst immunocompromised patients, this study was undertaken to analyse diagnostic difficulties of trichosporonosis and to reiterate the fact about the associated risk factors and outcomes of proven and probable invasive trichosporonosis.The aim of the study was to diagnose and identify risk factors and outcomes of proven and probable invasive *Trichosporon* species infections.

MATERIALS AND METHODS

This was a cross-sectional analytical study done at Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India, over a period of one year starting from March 2023 till February 2024. The present study is a part of a larger project. The Institutional Ethical Committee (IEC) approved the study and IEC number is CSP-MED/23/MAR/85/62.

Inclusion criteria: Samples growing dry yeast like colonies showing arthroconidia, blastoconidia and pseudohyphae in Gram stain and positive urease biochemical reaction were included in the study.

Exclusion criteria: Isolates grown from repetitive samples were excluded from the study.

All the clinical samples sent for routine diagnostics to the laboratory during the study period and archived samples were included (Total isolates, n=60). This study was a cross-sectional analytical study and was time-bound. All samples fitting the inclusion criteria received during the study period was taken up. Therefore there was no sample size calculation.

Demographic and clinical details of the patients were collected for analysing and correlating with results. These data have been obtained from electronic records of the institution. According to EORTC/IFICG and NIAID/MSG definitions [2], samples were categorised as proven invasive and probable invasive trichosporonosis.

1. **Proven Invasive Trichosporonosis:** Presence of at least one criteria:

a) Positive blood culture in patient with temporally related clinical signs and symptoms

b) Cerebrospinal Fluid (CSF) culture growing *Trichosporon* species, or

c) Biopsy specimens with positive culture including histopathological findings supporting *Trichosporon* species.

2. **Probable Invasive Trichosporonosis-** Presence of all the criteria:

a) At least one host related factor like treatment with immunosuppressive drugs, neutropenia, fever persisting even after treatment with broad-spectrum antibiotics;

b) One microbiological factor like culture positive or presence of the organism in a suspected sample;

c) One major clinical factor consistent with infection like radiological or cytobiochemical finding [1,2].

The clinical outcome of these patients was then followed-up.

A provisional identification of the genus *Trichosporon* was made phenotypically if the colony had a dry, cerebriform appearance with microscopic features of arthroconidia, blastoconidia and pseudohyphae and also hydrolyses urea. All the isolates were inoculated on SDA plate and incubated at room temperature (25°C) for 24 to 48 hours. Microscopic features were analysed using Gram stain and Dalmau technique. Genomic Deoxyribonucleic acid (DNA) was extracted from the clinical isolates and reference strains of *Trichosporon* species and *Candida* species according to the method of Mirhendi H et al., and Vijayakumar R et al., [4,5]. After provisional identification, further confirmation was done by PCR using *Trichosporon* genus specific primers [6] followed by *Trichosporon asahii* specific primers [6,7] whichever isolate that did not produce band of amplification for species specific PCR was subjected to IGS1 sequencing. Sequencing was done only for isolates which did not produce amplification bands for *Trichosporon asahii* specific PCR. In this study all 60 isolates were identified as *Trichosporon asahii*.

The reference strains used as quality control were *Trichosporon asahii* MTCC 6179, *Trichosporon jirovecii* MTCC 9036 and *Candida albicans* ATCC 90028. Antifungal susceptibility testing was done by broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) M27-A4 guidelines [8], only for the isolates requested by the treating physician (one isolate).

STATISTICAL ANALYSIS

All statistical analysis was performed using Microsoft Excel (2016) and descriptive statistics were presented as numbers and percentages.

RESULTS

A total of 60 (100%) isolates were tested in this study, out of the which majority were from urine [Table/Fig-1].

Sample source	n (%)
Urine	53 (88.3)
Blood	4 (6.7)
Pus	3 (5%)

[Table/Fig-1]: Sample source of clinical isolates.

Percentage of isolates from males was higher compared to females, the ratio being 2.5:1 [Table/Fig-2]. The ages ranged from 27 to 91, the median age being 65 years.

Gender	n (%)
Male	43 (71.7)
Female	17 (28.3)

[Table/Fig-2]: Distribution of gender among all isolates.

In this study 35 (58.3) isolates were obtained from patients admitted in the ICU with the presence of intravenous indwelling catheters in a majority of patients 54 (90%). Antibiotic usage was noted for everyone in the study population. The frequent co-morbidities include diabetes mellitus 40 (66.7%), hypertension 31 (51.7%) and cardiovascular diseases like myocardial infarction 20 (33.3%). Urinary catheterisation was also an important risk factor observed in majority of the cases 50 (83.3%). Other co-morbidities include renal pathologies like acute kidney injury and chronic kidney disease, cerebrovascular accident and decompensated liver disease.

Respiratory infection associated were pulmonary tuberculosis 5 (8.3%), bacterial pneumonia 3 (5%) and pulmonary aspergilloma 1 (1.7%). Conditions requiring long term hospitalisation like pancreatitis 1 (1.7%), meningitis and septic shock 4 (6.7%) and polytrauma were also observed [Table/Fig-3].

Risk factor/co-morbidity	n (%)
Intensive Care Unit (ICU)	35 (58.3)
Intravenous catheter	54 (90)
Antibiotic usage	60 (100)
Diabetes mellitus	40 (66.7)
Hypertension	31 (51.7)
Myocardial infarction	20 (33.3)
Urinary catheter	50 (83.3)
Malignancy	6 (10%)
Post-transplant status	3 (5%)
Renal pathology	13 (21.7)
Cerebrovascular accident	8 (13.3)
Decompensated liver disease	6 (10)
Respiratory infections	9 (15)
Pancreatitis	1 (1.7)
Meningitis	4 (6.7)
Septic shock	4 (6.7)
Polytrauma	2 (3.3)

[Table/Fig-3]: Percentage of risk factors and co-morbidities among clinical isolates.

All patients categorised as proven invasive trichosporonosis were admitted in the ICU and they also had diabetes and hypertension as a common risk factor. The patients categorised as probable invasive trichosporonosis was a mixture from the ICU and ward. Most of these patients had underlying co-morbidities and disease of other parts of the body.

Amongst the 60 (100%) patients, 4 (6.7%) patients had "Proven Invasive Trichosporonosis" and 2 (50%) amongst them succumbed to the infection. The first patient was a case of decompensated chronic liver disease and sepsis who was initially started on fluconazole and then escalated to voriconazole {Minimum Inhibitory Concentration (MIC)- 0.5 µg/mL}, since fluconazole was found to have high MIC of 64 µg/ml through antifungal susceptibility tests (MIC of other antifungals tested: amphotericin B- 8 µg/mL, itraconazole- 16 µg/mL). The other was a post renal transplant patient who had caecal perforation with peritonitis leading to polymicrobial sepsis and septic shock. Both these patients also had multiple cultures from different sites that grew gram negative bacilli. All the other patients were treated for their septicaemia and subsequently discharged with an uneventful course during the hospital stay. There were 56 (93.3%) patients who were categorised under "Probable Invasive Trichosporonosis" and amongst them 16 (28.6%) patients succumbed to the disease and the remaining recovered from the infection with an uneventful course [Table/Fig-4]. Repeated isolation from urine samples was required to consider *Trichosporon* species as a pathogen.

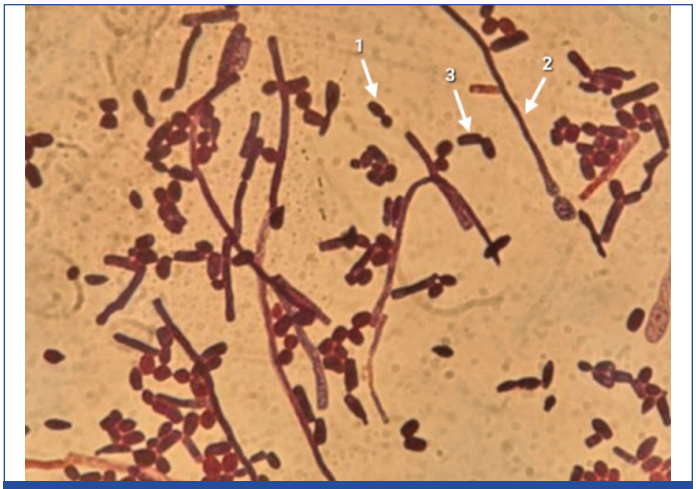
Proven invasive trichosporonosis		Probable Invasive Trichosporonosis	
Recovered	Succumbed	Recovered	Succumbed
2 (50%)	2 (50%)	40 (71.4%)	16 (28.6%)
Total=4		Total= 56	

[Table/Fig-4]: Outcome of proven and probable invasive Trichosporonosis.

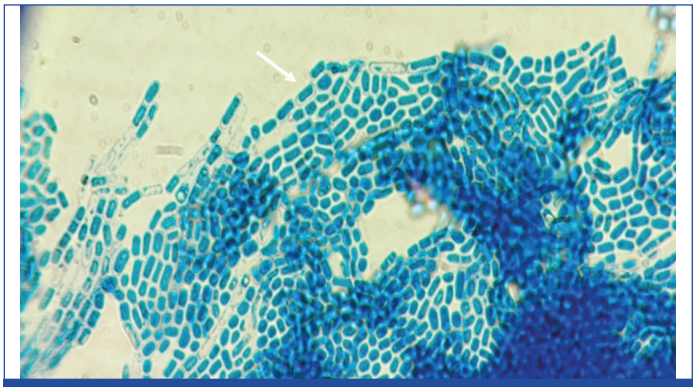
All the isolates grew on SDA as white to cream cerebriform colonies with radial furrows [Table/Fig-5] and microscopy showed blastoconidia, arthroconidia and pseudohyphae on Gram stain [Table/Fig-6] and barrel shaped arthroconidia by Dalmau technique [Table/Fig-7]. All the isolates hydrolysed urea. Hence, they were provisionally identified as belonging to the genus *Trichosporon*.

[Table/Fig-5]: Individual colony morphology on Sabouraud Dextrose Agar (SDA).

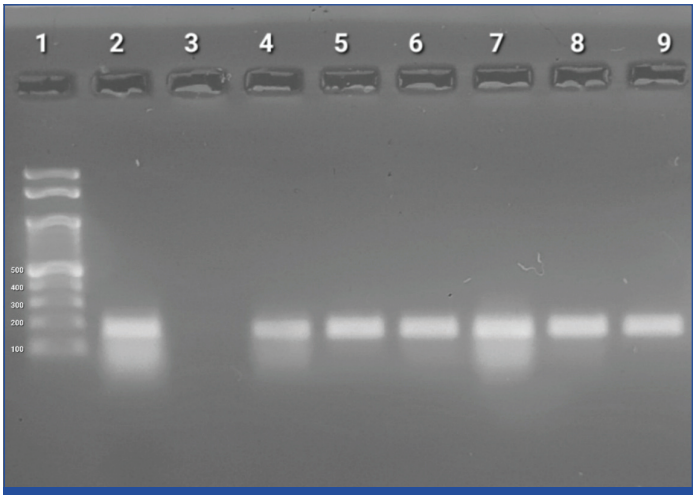
All the isolates were then further confirmed genotypically. For the *Trichosporon* genus specific PCR, all the test isolates and positive control (*Trichosporon asahii* MTCC 6179) produced amplification bands of approximately 170 base pairs. No band of amplification was observed for the negative control (*Candida albicans* ATCC 90028) [Table/Fig-8]. For *Trichosporon asahii* specific PCR, all the test isolates and positive control (*Trichosporon asahii* MTCC 6179) produced amplification bands at 430 base pairs. No band was seen for the negative control (*Trichosporon jirovecii* MTCC 9036) [Table/Fig-9].



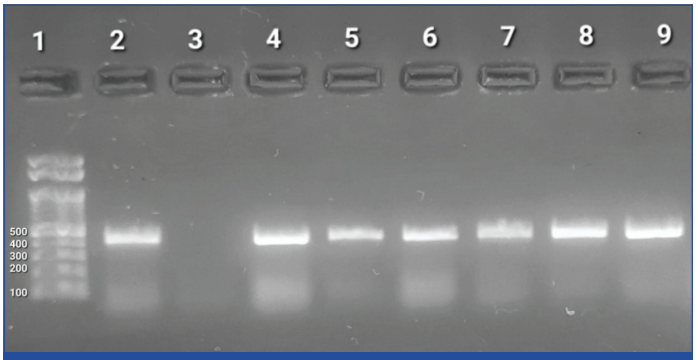
[Table/Fig-6]: Microscopic morphology- Gram stain (100x magnification).
*1: Blastoconidia; 2: Pseudohyphae; 3: Arthroconidia



[Table/Fig-7]: Dalmau technique with Lactophenol cotton blue staining (40x magnification).
*Arrow showing barrel shaped arthroconidia



[Table/Fig-8]: *Trichosporon* genus specific PCR.
*1- 100 bp ladder; 2- *Trichosporon asahii* MTCC 6179 (positive control); 3- *Candida albicans* ATCC 90028 (negative control); 4, 5, 6, 7, 8, 9- Representative clinical isolates



[Table/Fig-9]: *Trichosporon asahii* specific PCR.
*1- 100 bp ladder; 2- *Trichosporon asahii* MTCC 6179 (positive control); 3- *Trichosporon jirovecii* MTCC 9036 (negative control), 4, 5, 6, 7, 8, 9- Representative clinical isolates

DISCUSSION

Trichosporon species are opportunistic pathogens that are also part of the normal flora in humans. They are widely distributed in nature and have recently gained importance due to the increasing number of invasive infections. Trichosporonosis is usually difficult to diagnose as well as treat and is associated with high mortality rates [7]. *Trichosporon asahii* which is the more common species in this genus affects all individuals irrespective of their immune status. It is essential to diagnose the infection early and start the patient on the correct antifungal agent [7]. In present study, majority of the isolates were obtained from urine 53 (88.3%). In another study by Francisco EC et al., the most common source of the isolates was also from urine (43.3%) [9]. There are no guidelines available for the clinical interpretation of *Trichosporon* species when isolated from urine. They can be seen as part of the normal flora of the human skin, gastrointestinal tract and vagina [10]. Out of the total 60 (100%) isolates, 4 (6.7%) were from blood and these were considered as "proven invasive trichosporonosis" according to EORTC/IFICG and NIAID/MSG guidelines [1]. In another study, 16.7% of all their isolates were from blood [11]. All the isolates were identified as *Trichsoporon asahii* by phenotypic and genotypic methods. In a study by Chagas-Neto TC et al., 68% of the total isolates from blood were found to be *Trichosporon asahii.* The exact prevalence of *Trichosporon asahii* fungemia may be hard to estimate as they are not easily diagnosed in most laboratories [12]. Out of the total isolates 3 (5%) were isolated from pus and this was similar to another study where out of their total isolates 5.6% were from pus or wound swab [11].

In this study, isolates were higher in males 43 (71.7%) compared to females 17 (28.3%). This higher proportion in male population was also seen in a study by Li H et al., where 59.3% isolates were from males and 33.6% from females [3]. In another study, 60% of invasive *Trichosporon* species infections were seen in males compared to females. However, they found a reversal in superficial *Trichosporon* species infections where female population was more common than male [13]. The median age in this study was 65 years which differed from another study where the median age was 45 years (Mehta V et al.,). It was also noted from other studies that most cases of trichosporonosis is seen in age groups ≥ 66 years [13].

All the patients in present study group had at least one risk factor or co-morbid conditions. In our study, 35 (58.3%) isolates were obtained from patients in the ICU. This was comparable with a study by Mehta V et al., where 34% of the isolates were from ICU [13]. Another fact was that all patients with proven invasive trichosporonosis was admitted in the ICU signifying the role of ICU admissions in severe invasive fungal infections. Majority of the patients in this study also had invasive medical devices like intravenous indwelling catheter 54 (90%) and urinary catheter 50 (83.3%). Li H et al., found in their study that invasive medical equipment was a major risk factor accounting for 44.3% of the study population [3]. In another study, 62.8% of the patients with invasive *Trichosporon* species infection had history of urinary catheterisation [13]. Catheters can act as substrates where the organism attaches and forms biofilms which can eventually cause invasive infections [14]. For a probable invasive Trichosporon infection of the urinary tract, the organism must be repeatedly isolated from the sample as they can also be colonisers. ICU stay, administration of broad spectrum antibiotics, invasive medical devices and neutropenia remain the four main risk factors for *Trichosporon* species infections [3]. Diabetes was a common co-morbidity in this study accounting for 40 (66.7%) patients. In a study by Alboloshi GJ et al., 47.6% of the patients were diabetic [15]. Uncontrolled sugar levels in diabetic patients can lead to immune dysfunction predisposing them to infections. There is a decrease in T-lymphocytes as well as reduction in neutrophil activity, cytokine levels and increase in apoptosis of leukocytes [16]. Hypertension and cardiovascular diseases followed diabetes in prevalence 31 (51.7%) and 20 (33.3%), respectively). In other studies also this was comparable, with hypertension and chronic heart disease accounting for 38.1% and 33.3% respectively [15]. Immunosuppressive states like malignancy and renal transplantation were observed in 9 (15%) patients in this study whereas other studies have observed 10% of their study population being immunocompromised (Mehta V et al.,) [13]. There were 9 (15%) patients with respiratory pathologies out of the total study population and this was similar to the study by Li H et al., where they got 12.1% [3]. Other co-morbid conditions requiring long term hospitalisation and antimicrobial therapy were also observed. All the patients in this study were on antimicrobial treatment and some also on anticancer drugs. In a study by Liao Y et al., 84.05% were on antimicrobial use and 58.44% were on chemotherapy [17]. The risk factors observed in present study were similar to the ones in a study by Alboloshi GJ et al., where association with *Trichosporon* infection was also done [15]. Antimicrobial therapy can cause alteration of the microbial flora of the gastrointestinal tract and cause translocation of these organisms to the blood causing fungemia. Chemotherapy can induce mucositis and disruption of the mucosal barrier which can also favour invasion of organisms [18]. A common factor for the proven and probable invasive trichosporonosis patients in present study was the usage of antibiotics which was an important risk factor. This also signifies the importance of avoiding indiscriminate antibiotic usage.

Clinical outcome was followed for all the patients where 2 (50%) patients categorised as "Proven Invasive Trichosporonosis" and 16 (28.6%) patients categorised as "Probable Invasive Trichosporonosis" succumbed to the infection. Identifying *Trichosporon* species at an early stage is crucial for treatment especially for invasive infections because patients may go for a bad prognosis. Hence, early treatment with the appropriate antifungal agent can result in better outcome of the patient [19]. Mortality rates are also higher for invasive infections and can be from 40 to 60% depending on the patient's immunity status. In contrast to this localised infections like cellulitis have a better prognosis with surgical debridement and antifungal therapy [20]. Isolation of *Trichosporon* species from urine remains a challenge because there are no clear cut definitions for its clinical interpretation. They more commonly occur in patients who are on catheter and/or antibiotics or in the presence of urinary obstruction. If there is the presence of a host factor like antibiotic usage or neutropenia and a clinical factor like suggestive radiological or cytobiochemical findings along with microbiological isolation, these organisms can be considered to be a pathogen from sources like urine [1].

Phenotypic methods like macromorphology on SDA, microscopic appearance of arthroconidia, blastoconidia and pseudohyphae along with hydrolysis of urea provides a provisional identification of the genus. Due to the increasing incidence of patients presenting with missed yeast infections, accurate diagnosis by phenotypic methods alone may not be possible. By phenotypic methods it can still get misdiagnosed as other pathogenic yeasts like *Candida* species. Moreover these methods are time consuming, requires specialist training and can give inconclusive results [21]. Other commercially available identification systems based on assimilation of carbon and nitrogen compounds are also not reliable. Molecular methods like PCR are more reliable for the identification by making use of species specific primers. They are simple, rapid and specific. Their main advantage is the high sensitivity and specificity of the results obtained [22]. Phenotypic methods provides a suspicion of the genus which should then be confirmed by molecular methods which will help in the better prognosis. In present study, all the isolates were identified as *Trichosporon asahii* by genus specific and *Trichosporon asahii* specific PCR. Hence, there was no requirement for IGS1 sequencing. Amongst the *Trichosporon* species, *Trichosporon asahii* is the most common cause of trichosporonosis in immunocompetent as well as immunocompromised patients [22]. For isolates that do not produce bands of amplification for *Trichosporon asahii* specific PCR, sequencing of the DNA fragments must be done. IGS1 sequencing can be done for accurate identification of the species [23].

Invasive trichosporonosis is an opportunistic life-threatening condition with high mortality rates despite initiation of antifungal therapy. Accurate identification is essential because they are easily misdiagnosed and started on inappropriate antifungal agents [24]. In the case of fungemia with *Trichosporon* species, mortality rates can be as high as 77% [20]. In present study, as well 50% mortality rate was observed for patients with Trichosporonemia which is categorised under "Proven Invasive Trichosporonosis". This is much higher when compared to mortality rate of probable invasive cases, which was 28% in present study. Even if the mortality rate was lesser for probable invasive infections, it must be correctly diagnosed since these can progress to disseminated infection without appropriate treatment [25]. Hence, clinicians must keep a watch on patients with probable invasive trichosporonosis.

Limitation(s)

Since this was a time bound study and the fungi under consideration are rare, the sample size was only 60 isolates. Statistical analysis for comparing risk factors and finding association could not be carried due to lack of comparative group (control group matched for age, gender and risk factors). Follow-up could not be done for the patients who recovered from the infection and got discharged from the hospital.

CONCLUSION(S)

This study highlights the fact that *Trichosporon* species is an emerging pathogen not ignoring the fact that they may be a normal flora in the urinary tract. Most of the *Trichosporon* isolates in this study were urinary isolates and unless the patient has risk factors, it could just be a coloniser. Presence of *Trichosporon* species in blood has to be treated promptly. The outcome of these patients depends on accurate diagnosis and treatment. The risk factors play a very important role in the outcome as seen in present study. Managing the risk factors along with targeted antifungals and infection control will go a long way in improving the outcome.

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