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## Research Article

# TRICHOSPORONOSIS – AN ASSOCIATION WITH CLINICAL FACTORS AND ITS OUTCOME

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**Abstract-** Introduction: The genus *Trichosporon* usually cause superficial mycoses, but recently they have emerged as an important pathogen causing invasive infections in immuno-compromised patients. Invasive Trichosporonosis is mainly documented in patients with associated risk factors and co-morbid conditions like haematological malignancies, cardiac or renal disease, intravenous or central line catheter, prosthetic devices and implants, prior antibiotic therapy, stay in Intensive care unit. Objective: This study was undertaken to assess the risk factors and co-morbid conditions associated with Trichosporonosis and its clinical outcome. Materials and Methods: We considered around 72 clinical isolates for the study that were, dry yeast like colonies, urease positive, showed blastoconidia and arthroconidia in gram stain, and were suspected to belong to the genus *Trichosporon*. Later, they were confirmed genotypically using *Trichosporon* specific PCR. The demographic and clinical details were collected from all the patients for the study. Results: About 66% of the affected patients were males, with male to female ratio 2:1. All our patients had any one of associated risk factor. Indwelling catheter was present in most of the patients (97.2%) in our study. Bladder catheterisation was the predominant risk factor among patients who grew *Trichosporon* sp. in urine sample (83.7%). Majority of the patients (52.8%) had associated bacterial infections. Discussion and conclusion: The associated risk factors increase the colonisation of this genus by breaking mucosal barrier, later leading to establishment of infection.

**Keywords**- Trichosporonosis, risk factors, indwelling device.

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## Introduction

The members of the genus *Trichosporon* are mainly found in the soil. They can colonize and proliferate in different parts of the human body, including the gastrointestinal system, respiratory tract, skin and vagina [1,2].*Trichosporon*sp can cause an extensive spectrum of clinical manifestations. They can mainly manifest as superficial cutaneous infections in immuno-competent individuals, or severe systemic infections in immuno-compromised patients [3,4]. Invasive infections due to *Trichosporon* species are usually uncommon. However, in the recent past the genus *Trichosporon* has emerged as an important opportunistic pathogen in immuno-compromised patients [5-7]. Invasive trichosporonosis has also been reported in immuno-competent patients with associated risk factors, including patients with prosthetic valves, underlying peritoneal dialysis, intravenous catheters and urinary catheters [8,9]. The genus *Trichosporon* is often under-reported since they are clinically misdiagnosed as other types of yeast, particularly *Candida* non albicans sp [10]. The conventional phenotypic characterisation techniques which are routinely used in most laboratories for yeast identification are time consuming, and are often inaccurate for species level identification of *Trichosporon* [11,12]. Hence, timely and precise diagnosis remains challenging. Mortality with *Trichosporon* sp remains high (53% - 76%), because of delayed diagnosis and the lack of an ideal treatment plan [13,14]. Due to low incidence, difficulty in diagnosis and under-reporting, mostly *Trichosporon* infections are limited to individual case reports or small case series. There has been no complete analysis to improve our understanding about the epidemiology, associated risk factors with outcomes and therapeutic aspects of this genus [14]. Hence, this study was undertaken to enhance our knowledge of this uncommon infection, by analysing demographic details, associated risk factors and treatment outcome.

## Materials and methods

**Type of study:** Hospital based descriptive study

### Strains and Clinical source

A total of 72 clinical isolates suspected to belong to the genus *Trichosporon*, collected from various clinical samples of different patients from Sri Ramachandra Medical College and Research Institute & few other hospitals in Chennai and one hospital in Lucknow, between July 2011 & June 2016 were considered for our study. Yeast-like colonies were observed for microscopic characteristics by Gram staining and Dalmau technique [15]. The isolates which showed the budding yeast cells, hyphae & arthroconidia by microscopy and were urease positive, were provisionally identified as *Trichosporon species* [16].The identification was further confirmed using molecular techniques. The isolates were preserved at -20°C on skimmed milk medium [17] until use.

### Sample source

Majority of our isolates were from urine *i.e*., 43(59.7%). Around 12 isolates were from blood. For one patient, *Trichosporonsp* was isolated from two set of blood cultures and one urine culture. About 7(9.7%) of the isolates grew from samples collected by percutaneous nephrolithotomy (PCN). Out of the 5(6.9%) respiratory isolates, three were sputum, one bronchoalveolar lavage, and one tracheal aspirate. Around 4(5.5%) of the *Trichosporonsp* were isolated from pus, and one from peritoneal dialysis fluid [Fig-1].

**Demographic and clinical data**

Demographic data, treatment regimens and the outcome of each incident of Trichosporonosis were recorded. Factors previously reported as predisposing factors to *Trichosporo*n infections were also included in the recording form.

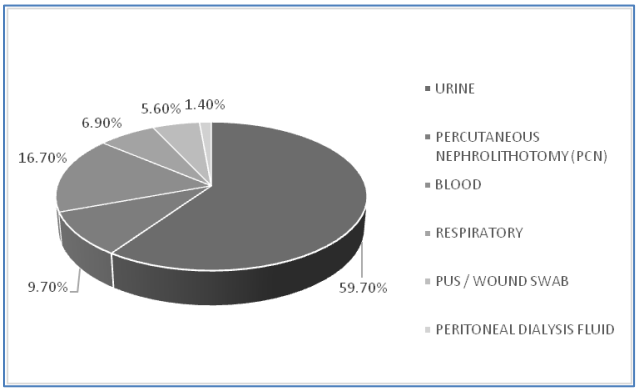



**Fig-1 Sample Source of Clinical isolates**

**Molecular identification**

**DNA extraction**

Genomic DNA from clinical isolates, reference strains of *Trichosporon* species and reference strains of *Candida* species were isolated by the method of Vijayakumar, *et al*., (2011) [18]. Briefly, 400 µl of lysis buffer (10mM TRIS), (pH - 8), 1mM EDTA (pH - 8), 3% SDS and 100 mM NaCl) was taken in a 1.5-ml centrifuge tube. A loop full of *Trichosporon* culture was suspended in the lysis buffer and heated at 100°C in water bath for 1 min. An equal volume of Phenol: Chloroform was added to this suspension and mixed well. It was then centrifuged at 10,000 rpm for 10 min. The aqueous layer was transferred to a fresh centrifuge tube and the step was repeated again by adding chloroform to the supernatant. The DNA was precipitated with cold isopropyl alcohol, centrifuged and washed with 70% ethanol and dried. The pellet was later re-suspended in 30 µl of Tris-EDTA (TE) buffer and stored at -20°C until use.

***Trichosporon* specific PCR**

The first set of primers used were *Trichosporon*genus specific primers (TRF - 5'AGAGCCTACCATGGTATCA 3' TRR-5'TAAGACCCAATAGAGCCCTA3') [19]. They would precisely amplify only *Trichosporon* species, by aligning with the small subunit (SSU) of ribosomal DNA (rDNA) sequences, since this region is not conserved in other medically important yeasts. The PCR master mix was prepared containing 25µl of PCR mix (Takara, Japan), 1 µl of forward (TRF) and reverse primer each (TRR) (GeNei, Bangalore), 1 µl of template DNA and the volume made up to 50 µl with sterile nuclease-free water.The reaction mixtures were amplified in a thermal cycler (Veriti 96 well, Applied Biosystems, USA), with the following program: 95°C for 7 min, followed by 30 cycles consisting of 95°C for 30s, 54°C for 30s, and 72°C for 30s, with a final extension period at 72°C for 10min [19]. After thermal cycling, 10µlof the amplified product was run on a 1.5% (wt/vol) agarose gel, stained with ethidium bromide, and visualized with UV light.

**Reference strains**

*T. asahii* MTCC 6179, *T. asteroides* MTCC 7632, *T. cutaneum* var. *cutaneum* MTCC 1963,*T.jirovecii* MTCC 9036, *Candida albicans* ATCC 90028.

**Results**

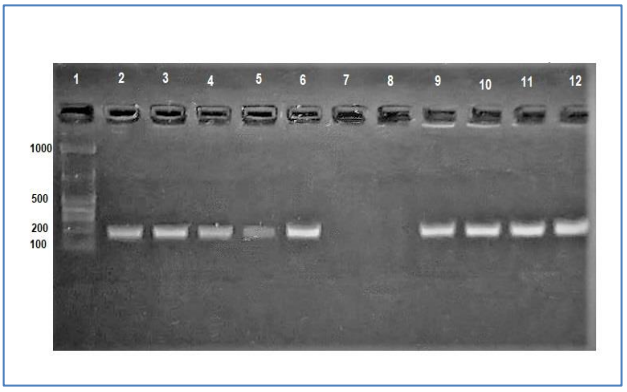
**Molecular identification**

PCR was performed with *Trichosporon* genus specific primers to double check for accurate identification of the genus [19]. This pair of primer is *Trichosporon* specific and amplified part of the nucleotide sequences of the rDNA small subunit (18S). DNA bands of approximately 170bp were obtained for all the isolates tested and for *Trichosporon* reference strains. In addition, there was no amplification of DNA isolated from *Candida albicans* ATCC 90028 (negative control) [Fig-2]. Therefore, all our strains were confirmed to belong to the genus *Trichosporon*.

**Demographic and Clinical Data**

**Age and sex distribution**

In our study, out of the total 72 patients males (48/72 *i.e.,* 66.7%) were more commonly affected than the females (24/72 *i.e.,* 33.3%). Male to female ratio was 2:1. *Trichosporon* infections were significantly high in elderly age among men in this study *i.e.*, 41-60 years (37.5%) &>60 years (36.1%) age group, whereas *Trichosporon* infection rates were high among women in reproductive age group(p = 0.23; p value was calculated by chi square test. It was <0.05, which showed significant association between age interval and gender of the patients). The median age of patients in the present study is 53 years (ranging from 1 day to 90 years) [Fig-3]. Two of our patients were neonates who had invasive Trichosporonosis, in whom *Trichosporon*sp grew in blood.




1- 100bp ladder
2- *T.asahii*MTCC 6179
3- *T.asteroides* MTCC 7632
4- *T.cutaneum* MTCC 1963
5. *T.jirovecii* MTCC 9036
6, 9, 10, 11, 12 – Representative clinical isolates
7- *Candida albicans* ATCC 90028
8 – Blank control

**Fig-2 *Trichosporon* specific PCR**

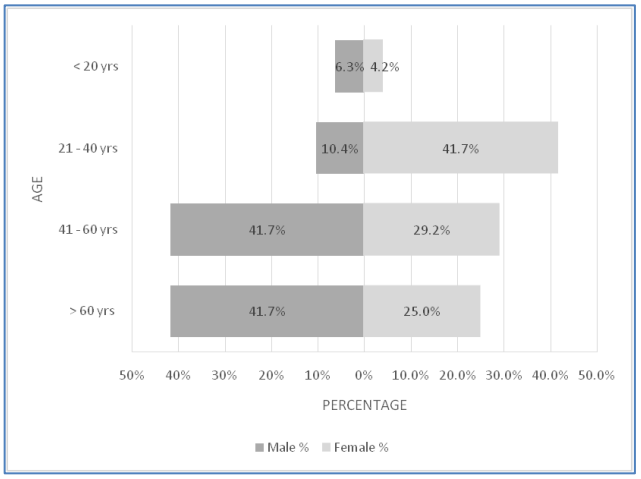



**Fig-3 Distribution of patients by age group and sex**

**Risk factors / Co-morbid conditions**

All the patients in our study were associated with any one of the underlying co-morbid conditions among which the predominant ones were, diabetes mellitus (56.9%), ICU admission (51.4%), renal failure & post-surgery (29.1%) and hypertension (25%). Few of our patients had intense immunosuppressive states like malignancy (9.7%) and post-transplant / prosthetic valve recipients (5.6%). Other associated co-morbid conditions which require long term hospitalisation were also observed as associated risk factors among our patients like, cardiac failure (8.3%), cerebrovascular accident (6.9%) and acute pa ncreatitis (2.8.%) [Fig-4]. Five of our elderly patients (6.9%) were under ventilator support. Majority of the patients (97.2%) had an indwelling intravenous catheter in our study. Out of the 43 urine samples which grew *Trichosporon*sp in our study, majority of the patients *i.e.,* 36 (83.7%) were catheterised, contributing to its role as a predominant risk factor in our study.

**Associated infections**

Majority of the patients in our study *i.e*., 48 (66.7%) of them had one associated infection with bacteria, fungus or virus. In around 21(29.2%) patients, details of the co-infections were not available. Only 9 (12.5%) of the patients did not have any associated co-infection. Bacterial infection was the most frequently associated one occurring in 32(52.8%) of the patients, next to which comes the fungal infection (9 *i.e*., 12.5%), and then the viral infection (1 *i.e*., 1.4%) respectively [Fig-5].

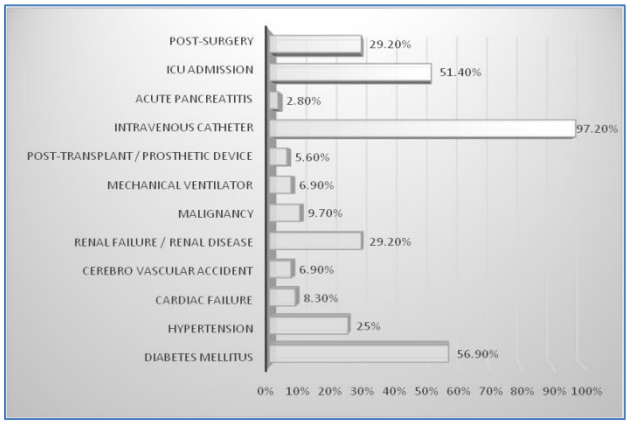



**Fig-4 Distribution of underlying risk factors/ Co-morbid conditions in study group**

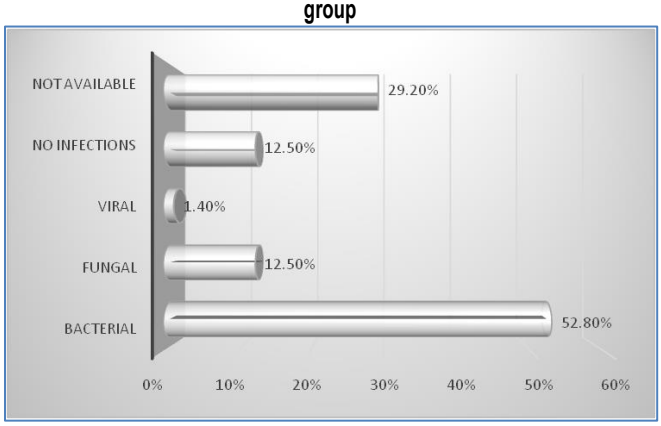



**Fig-5 Other associated infections in study population**

**Antimicrobial / Drug treatment**

All the patients in the current study were under antimicrobial treatment, anti-cancer chemotherapy or steroid treatment. The antimicrobial agents most commonly associated with *Trichosporon* infection were Carbapenems (33.3%) and inhibitor combinations (38.9%). Receipt of antifungal treatment was the next commonly documented risk factor seen in 27(37.5%) of the patients. The other antimicrobial and immuno-suppressive agents recognised as risk factors in the present study are shown in [Fig-6].

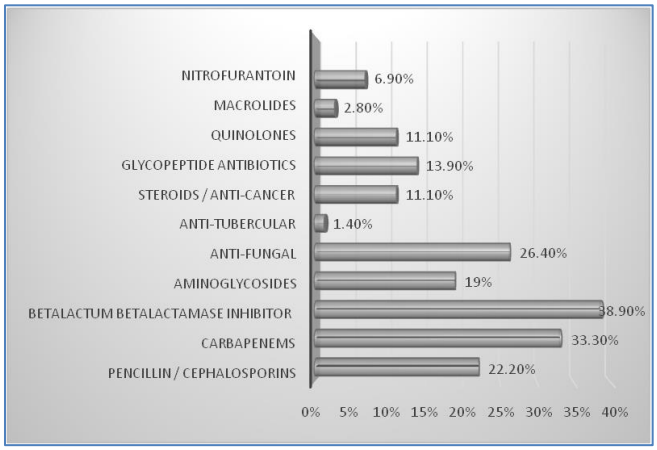



**Fig-6 Underlying antimicrobial / drug treatment in study population**

**Clinical outcome**

Most of the patients (43 *i.e*., 59.7%) were discharged. Around three patients in our study group had disseminated Trichosporonosis, and they succumbed to infection. In about 26 (36.1%) of the patients, the outcome was not known since they got discharged against medical advice, or did not turn out for follow up.

**Discussion**

*Trichosporon sp* has been so far reported as the second most common cause of disseminated yeast infections in humans, next to the genus *Candida* [20]. They are emerging opportunistic pathogens causing invasive infections in immuno-compromised patients [20,21]. Such infections are usually difficult to diagnose, do not respond to treatment with routinely used antifungal agents and associated with high mortality rates [21,22]. In this study, we have genotypically charaterised 72 *Trichosporon* clinical isolates collected from patients and assessed their risk factors, associated co-morbid conditions, other infections and their clinical outcome. Urinary isolates were majority in number [43 (59.7%)] in our study. Since there are no clear and precise guidelines for the clinical interpretation of *Trichosporon* sp. recovery from urine, urinary tract infections caused by this organism pose a challenge for clinicians [23]. Majority of the patients *i.e*. 38 (88.3%) in whom urinary isolates grew *Trichoporon* sp, were catheterised. Since *Trichosporon* species may constitute a part of normal flora of human skin and perigenital area, it is possible that this fungus colonised the catheter from the skin flora during catheterisation, and also would probably evolve as invasive trichosporonosis [24]. We had around 7 PCN samples which grew *Trichosporon*. Studies suggest that positive stone culture and sensitivity collected by nephrolitholotomy are superior predictors of impending urosepsis than bladder urine [25]. Therefore, it is recommended to collect these specimens on a routine basis in patients with renal stones. In our study, 12(16.7%) isolates were from blood. We adapted definitions by European Organization for Research and Treatment of Cancer/Invasive Fungal Infection Cooperative Group (EORTC/ IFICG) and National Institute of Allergy and Infectious Disease Mycoses Study Group (NIAID/MSG) for the clinical interpretation of *Trichosporon* sp. from blood specimen as "proven invasive Trichosporonosis" proposed by Girmenia and collaborators [7]. These criteria require isolation of *Trichosporon* species in blood cultures of patients with temporally related clinical signs and symptoms of infection.

In the present study, 5 *i.e*. 6.9% of the isolates grew *Trichosporon* in respiratory sample. In another study by Girmenia, *et al*., 6 out of 52 cases *i.e.,* 11.5% of the isolates grew *Trichosporon* sp in respiratory sample which is close to the isolation rate in our study [7]. Out of the 4 (5.6%) wound/pus specimens, 3 grew *T.asahii* and in one *T.cutaneum* was isolated. This finding was almost similar to another study by Sheng-Yuan Ruan, *et al*., where out of the 43 clinical *Trichosporon* isolates 11 were from wound specimens, out of which 4 were *T.asahii* and one was *T.cutaneum* [26]. We had one patient in whom peritoneal dialysis fluid grew *Trichosporon* sp. Peritonitis is one of the most common and serious complications of peritoneal dialysis, and a case of peritoneal infection due to *Trichosporon* sp. was documented by Khanna, *et al*., in 1980 [27]. Analysis of the clinical data of patients in our study showed that, *Trichosporon* infections were more common in males, with the male-to-female ratio of 2:1. This finding is similar to that reported in other studies [13,21]. In our study the median age group of the patients was 53 years. In another study by Kontoyiannis, *et al.*, the median age group reported was 44 years [13]. Diabetes mellitus was the most common risk factor observed in 56.9% of the patients. In another study by Wei sun, *et al.*, though diabetes was the most common risk factor the percentage reported was little less *i.e*. 34.7% [28]. This difference may be due to the variations in the prevalence of diabetes mellitus in different geographical regions. High blood sugar level is associated with impaired leucocyte function, and also provides a favourable environment for the growth of the yeasts, including *Trichosporon* sp. The next most commonly associated risk factor in our study is ICU admission accounting for 51.4% of the total cases. Several documented cases of trichosporonosis in aged and critically ill patients have been linked with ICU patients in different hospitals by previous researchers, similar to the findings of our study [24,29]. All the associated co-morbid conditions almost contribute to immune-suppression. Since *Trichosporon*

sp is a normal colonizer of the skin and mucous membrane, significant host compromise of both anatomic and neutrophilic defences contributes to the establishment of infection. In most of the patients, the source of the invasive organism is their own flora. In the present study, *Trichosporon* infections in patients are most frequently associated with bacterial infections (52.8%). Patients who are at a risk of developing *Trichosporon* infections are also vulnerable to systemic bacterial infections, and frequently receive antimicrobial therapy. This can result in imbalance of microbiota and this along with concomitant bacteremia can aggravate the patient's condition, leading to invasive infections in such patients. Hence, bacterial co-infections should be considered seriously and early initiation of prompt treatment is mandatory. Treatment with antimicrobial agents was observed in almost all the patients in the current study, among which treatment with carbapenems (33.3%) and inhibitor combinations (38.9%) were the most commonly recognised risk factors. In another study by Wei sun, *et al*., treatment with antimicrobials was the most commonly documented risk factor [28]. The broad spectrum antibiotic destroys the bacteria at the pathogenic site of infection and hence the yeast gets a chance to proliferate. This in turn leads to the establishment of yeast infection. Catheterisation was a major risk factor identified in our study group where 83.7% of the patients were catheterised. This finding was similar to the finding of another study by Pradhan, *et al*., 2017 where 95% of the patients had indwelling catheters which was a major recognised risk factor [30].

## Conclusion

Mucosal colonisation and consequent invasion by *Trichosporon* species is predominantly due to break in mucosal barriers. Factors like indwelling catheters, mechanical ventilation, prior infections, and use of broad spectrum antibiotic treatment for documented or presumed bacterial infections all contribute to break in the mucosal barrier, thus leading to the establishment of *Trichosporon* infection. Therefore, invasive *Trichosporon* infections may occur in immuno-competent individuals suffering from chronic illness or disruption of skin and mucous membranes.

**Application of research:** A knowledge of *Trichosporon* infections and its associated risk factors helps to understand the pathogenesis of this organism, and alerts the physician about the potential role of this yeast in causing invasive infections.

**Research Category:** Mycology

**Abbreviations:**

EORTC/IFICG: European Organization for Research and Treatment of Cancer/ Invasive Fungal Infection Cooperative Group

NIAID/MSG: National Institute of Allergy and Infectious Disease Mycoses Study Group)

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**Research Guide or Chairperson of research: Dr. Anupma Jyoti Kindo**

University: Sri Ramachandra Medical College and Research Institute, Chennai, 600116, Tamil Nadu

Research project name or number: Nil

**Author Contributions:** All author equally contributed

**Author statement:** All authors read, reviewed, agree and approved the final manuscript

**Conflict of Interest: None declared**

**Institutional Ethical Committee Approval Number:** REF: IEC-NI/12/MAR/27/14

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