Complicated urinary tract infection by *Trichosporon loubieri*

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A B S T R A C T

We report two cases of complicated Urinary Tract Infection, one with nephrostomy tube left in-situ and other with bladder outlet obstruction, caused by *Trichosporon loubieri*. Both patients responded well to antifungal treatment along with change/removal of catheters. In both the cases, correct identification of *T. loubieri* was done by IGS1 sequencing. Prompt identification and timely management headed to good clinical outcome. Hence, clinicians should be aware of *T. loubieri* as an emerging fungi causing human infections.

1. Introduction

*Trichosporon* species are emerging opportunistic basidiomycetous yeasts [1]. The taxonomy of the genus *Trichosporon* is progressing fast with increased number of species causing disseminated trichosporonosis [2]. *T. loubieri* is a rare species which has been reported recently as a cause of disseminated trichosporonosis, which is associated with high mortality regardless of antifungal therapy [3–5]. Since *Trichosporon* species have different antifungal susceptibilities, it is important to speciate them accurately for specific therapy [6]. Identification based solely on carbon and nitrogen assimilation profiles may not be totally reliable [2]. Molecular methods like *Trichosporon asahii* specific Polymerase Chain Reaction (PCR) could identify only *T. asahii* isolates. Hence, sequencing of DNA fragments appeared to be a better approach for identification of *Trichosporon* species other than *T. asahii*. However, Internal Transcribed Spacer (ITS) sequencing was unable to differentiate several closely related species of the genus *Trichosporon* and Intergenic Spacer (IGS1) sequences unambiguously identified all *Trichosporon* isolates [7]. Therefore, DNA amplification of the IGS region and nucleotide sequencing can be used in the identification of uncommon *Trichosporon* species like *T. loubieri*. To the best of our knowledge, very few cases of human trichosporonosis caused by *T. loubieri* have been reported worldwide so far. We herewith report two cases of complicated urinary tract infection due to *T. loubieri*.

2. Case

2.1. Case I

The first patient was a 65-year old male who came to the urology department with complaint of left loin pain that was radiating to the back for the past two months. The patient also had decreased urine output and increased frequency of micturition for the past two months. On examination, the left testes was hard in consistency with grade III ascites. Urine culture sent on day 0 had growth of *Escherichia coli* of >100,000 cfu/ml. The serum creatinine level was 1.4mg/dL. Ultrasonogram (USG) of the abdomen showed left Hydroureteronephrosis of around 9.8 mm calculi in the upper ureter and prostate of 32cc with no intravesicular extension. Computed tomography of kidneys, ureters and bladder (CT KUB) also revealed left pelviureteric junction calculi of 1.4cm (1230 Hu) and vesical calculi of 5mm (800 Hu) (day +1). On day +2, the patient underwent Trans Ureteral Resection of Prostate (TURP) along with Cystolitholapaxy and left Double J stenting. The patient was treated with Tablet Finasteride 5mg, Tablet Magnesium & Potassium Citrate, Tab Tramadol and Tab Cefpodoxime 200mg + Clavulanate Potassium 125mg, and was discharged (day +3). After a week (day +10), the patient came with complaints of severe left loin pain. Left percutaneous nephrolithotomy was done for calculi in left pelvi ureteric junction and was discharged with nephrostomy tube in situ (day +13). After a week (day +20), the patient came to the urology OPD for follow up with complaints of haematuria and burning micturition. Urine culture sensitivity done on day +20, showed growth of dry yeast like colonies of ≥100,000 cfu/ml

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with no bacterial growth. The culture isolate showed blastoconidia, pseudohyphae, arthroconidia on gram stain and was also urease positive, and hence was considered as belonging to the genus \textit{Trichosporon}. Identification of the culture till species level was done using IG51 PCR and sequencing. The culture isolate was finally identified as \textit{Trichosporon loubieri}. The MIC values by broth microdilution method for amphotericin B was 4 \(\mu\)g/ml, fluconazole 2 \(\mu\)g/ml, itraconazole 2 \(\mu\)g/ml, voriconazole 0.125 \(\mu\)g/ml, posaconazole 0.25 \(\mu\)g/ml, ravuconazole \(\leq 0.125 \mu\)g/ml and caspofungin 8 \(\mu\)g/ml. The patient was started on fluconazole 200mg/day for 2 weeks and the nephrostomy tube was changed (day 21). The patient got well and discharged. The nephrostomy tube was removed on day +28.

2.2. Case II

The next patient was one month old male infant who was brought to the Urology OPD with complaints of oliguria for three days. The baby was born with birth weight of 2.9 kg by Lower Segment Caesarean Section (LSCS) with mild respiratory distress, after which the baby was fine with the resuscitative measures and was discharged. The laboratory values (day 0) were: Haemoglobin-10.9 g/dl, Total leucocyte count- 11000/mm3 (Neutrophil-52%, Lymphocyte-4%, Monocyte-1%, Eosinophil-0.3%), Mean Corpuscular Volume (MCV)- 60\(\mu\)m3, Serum Creatinine- 1.3mg/dl, Serum Urea 30 mg/dl, Urine analysis: specific gravity-1.005, protein +++, Gram stain of urine: plenty of pus cells, gram negative bacilli, and few budding yeast cells. Urine culture had growth of \textit{Escherichia coli} with a colony count of > 100,000 cfu/ml. USG abdomen showed bilateral hydrenephrosis with thickened bladder wall. Voiding cystourethrogram showed posterior ureteric valves (PUV) with right sided vesicoureteric reflux grade IV (day +1). Urinary catheter was placed to improve the draining of kidney (day +1). The patient was treated with cefoporazone sulbactam 40 mg and amikacin 30mg two times a day for 5 days (day +1). The patient continued to have fever. Second urine sample was collected and sent for culture (day +5). Direct microscopic examination of urine showed plenty of pus cells, moderate budding yeast cells and few gram negative bacilli. The urine culture showed growth of dry yeast like colonies (100000 cfu/ml) with no bacterial growth. Gram stain from the culture grown on Sabouraud dextrose Agar (SDA) showed budding yeast cells, pseudohyphae and arthroconidia. The culture isolate grew after 48 hrs, and was finally identified using IG51 sequencing as \textit{Trichosporon loubieri}. Antifungal susceptibility testing by broth microdilution method showed MIC values for amphotericin B 4 \(\mu\)g/ml, fluconazole 4 \(\mu\)g/ml, itraconazole 2 \(\mu\)g/ml, voriconazole \(\leq 0.125 \mu\)g/ml, posaconazole 0.25 \(\mu\)g/ml, ravuconazole \(\leq 0.125 \mu\)g/ml and caspofungin 8 \(\mu\)g/ml. The patient was started with voriconazole 20mg two times daily for five days. He was afebrile. Endoscopic valve ablation was performed after one week and renal function normalized. The infant was discharged home in good condition.

Both the isolates produced showing dry, white colonies with irregular foldings and central umbonation on SDA (Fig. 1). Gram stain from the colonies showed blastoconidia, pseudohyphae, arthroconidia and typical fusiform giant cells (Fig. 2) characteristic of \textit{T. loubieri} as mentioned by Gueho et al.\cite{8}. The enzyme urease was produced in Chris- tensen’s urease medium, which is a characteristic feature of this genus. Hence, both the isolates were provisionally identified as belonging to the genus \textit{Trichosporon}. Conventional phenotypic methods like sugar assimilation studies misidentified both these isolates as \textit{T. asahii}. PCR was performed initially with \textit{Trichosporon} genus specific primers [9] (TRF (forward) - 5’AGAGGCTACATGTTGATCA 3’ TRR (reverse) - 5’ TAAAGACCATATGACCTTATA 3’) to double check for accurate identification of this genus. This primer pair is \textit{Trichosporon} specific and it amplified part of 18S rDNA. Both the test isolates and positive controls (\textit{T. asahii} MTCC 6179, \textit{T. asteroides} MTCC 7632, \textit{T. cutaneum} var. \textit{cutaneum} MTCC 1963) produced bands of approximately 170 bp size (Fig. 3). There was no amplification in the negative control (\textit{Candida albicans} ATCC 90028). Later, \textit{T. asahii} specific PCR was performed using \textit{T. asahii} specific primers (TASF - 5’GGATCTATGATGTTGCTTTATA3’ TASR - 5’AGGACCTTCAACAATGAC3’) [10]. Both the isolates did not produce an amplicon, revealing the phenotypic misidentification of these isolates. Hence, these isolates were taken up for further speciation by IG51 sequencing (Agrigenom, Kerala). PCR and sequencing was done using the 26F (5’ATCCTTTGTGACGACTTGA-3’) and 5SR (5’AGCTGACTCGAGATCGG-3’) primers [7]. All the PCRs were performed with reaction conditions standardized in our laboratory as shown in Table 1. Phylogenetic analysis was done by comparing the obtained nucleotide sequences with the nucleotide sequences of \textit{Trichosporon} reference strains obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank/). The reference strains used are given in Table 2. All the phylogenetic analysis was done using MEGA Software, version 7.0. All the sequences were aligned and phylogenetic tree was constructed using Maximum parsimony clustering. Its stability was assessed by parsimony bootstrapping with 1000

![Fig. 1. Colony morphology on SDA with antibiotics showing dry, white colonies with irregular foldings and central umbonation.](image)

![Fig. 2. Microscopic morphology of \textit{T. loubieri} on gram stain showing hyphae with arthroconidia and typical fusiform giant cells (100 x magnification).](image)
Both these isolates were identified according to the CLSI M27-A3 document. Antifungal susceptibility testing was also performed for these isolates by broth microdilution method. Testing yeasts that cause invasive infections [11] (CLSI Order # 56600, 'broth microdilution method according to the CLSI M27-A3 document'.

Amplicon from these isolates was grown in urine culture and the sequence was deposited in the GenBank database (Accession Nos. MG 912612 and MG 912613). An- tained sequence was 536bp for both the isolates and were deposited in the GenBank database.

The length of the obtained sequence was 536bp for both the isolates and were deposited in the GenBank database. Both the isolates had identical sequence and clustered together with high genetic relatedness (bootstrap ≥ 99). Hence, both these isolates were identified as T. loubieri. The length of the obtained sequence was 536bp for both the isolates and were deposited in the GenBank database. Both the isolates had identical sequence and clustered together with high genetic relatedness (bootstrap ≥ 99). Hence, both these isolates were identified as T. loubieri. The length of the obtained sequence was 536bp for both the isolates and were deposited in the GenBank database.

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infections, though few cases of human trichosporonosis have been reported [3–5,17,18]. Among these reported cases, three patients who had fungemia due to T. loubieri, succumbed to infection. The prognosis was good in other two patients who had left kidney infection [17] and skin and soft tissue infection without fungemia [18]. Similarly in these two cases blood cultures were negative for both the patients, they responded well to treatment and got discharged. This can also be attributed to early identification and rapid treatment of these patients which resulted in good clinical outcome.

T. loubieri which is a new and rare species causing human infection could not be identified by conventional phenotypic methods, and was misidentified as T. asahii in both cases. Yeast identification which is usually done based on physiological and biochemical tests may be laborious, leading to inconclusive presumptive identifications, mainly for uncommon or emerging fungi [19]. Whereas, molecular techniques for identification of yeasts are fully discriminative even for closely related species with high sensitivity and specificity [20]. Recently, sequence analysis of the rRNA IGS1 region, which is a more powerful tool for distinguishing between phylogenetically closely related species, is used for identification of Trichosporon spp. [7]. T. loubieri isolates in these two cases were unambiguously identified by IGS1 sequencing.

Both of the T. loubieri isolates had high MICs to amphotericin B (4 & 4 μg/ml) and caspofungin (8 μg/ml). Trichosporon infections when treated with these antifungal agents, may result in poor clinical outcome because of their high MICs. Hence, correct identification of the Trichosporon species using molecular methods with their antifungal susceptibility pattern, helps the clinician in providing appropriate treatment.

In conclusion, our cases highlight the need of molecular sequencing studies to identify Trichosporon to the species level. It also throws light on the fact that trichosporonosis can also be caused by rarer and emerging Trichosporon sp. like T. loubieri. These infections, if not identified accurately and treated promptly, may lead to dissemination with high mortality rates. Hence physicians and the laboratory personnel must be aware of the potential role of this species in causing human infections.

Conflict of interest

‘There are none.’

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