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Cryptococcus Laurentii – A Human Pathogen, Risk Factors, Clinical Manifestations, Treatment and Their Antifungal Susceptibility

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Abstract **Original Research Article**

A retrospective study, carried out on 29 patients with *Cryptococcus laurentii* infection admitted to a tertiary care hospital, during a study period of one year. The demographic profile, clinical presentation, risk factors/predisposing underlying condition responsible for development of the disease and final outcome of these patients were analyzed. In addition MIC of the antifungal drugs against *Cryptococcus laurentii* isolates was analyzed. It was observed that *C.laurentii* can involve diverse organ system with varied clinical presentation. Disease can range from a mild localized skin infection to a fulminant septicemia or peritonitis. Debilitated individuals or persons with impaired immunity or with invasive devices are more prone to infection with *C laurentii*. Antifungals, specifically fluconazole and Amphotericin B remains the mainstay of the treatment, however antifungal resistance should be evaluated for these isolates to avoid the treatment failure.

Keywords: Cryptococcus laurentii, immune-compromised, risk factors, clinical presentation. Treatment, antifungals, MIC.

INTRODUCTION

The Cryptococcus genus consists of >70 species of true encapsulated yeast [1]. *Cryptococcus neoformans*, remains a common cause of meningitis in immuno-compromised individuals and is rather considered as one of the AIDs-defining illness [2, 3]. Non-neoformans Cryptococcus species conventionally were considered non-pathogenic for the human beings. However, recently *Cryptococcus laurentii*, has been incriminated in different clinical manifestations involving skin, lungs, pleura, bloodstream, Gastro-intestinal tract and central nervous system (CNS), as an opportunistic pathogen. It is probably due to growing number of patients with immune-compromised conditions [4-6]. The major risk factors for *Cryptococcus laurentii* infection includes malignancy, neutropenia, lympho-proliferative disorders, prolonged use of immunosuppressant, invasive catheter devices and organ transplantation or any other immuno-compromised condition including AIDS[3, 6].

With the increased knowledge towards understanding the pathogen in terms of its pathogenesis and clinical presentation, will help in early recognition and identification of the organism, along with its antifungal susceptibility, which will further, help in reducing the treatment failure, improved patient outcome and reduced hospital stay.

Therefore, a retrospective analysis of all the patients with *Crytococcus laurentii* infection were carried out including their demographic profile, clinical presentation, risk factor involved and ultimate outcome of the patient. Further all the isolates of C laurentii were characterized in terms of their antifungal susceptibility with MIC.

METHODS

A retrospective study was conducted over a period of 1 year, including all the patients from whom *Crytococcus laurentti* was isolated, from any of the clinical sample. All the blood and body fluid (Bile, pleural fluid, peritoneal fluid etc) samples received in the Department of Microbiology, from these patients were processed in automated BACTEC or BacT/ALERT system. Smears were prepared from the positive culture bottles and Gram staining of the smears was done. Simultaneously all the positive bottles were sub-cultured on blood agar and MacConkey agar plates. In addition subculture was made on Sabouraud dextrose

agar (SDA) slants if yeast is suspected. The plates were incubated at 37°C for 18-24 hours and Sabouraud dextrose agar (SDA) slants were incubated both at 37°C and 25°C, in BOD incubator for 4 weeks. The cultures were examined at regular intervals for any growth. The growth of *Cryptococcus laureate* was identified on the basis of colony morphology, microscopy and on VItek - 2 system using YST and antifungal susceptibility was done by using AST YS08 cards. The results of antifungal susceptibility were interpreted using the breakpoints mentioned in Table 1, since breakpoints for *C laurentii* have not been evaluated yet.

The other samples including urine, drained pus, endotracheal secretions etc were directly inoculated on SDA slants and were incubated at both 37°C and 25°C in BOD incubator for 4 weeks and if the growth of yeast is obtained, it was identified and characterized as mentioned above.

Table-1: The Minimum inhibitory Concentration- µg/ml, breakpoints used for *C. laurentii*

	Minimum inhibitory Concentration (MIC)/µg/ml			Defined by
Antifungal	**Sensitive**	**Intermediate**	**Resistant**	
Amphotericin B	≤1	2	≥4	EUCAST (For Cryptococcacae)
Fluconazole	≤4	8	≥16	EUCAST(For Cryptococcacae)
Micafungin	≤2	-	-	MANUFACTURER (For Cryptococcacae)
Caspofungin	≤0.25	0.5	≥1	CLSI (for C.albicans)
Voriconazole	≤0.12	0.25-0.5	≥1	CLSI (for C.albicans)

Patient's demography, clinical signs and symptoms at the time of presentation, underlying disease and outcome were analyzed.

RESULTS

A total of 31 isolates of *Cryptococcus laurentii* were obtained from 29 patients. Duplicate isolates were not included in the analysis of data. Table 2 depicts the demographic profile, underlying disease/risk factors, clinical manifestations, treatment and outcome of the patients with *C.laurentii* infections. The average age of the patients was found to be 46.4years ±18.4 and male outnumber the female patients. It was observed that the *Cryptococcus laurentii* has been isolated from different clinical samples obtained from different organ system. The blood stream and urinary tract infections were the most common infection, followed by respiratory tract infection and peritonitis.

Most common risk factor or predisposing condition for the development of C. laurentii infection was found to be abdominal surgeries like repair of oesophageal perforation, laproscopic cholesytectomy, endoscopic retrograde cholangiopancreatography(ERCP) for cholelithiasis, exlaprotomy, gastrojejunostomy. These patients presented with blood stream infection or peritonitis. In one patient bile was found to be culture positive for C. laurentii. Urinary tract infections were more commonly observed either with diabetic, neutropenic or with catheterized patients. The patients with malignancies, neutropenia or on immunosuppresents primarily presented with blood stream or respiratory infections.

Antifungal susceptibility pattern of all the 29 isolates were determined on VItek-2 system using AST YS08 cards. The data was analyzed using the breakpoints as mentioned in Table 2 and has been depicted in Table 3. For voriconazole and caspofungin the breakpoints have not been defined for family cryptococcaceae, hence breakpoints for *C albicans* were used to analyse the susceptibility pattern. Based upon the antifungal susceptibility pattern Amphotericin B was found to be most effective (86%) drug against *Cryptococcus laurentii* followed by azoles (69%), both voriconazole and fluconazole. However *C laurentii* has been found extensively resistant to antifungals with very high MICs as shown in Table 3. 9/29 (31%) of the *C. laurentii* isolates showed resistance to fluconazole with MIC ranging between 16 to ≥64µg/ml with 4 isolates showing an MIC of ≥64µg/ml. However 14% of the isolates were found resistant to resistant to Amphotericin B with MIC ranging from 4 to ≥16µg/ml. Only 1 isolate showed an MIC of ≥16µg/ml against Amphotericin B.

Only 21 out of the total 29 patients were put on antifungals, either azoles or amphotericin B Mortality was observed in 10.4% of the patients and another 10.4% of the patients went DAMA and could not be followed up.

DISCUSSION

Cryptococcus laurentii has been isolated from the environment in varying habitats including soil, vegetable peels, and wild pigeon droppings and as a contaminant during the fermentation processes [2, 5]. Until recently, the organism was considered a rare pathogen for humans. However quite a few number of clinical cases with varied clinical manifestation has been reported in the literature during the past few years specifically in immune-compromised patients. The clinical manifestations varied from mild localized skin infections to fulminant fungemia. Other clinical manifestation includes peritonitis, respiratory tract infections and urinary tract infections [5-9]. In the present study the organism has been isolated from various clinical samples including urine, endotracheal secretions, pleural fluid peritoneal fluid, pus, bile and blood, indicating involvement of the organism in

diverse clinical manifestation. The data is corroborated by previous studies, indicating the isolation of organism from various sites of infection including skin, respiratory tract, peritoneal fluid, CSF or blood [5, 7-9].

However, in the present study it was observed that all the patients had one or more underlying conditions or risk factors, which predisposed the individual to *Cryptococcus laurentii* infection. Most of the patients presenting with peritonitis or blood stream infection had undergone abdominal surgeries or had central /peripheral venous catheters in place. Risk factors identified. In the patient presenting with urinary tract infections prolonged catheterization, diabetes mellitus and neutropenia. Predisposing conditions leading to respiratory tract infections included prolonged use of steroid, diffused cerebral atrophy/patient on ventilator. In the present study, keeping in view the varied clinical presentations and probable risk factors, the importance of hospital environment cannot be ruled out.

Various therapeutic options are available for the treatment of *Cryptococcus laurentii* infection including Amphotericin B, Fluconazole or flucytosine. There have been many reports available in the literature, of successful treatment of *Cryptococcus laurentii* fungemia, respiratory infections and other clinical syndromes with Ampotericin B, fluconazole and a combination of Amp B and flucytosine [9-11]. In the present study also 21/29 patients were put on antifungals and most of these patients responded well to the treatment. The treatment failure in the remaining patients may be attributed to the antifungal.

In the present study, *C laurentii* has been found extensively resistant to antifungals with very high MICs. 31% of the *C laurentii* isolates showed resistance to fluconazole with MIC ranging between 16 to ≥64µg/ml. Out of these, 4 isolates showed an MIC of ≥64µg/ml. However 14% of the isolates were found resistant to Amphotericin B with MIC ranging from 4 to ≥16µg/ml. Only 1 isolate showed an MIC of ≥16µg/ml against Amphotericin B. Contrary to these, Ryder *et al.* [12] reported MIC of fluconazole against 7 isolates of *C. laurentii* varied between 1-4µg/ml. However Averbuch *et al.* [5] has reported an MIC of 50µg/ml for fluconazole against a *Cryptococcus laurentii* isolate from a patient of fungemia. Further they have also reported three blood culture isolates with an MIC ranging between 0.037 -0.25 µg/ml for Amphotericin B. However the breakpoints for *Cryptococcus laurentii* are not available, hence the susceptibility pattern of these isolates was analyzed using the breakpoints as mentioned in methodology. Using the above mentioned breakpoints it has been observed that clinically relevant isolates of *C.laurentii* shows varied susceptibility to all the antifungal tested.

To conclude, C. laurentii, which was considered a saprophyte until recently, may get involved significantly in diverse mild to life threatening clinical manifestations, specifically in immune-compromised or debilitated patients. It can also be an important hospital acquired pathogen specifically in urinary catheterized patients or in patients with central venous catheters, causing urinary tract infections and blood stream infections respectively. However, antifungal susceptibility testing needs special emphasis and there is a need to define the specific breakpoints for *Cryptococcus laurentii*.

Table-2: Patient Characteristics, underlying disease/risk factors, clinical manifestations, treatment and outcome of the patients with *Cryptococcus laurentii* infections

Characteristic	No of patients N=29
mean age in years+SD --- 46.4years ±18.4	
Sex (M/F)	16/13
Underlying diseases/ Risk factor	4 (14%)
• Diabetes mellitus	8 (27.4)
• Abdominal surgery	5 (17%)
• Prolonged steroid usage	2 (7%)
• Hemiplegia	2 (7%)
• Malignancy	2 (7%)
• Neutropenia	6 (20.7%)
• Urinary catheter	4 (14%)
• Hypertension	2 (7%)
• Diffuse cerebral atrophy	2 (7%)
• Intravenous devices	
Clinical manifestation	
• Blood stream infection/septic shock	13 (44.8%)
• Respiratory infection	4 (14%)
• Biliary tract infection	1 (3.5%)
• Urinary tract infection	6 (20.7%)
• Skin infection	2 (7%)
• Peritonitis	3 (10.3%)
Antifungal Treatment	21 (72.4)
Outcome	
Death	3 (10.3%)
DAMA	3 (10.3%)
Discharged in stable condition	23 (79.4%)

Table-3: Antifungal susceptibility pattern of the *C.laurentti* isolates (n=29), based upon the MIC breakpoints given in Table 1

Fluconazole			Voriconazole			Amphotericin B			Caspofungin			Micafungin		
	MIC	No of isolates		MIC	No of isolates		MIC	No of isolates		MIC	No of isolates		MIC	No of isolates
Sensitive 20/29 (69%)	≤0.125	2	**Sensitive 20/29 (69%)**	≤0.125	20	**Sensitive 25/29 (86%)**	≤0.25	6	**Sensitive 17/29 (59%)**	≤0.25	17	**Sensitive 17/29 (59%)**	≤0.65	17
	1	16	**Interme – diate 4/29 (14%)**	0.25	4		0.5	13	**Interme-diate 4/29 (14%)**	0.5	4	**Resistant 12/29 (41%)**	1	4
	2	2	**Resistant 3/29 (10.9%)**	1	3		1	6	**Resistant 8/29 (27%)**	≥4	8		≥4	8
Resistant 9/29 (31%)	16	2		2	2	**Resistant 4/29 (14%)**	4	3						
	32	3		≥4			≥16	1						
	≥64	4												

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