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CANDIDA HAEMULONII IN BLOOD - A NEW CHALLENGE

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ABSTRACT

Currently, a series of cases with invasive *Candida haemulonii* is occurring and this is an emerging yeast pathogen for which the optimal strategy of patient management has yet to be elucidated. In this study, blood culture was done from clinical sepsis cases by BACTEC (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md) method. Yeast isolates were identified by Gram staining, germ tube test and colony morphology. Antifungal susceptibility was determined by minimum inhibitory concentration (MIC) value. DNA sequencing of the strains were also performed. Candida haemulonii was isolated in 8 cases from November 2014 to August 2015. All isolates showed high level resistance to Amphotericin B, variable resistance to Flucytosine and Fluconazole, susceptibility to Echinocandins and Voriconazole. Majority of the cases had central venous catheter, prolonged broad-spectrum antimicrobial use and two also had parenteral nutrition. While, 2 cases were successfully treated with Caspofungin and Voriconazole, 4 patients could not survive but showed some improvement both by clinical and sepsis marker study (CRP, procalcitonin) with initiation of antifungal treatment. DNA sequencing showed that the isolates were *Candida auris.* Thus, clinically the impact of this species has been attained to some extent in this study which is immensely vital considering the very high mortality rate.

Keywords: Candida haemulonii, yeast, MIC, antifungal treatment

INTRODUCTION

Candida haemulonii (syn. *Torulopsis haemulonii*) is a rare yeast species isolated from human clinical sources. Originally it was isolated from the gut of a blue-striped grunt fish (Haemulon scirus) in 1962 (van Uden *et al.,*1962). Lavarde (1984). first reported isolation of this yeast from the blood of a patient with renal failure (1984). Since then, several cases have been described in the literature, including catheter-related fungemia (Kim *et al.,* 2011), bloodstream infections (Rodero *et al.,* 2002; Ruan *et al.,* 2010), and osteitis (Crouzet *et al.,* 2011) and outbreaks in intensive care units (Khan *et al.,* 2007). A striking antifungal susceptibility pattern has been observed, which shows high MICs of amphotericin B (AMB) and fluconazole (FLC) (ranges, 0.5 to 32 and 4 to 64 mg/litre, respectively), which has often been associated with clinical failure (Crouzet *et al.,* 2011; Khan *et al.,* 2007; Kim *et al.,* 2009; Rodero *et al.,* 2002, Ruan *et al.,* 2010).

Lehman *et al.*, (1993) studied 25 strains of *Candida haemulonii* complex from different geographic origins and clinical sources and described two genetically distinct *C. haemulonii* groups, I and II which were renamed as *C. haemulonii* and *C. duobushaemulonii* respectively.

In recent years, two species related to *C. haemulonii* have been described, namely, *Candida pseudohaemulonii* and *Candida auris*, which are phylogenetically closely related to *C. haemulonii* in the *Metschnikowiaceae* clade (Kurtzman, Fell & Boekhout, 2011). Sugita *et al.,* (2006) described *C. pseudohaemulonii*, which was isolated from the blood of a Thai patient. This species is as resistant to AMB and azole agents as are the two genetic groups of *C. haemulonii* (Sugita *et al.,* 2006). The second related species, *C. auris*, was described in 2009 by Satoh *et al.*, and isolated from the external ear canal of an inpatient in a Japanese hospital.

Recently, *C. haemulonii* and closely related species have caused outbreaks in South Korea and Kuwait (Khan *et al.,* 2007; Kim *et al.,* 2009). The reasons for their emergence are not clear, but they may be related to selective pressure as a result of the commonly applied FLC or AMB therapy. *C. haemulonii* and *C. pseudohaemulonii* were isolated from patients with central venous catheter-related fungemia, whereas the *C. auris* strains were isolated from the ear canals of inpatients (Oh *et al.,* 2011). Recently researchers discovered that the *Candida haemulonii* isolates been identified in Vitek2 system were *Candida auris* when compared with DNA sequencing data (Kathuria *et al.,* 2015).

Table1: 25 strains of Candida haemulonii complex (Lehman et al. 1993)

	C. haemulonii	*C. Duobushae-mulonii*	*C. haemulonii var vulnera*	*C. auris*	*C. Pseudohaemulonii*
L-Sorbose assimilation	-	+	-	-	+
Arbutin assimilation	-	+	-	ND	ND
L-Arabinose assimilation	-	w	-	-	V
L-Rhamnose assimilation	+	w	+	-	+
Melezitose assimilation	+	+	d	+	+
D-Gluconate assimilation	+	+	+	-	+
Xylitiol assimilation	w/d	+	+	-	+
Glycerol assimilation	+	+	+	-	+
Growth at 37 deg C	-	+	+	+	+
Growth at 40 deg C	-	-	-	+	-
Growth in 60% glucose	-	+	-	-	ND

Till date few cases with high mortality have been reported in India from Chennai in patients requiring long term hospitalization. This is the first report from a neurological and neurosurgical tertiary care centre in India.

MATERIALS AND METHOD

Blood culture was done from clinical sepsis cases by BACTEC method. Yeast isolates were identified by Gram staining, germ tube test, colony morphology on Candida chromagar plate, growth at 37 and 40 deg C, growth in 60% glucose solution and by Vitek YST system. Antifungal susceptibility was determined MIC value by Vitek2 YST method. Clinical, laboratory and microbiological correlation was made. Strains were sent to NCPF, Chandigarh for DNA sequencing.

RESULT

Candida haemulonii was isolated in 8 cases from November 2014 to August 2015 (Table 2). All isolates showed high level resistance to Amphotericin B (MIC 8 mcg/ml) and variable resistance to Flucytosine and Fluconazole. All isolates were susceptible to Echinocandins and Voriconazole (Table 4 and Figure 1). No other pathogen could be demonstrated except in 2 cases. Majority of the cases had central venous catheter, prolonged broad-spectrum antimicrobial use and 2 also had parenteral nutrition (Table 2). 2 cases were successfully treated with Caspofungin and Voriconazole but 4 patients could not survive. However the deceased patients did show some improvement both by clinical and sepsis marker study (CRP, procalcitonin) with initiation of antifungal treatment (Table 2). The isolates were identified as *Candida auris* by DNA sequencing at NCPF, Chandigarh.

Table 2: Distribution of the cases

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Gender/Age(yr)	M/74	M/65	F/53	M/52	F/58	M/70	M/72	M/12
Antibacterial drug use	Piperacillin-tazobactum/ Ceftriaxone/ Meropenem/ Tigecycline/ Colistin	-	Piperacillin-tazobactum	Meropenem/ Vancomycin	Linezolid/ Acyclovir/ Meropenem/ Amikacin	Piperacillin-tazobactum/ Levofloxacin/ Meropenem/Tigecycline/ Linezolid	Amoxy-Clav/ Meropenem/ Linezolid/ Piperacillin-Tazobactum	Ceftriaxone/ Meropenem

TLC (Neutrophil %)	9500(84) 17800(92) 18900(94) 23000(90) 34500(94)	22800 8000(88) 4500	23200(79)	15700(93) 13600 12100 11500	11700 14600 14800(89) 22200(93)	10000(80) 9400(78) 11400(90)	7500 8600 10100 10300(85)	5200 10600 14800(80) 17200(88)
CRP/Procalcitonin	72-234-298/7.5	163/1.59	230/18.1	-	155-86.3/31.5	109.7	-/<0.25	65-10/55.2
Fever	+	-	+	-	+	-	+	+
Nutritional status	Poor	Poor	Poor	Very poor	Very poor	Very poor	Poor	Very poor
Parenteral nutrition	-	-	- (Enteral Albumin)	- (Enteral Albumin)	+	- (Enteral Albumin)	-	+
Other infection	-	-	Prev. Blood - *A.baumannii*	-	-	-	ET suction - *A.baumannii*	Urine-*E. coli*
Antifungal use	Fluconazole	AmphoB Anidulafungin Voriconazole	-	Oral Voriconazole	Fluconazole 20 days Inj Caspofungin	-	-	Caspofungin
Outcome	Death Stay 1 month After diag stay 15 days	Death Initial improvement with Anidulafungin Stay 12 days After diag stay 10 days	Death Stay 2 days	Cured Stay 1 month After diag stay 23 days	Transferred out Stay 1 month 10 days After diag stay 30 days	Death Stay 1 month After diag stay 5 days	Transferred out Stay 1 month After diag stay 3 days	Cured Stay >3.5 months
Co-morbidity	Diabetes, RTA	Renal Failure/ Orbital apex syndrome on AmphoB	Haemorrhagic CVA	Haemorrhagic CVA	Diabetes/ Hypothyroid/ ARF/ Vasculitis	Cervical vertebral fracture	Malaena/Hypothyroid/ Heaptic encephalopathy/ AF	Brain tumor surgery
Central line days	13	60	30	-	17	-	8	-

Table 3: Biochemical characteristics of the isolates

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**	**Case 8**
L-Sorbose assimilation	-	+	-	+	-	-	-	-
Arbutin assimilation	-	+	-	+	-	-	-	-
L-Arabinose assimilation	-	+	+	+	+	+	+	+
L-Rhamnose assimilation	-	+	-	+	-	-	-	-
Melezitose assimilation	+	+	+	+	+	+	+	+
D-Gluconate assimilation	+	+	+	+	+	+	+	+
Xylitiol assimilation	+	+	-	+	+	+	+	-
Glycerol assimilation	+	+	-	+	+	+	+	-
Growth at 37 deg C	+	+	+	+	+	+	+	+
Growth at 40 deg C	-	-	-	-	-	-	-	-
Growth in 60% glucose	-	+	+	+	+	+	+	+
Speciation	***C.vulnera***	***C.duobu shaemuloni***	***C.duobu shaemuloni***	**C.duobu *shaemuloni***	***C.duobu shaemuloni***	***C.duobu shaemuloni***	***C.duobu shaemuloni***	***C.duobu shaemuloni***

Table 4: Antifungal MIC (mcg/ml) distribution among the isolates

case no.	Flucytosine	Fluconazole	Voriconazole	Amphotericin B	Caspofungin	Micafungin
1	<=1	**8**	<=0.12	**8**	<=0.25	0.06
2	8	**32**	0.25	**8**	0.5	0.12
3	8	**32**	0.25	**8**	<=0.25	0.12
4	8	**>=64**	0.25	**8**	0.5	0.12
5	<=1	**16**	<=0.12	**8**	0.5	0.12
6	32	2	<=0.12	**8**	<=0.25	0.12
7	<=1	**16**	<=0.12	**4**	<=0.25	<=0.06
8	4	**>=64**	0.25	**8**	<=0.25	<=0.06

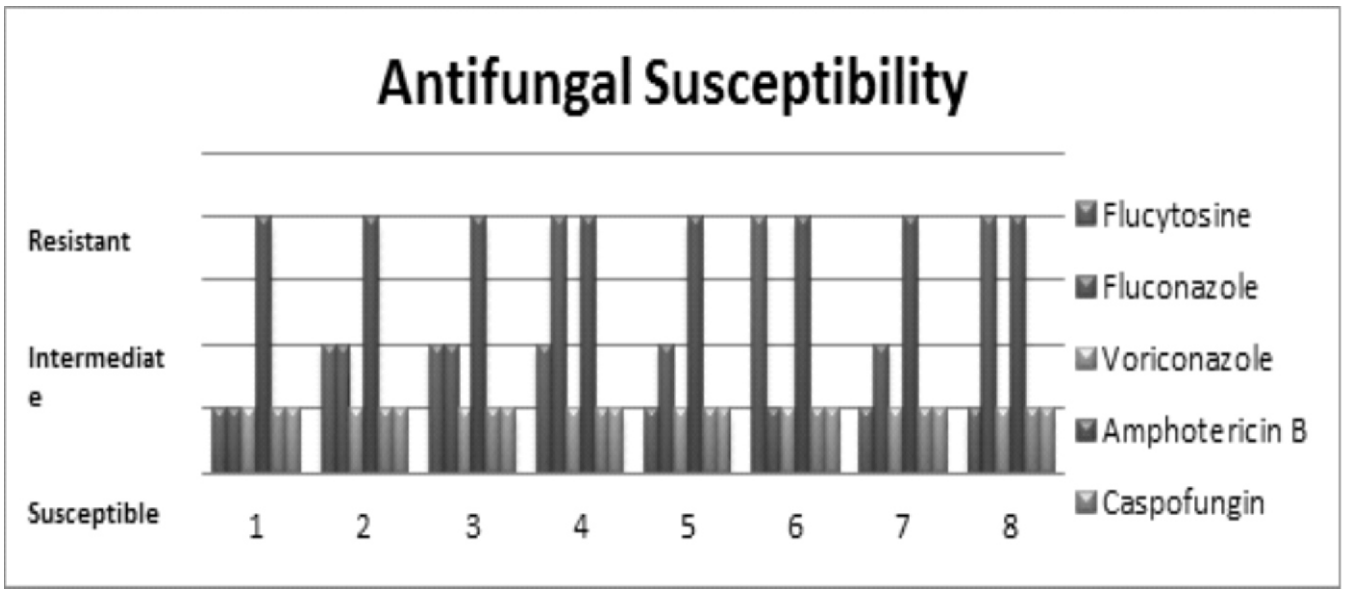

Figure 1: Antifungal susceptibility profile of the isolates

DISCUSSION

Considering the recent case series of invasive *C. haemulonii* infections, *C. haemulonii* is considered to be an emerging yeast pathogen for which the optimal strategy of patient management has yet to be elucidated. Antifungal resistance is the focus of attention in the management of invasive candidiasis. In previous cases, most *C. haemulonii* were resistant to both fluconazole and amphotericin B (Khan *et al.,* 2007; Rodero *et al.,* 2002). Clinically, when amphotericin B was administered empirically, it failed to eradicate *C. haemulonii* candidemia (Khan *et al.,* 2007). The majority of cases in which fluconazole was administered empirically also failed to eradicate candidemia (Khan *et al.,* 2007; Ruan *et al.,* 2010). Although fluconazole administration has been shown to resolve candidemia, these cases could be considered transient candidemia combined with the removal of the intravenous catheter. Therefore, the resistance of *C. haemulonii* poses a therapeutic challenge for the treatment of invasive candidiasis.

In our cases 2 cases were POA (point of admission) infection cases that had undergone hospitalization along with invasive device placement such as central venous catheter etc. Case 2 was already getting Amphotericin B as treatment for suspected fungal orbital apex syndrome to which *C. haemulonii* is resistant. Switching over to Anidulafungin, a Caspofungin antifungal, showed clinical improvement; however patient did not survive. Similarly case 5 did not respond with Fluconazole, and

situation worsened. These correlate well with the antifungal susceptibility pattern obtained *in vitro*. Successful treatment with Caspofungin in case 8 and with Voriconazole in case 4 supports this fact.

We also tried to speciate the isolates by using retrieved Vitek results. Majority of the isolates belonged to *C. haemulonii* biogroup 2 or *C. duobushaemulonii*. Only one isolate gave biochemical picture like that of *C. haemulonii var vulnera*.

In our cases poor nutritional status along with broad spectrum antibacterial use and invasive devices &/or parenteral nutrition could have given opportunity to *C. haemulonii* to invade bloodstream. In few cases we were able to isolate *C. haemulonii* from the central venous catheter tip which strongly points towards Central line associated bloodstream infection (CLABSI). In other cases clinical improvement did occur along with fall in sepsis markers with appropriate antifungal treatment. Only in one case that had melena, it could have been a contamination or asymptomatic transient candidaemia resulting from thinned out deranged intestinal wall. Like previous reports our patients had high mortality rate also.

However, identification of the *Candida auris* seems difficult for Vitek2 automation (Kathuria *et al.,* 2015). DNA sequencing of the isolates confirmed their actual identity as *Candida auris*. Seen from a realistic clinical point of view this finding could be of research interest but practical implication and utility remain same as the antifungal susceptibility matters. Early and prompt diagnosis facilitates clinical satisfaction.

CONCLUSION

To date, very little is known regarding the clinical characteristics and antifungal susceptibility profiles of clinical isolates of *Candida* species closely related to *C. haemulonii*. We had an opportunity to face with them and have presented our experience data. It was felt that proper mycological diagnostics is the answer to battle against the emerging fungal pathogens. In conclusion, as found in the previous case reports, fluconazole and amphotericin B are not reliable empirical antifungal agents for the treatment of *C. haemulonii* candidemia. Echinocandins may be an appropriate empirical choice of antifungal agent for invasive *C. haemulonii* infections. Whether they are taxonomically or by genetic analysis belong to *Candida haemulonii* or *Candida auris*, remains question to researchers but clinically the impact is much more important considering the very high mortality rate. Probably it is going to be a "big shot" in the spectrum of the healthcare associated infections.

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