6.0 DISCUSSION

One of the major environmental agents of human mycotic disease is the dimorphic fungus, *Cryptococcus neoformans*. These fungi are virulent in diverse animal species without requiring them for replication and survival (Casadevall et al., 2003). These authors have presented a list of virulence factors in the yeast that function both in pathogenesis and in the environment thus arguing the “dual” use for these factors.

Opportunistic infections caused by this fungus in immunocompromised patients such as AIDS are life threatening complications. Cryptococcosis is one of the most commonly recognized opportunistic infection in such patients. In recent years a sharp rise in the incidence of Cryptococcosis has been observed and this has paralleled with the sharp increase in the incidence of AIDS all over the world (Levit, 1991). Zuger et al (1986) reported Cryptococcosis in 90% of the patients with the AIDS defining opportunistic infection in HIV +ve patients. The duration of survival and quality of life of these patients depended to a large extent on effective diagnosis, treatment and prevention of such infections.

Clinical Observations: - Samples of CSF were considered primary source and samples from extra neural sites from some of these patients were considered as secondary. Culture of the fungus from these samples was used as the gold standard to confirm the infection.

The prevalence of Cryptococcosis in patient with AIDS varied according to geographical regions. The prevalence of Cryptococcosis in HIV infected patients is 3 to 6 % in Europe, 6 – 10% in the U.S.A. and 10 to 30% in some tropical
countries particularly in central black Africa, predominant with meningeal infections (Zuger et al., 1986; Holmberg and Meyer, 1986; Dismukes, 1988)

A number of reports on human Cryptococcosis has been reported from India (Balakrishna Rao et al., 1952; Sinha and Barua, 1960; Aikal et al., 1967; Basu Mallik et al., 1967; Talular and Meera, 1986, Aher et al., 1996). Reports from this part of the country have been increasingly recorded (Subramanian et al., 1985, Sharma et al., 1985; Rajkumar et al., 1992, Asha et al., 1995) and rarely from AIDS patient (Kumarasamy et al., 1995). Banerjee (2005) reported that HIV co-infection with Cryptococcus, has also increased from 20 % in 1992-1996 to 37 % in 1996-2000 to 49 % in 2000-2004.

In the present study, we report (Table 6) low incidence (8%) of pulmonary Cryptococcosis, while meningeal Cryptococcosis was detected in 61/143 (43%) samples. Of these 45/64 were from HIV patients (70%). Other disposing factors for Cryptococcosis were recorded in 30% of the samples (19/64; Table 7 & 8).

Analysis of clinical data revealed that, the HIV infection (61%) is the major form of predisposing condition for Cryptococcosis (Table 7). Before the AIDS era, Cryptococcosis taking a disseminated course with CNS involvement was very rare. It occurred chiefly against a background of primary condition such as Hodgkin’s disease Sarcoïdosis, tuberculosis, Systemic corticosteroid therapy and organ transplants. Since 1989 AIDS has become the most frequent predisposing condition for Cryptococcosis (Kovacs et al., 1985; Zuger et al., 1986; Staib, 1986; Dismukes, 1988; Chuck and Sande, 1989). Chakrabarti et al. (1995) however reported that renal transplantation and steroid therapy were the major predisposing conditions other than HIV infection.
In the present study from southern region of India the prevalence of Cryptococcal meningitis was found to be 45/143 (31.4 %). Another study from Vellore (South India) by Koshi et al. (1989) showed prevalence of 9.6% in patients without AIDS, but with liver cirrhosis. Kumarasamy et al. (1995) studied the spectrum of opportunistic infections in 100 AIDS patients and one patient (1%) was found to be having cryptococcal meningitis.

Fever and headache, the most common symptoms were reported by 80-90 % of patients. Other non specific symptoms including nausea, vomiting and malaise was observed in less than 50% of patient. Alteration of mental status in 20% and photophobia in 19 – 28% was common (Dismukes, 1988). Some times, headache (Kovacs et al., 1985, Mitchell et al., 1995) or fever, (Rozenbaum and Goncalves, 1994) were the only clinical symptom observed. Neck stiffness more frequently occurred in the non-immunocompromised patients (75%) than patients with AIDS (32.8%) or other predisposing factors (47.4%) as reported by (Rozenbaum and Goncalves, 1994). In the present study the major symptoms observed were head ache (92.1%), fever (73.4%), malaise (50%) and stiff neck (34.3%). Thus the findings in the present study, agreed with the observation made by earlier investigators.

**Laboratory findings**

Cryptococcosis in AIDS patient is usually associated with profound immunodeficiency and with the CD4 count almost invariably less than 100 cells/mm³ (Crowe et al., 1991). The analysis of immune function marker such as CD4 cell counts in the present study was 109±84.3 cells/ mm³ for 64 patients. Review of retrospective study by Brandt et al. (1996) indicated a range 3-155 with the mean of 25 cells/ mm³ of CD4 cell counts were associated with cryptococcal meningitis. For example in the series of 18 patients with AIDS and cryptococcal
meningitis, reported by Kovacs et al. (1985), the leucocytes count was <5cells/mm³ in 11 of 17 patients tested, the protein level was < 45mg/dl in 5 of 16, the glucose level was > 40 mg/dl in 11 of 16, and yet in these patients, the CSF indices were absolutely normal. In another series, of 22 patient reported by Zuger et al. (1986), the leukocyte count was <20/mm³ in 15 of 22, protein <40mg/dl in 14 of 22, and glucose >50mg/dl in 14 of 22 patients. Thus, the clinician should not be misled by normal or only slightly abnormal CSF indices in AIDS patients with Cryptococciosis. Diagnostically reliable specific tests such as culture, detection of capsular antigen of Cryptococcus neoformans etc. must be resorted to.

**Microbiology and Culture for diagnosis**

Early diagnosis and treatment of less life threatening forms of the disease such as pulmonary disease could help the patient less the impact of the disease for any given individual. Pulmonary Cryptococcosis has been reported for only a small number of patients with AIDS, despite the fact that lungs are probably the major route of entry of the organism (Wasser and Talavera, 1987). This low frequency may be artifactual in that conventional fungal sputum cultures from these patients frequently yield Candida albicans, because of oral contamination of the specimens with Candida albicans colonization and disease (thrush) are extremely common in AIDS patients (Piot and Colebunders, 1987).

Moreover, the colony morphologies of *C. albicans* and *C. neoformans* may appear identical on conventional primary isolation media in the first day or two of growth. *C. albicans* may occur in great numbers on the plate, thereby obscuring the presence of *C. neoformans* colonies. The present study also showed that, the direct plating on to selective media may increase the sensitivity supporting the earlier study by Denning et al., (1990). This study also demonstrated the
specificity of Staib’s agar over another selective medium sunflower seed agar (Table 11).

Of 3 media NSDA, SSA and SBA, SBA could recover isolates from maximum number of clinical samples in this study (tables 11). So if culture on SAB were considered as Gold Standard, then % recovery of isolates in the different clinical samples primary and secondary was 8% in pulmonary samples, 43% in CSF, 32% in blood as seen from (table 11). Among the various presumptive microbiological tests on these samples culture on SAB was taken as 100% (sensitivity; Table14).

The width or volume of the capsule varies with different strains and can be controlled in culture using simple methods as described by Zarogoza et al. (2004). They showed that culture of the yeast in mammalian serum or 1/10th diluted SD broth in MOPs buffer at pH 7.3 efficiently induced capsule growth. High CO₂ levels or low iron was also shown to increase capsule size. While these conditions can be induced during culture, direct microscopy by negative staining on the clinical samples was done here. Sensitivity of results for staining (negative) varied between 66- 33% for different samples (Table 14).

The occurrence of C. neoformans in the environment

Emmons (1951 and 1955) found fecal matters of the pigeon colonized by C. neoformans. The association of C. neoformans with bird’s excreta was explained as the utilization of substances such as purines, urea and creatinine in highly concentrated form by C. neoformans due to its osmophillic capacity. It was further more stated that, creatinine was exclusively utilized by C. neoformans in contrast to the other species of the genus Cryptococcus (Staib, 1963). Many studies have shown that excreta of pigeon and less frequently that of other birds
serves as an important natural reservoir of *C. neoformans* especially the serovars B/C. The recovery of *C. neoformans* from pigeon excreta from other parts of India is also well documented (Gugnuni *et al.*, 1973; Pal, *et al.*, 1979; Pal; 1990).

Isolates of *C. neoformans* var *neoformans* are most readily isolated from droppings of pigeons and other avian species. But the ecological niche of var *gattii* was an enigma until 1990, when Ellis and Pfeiffer isolated the fungus from *E. camaldulensis* in Australia and var *gattii* was never isolated from birds droppings. In India, although several investigators isolated *C. neoformans* from environmental sources but seldom has the isolates been characterized. In the present study, also all the isolates from pigeon droppings were identified as var *gattii*. Bennett *et al.* (1977) reported the prevalence of serotypes among the isolates from environmental sources from USA and the majority of the isolates (84.6%) were serotypes A, 14.5% serotype D and 0.8% were of untypable strains. The study on the environmental isolates from North Tamilnadu revealed that out of the 13 isolates, (02) 3% were serotype A, and (11)16.4% were serotype B/C respectively.

The results of the present study also revealed the association of *C. neoformans* with the environmental specimens (Table 12 & 13) in 42 specimens of pigeon droppings collected from 6 different locations in this part of Chennai (North Chennai). The percent of samples from which growth of *C. neoformans*, was recovered from pigeon droppings was only 21.4 % in the present study. This was less when compared with another study from Vellore (54.5%; Asha *et al.*, 1995). This difference may probably be due to the differences in the seasonal variation or specimen collection sites. Apart from pigeon droppings, cryptococci was isolated from the Eucalyptus tree in 16% of the specimens. In a previous study from North India (Chakrabarti *et al.*, 1997), 696 eucalyptus tree
specimens were tested, and in 5 (0.7%) specimens the growth of *Cryptococcus neoformans var gattii* was isolated.

Isolation of the yeasts from the environment also showed recovery of maximum No. isolates on SAB than other media used (Table 13). These yeasts were found both in pigeon droppings and dried plant material.

Growth of the yeast colonies on various media are used routinely for identification of the genus, species or variety. Identification of the colonies on SAB was easily used to identify *C. neoformans* from other common yeasts like *Candida, Torulopsis* etc. On this medium, isolates produced white, mucoid and elevated colonies typically colouring the medium with brown pigment. This pigment (melanin) resulting from the activity of CN LAC1 gene (Zhu and Williamson, 2006) is a known virulence factor. Isolates obtained from the CSF samples showed this reaction on SAB medium confirming their ability to be infectious.

Media containing seed extracts of *Guizotia abyssinica* (Staib, 1962), Sunflower seeds (Pal and Baxter, 1985) or mustard seeds (Nandakumar, 2006) were used to show that *C. neoformans* spp. selectively produced a brown pigment unlike other yeasts like *Candida* spp. Media containing polyphenolics or polyaminobenzene compounds induce the phenoloxidase in this fungus resulting in the brown pigmentation. A similar coloration is also seen in caffiec acid and o-diphenol. Ferric citrate agar (Kaufmann and Merz, 1982).

Typical reactions on urea agar, inability to utilize nitrate, inability to ferment sugars, are tests by which Cryptococci are discernible from other yeasts (Tables 15; Figs.5, 6, 7).
Biotyping of the isolates

The characteristics of *C. neoformans* that differentiate it from other yeast species are its polysaccharide capsule and urease activity. However, isolates lacking a capsule or urease activity are not uncommon (Ruane et al., 1988). Similarly, although melanin formation is a stable character of *C. neoformans* and phenoloxidase positive has also been reported (Kabasawa et al., 1991). However there was no melanin negative strain observed in the present study, although varying intensity of the melanin production was observed with different isolates.

The prevalence of varieties and serotypes of *C. neoformans* from clinical sources:

Serotyping of the strains isolated form human samples or environment is important to understand prevalence of the dominant types or understand the natural reservoirs of such variants. For instance *C. gattii* is found mostly in the tropical or sub-tropical climates rarely infect humans (Chaskes et al., 2006). With increasing incidence of Cryptococciosis in immunocompromised patients, the detection of multiple cases of *C. gattii* is alarming (Chaskes et al., 2006; Banerjee, 2005). Serotyping using factor serum (Ikeda et al., 1996) requires use of kits not readily available or is expensive and requires training for routine lab use.

Kwonchung and Bennett (1984) studied identification into varieties and serotypes of 725 clinical isolates of *C. neoformans* from various parts of the world. Isolates from regions with temperate climate such as USA (excluding southern California and Hawaii), Europe and Japan belonged mainly exclusively that 96% of the clinical isolates from America and 57% of isolates from China were var *neoformans*. 
Other investigators with small numbers of clinical isolates have reported similar trends (Bennett et al., 1977; Muchmore et al., 1980; Mishra et al., 1981; Swine, 1984; Swine et al., 1986).

In contrast, there was a high frequency (35 to 100%) of species *C. gattii* in some tropical and subtropical regions such as southern California, Australia, Southeast Asia, Brazil and central Africa (Kwon-chung and Bennett, 1984). It was suggested that the presence of var *gattii* predominantly in tropical and subtropical regions may be a consequence of the association of this variety with Eucalyptus trees, as these trees are not found in temperate climates (Ellis and Pfiffer, 1990).

According to a recent study by Dromer et al. (1994), the serotype A of var *neoformans* is most common throughout the world and serotype D is uncommon in USA, but is more frequent in Europe, particularly in Italy (49%) and in France (>20%) and nearly all the isolates from southern California belong to serotype C (Shadomy et al., 1987).

In India from northern region, the study (Padhye et al., 1993) with 18 clinical isolates revealed that, 15 isolates (83.3%) were var *neoformans* and remaining 3 isolates (16.7%) were *C. gattii*. The serotyping revealed that of the 18 isolates, 13 (72.2%), 2 (11.1%) and 3 (16.7%) were serotype A, AD and B respectively. From southern region Asha et al. (1995) reported that, out of 11 clinical isolates 10 (91%) were var *neoformans* (serotype A) and one (9%) was *C. gattii* (serotype B).

In the present study, the Biotyping results based on growth with specific media revealed that 83%, 12.5% and 4.6% of the isolates were serotype A, D, and B/C respectively (tables 16 & 17). Thus, the epidemiological situation of
C. neoformans in India is similar to that of the USA and Japan (Bennett et al., 1977, Hironaga et al., 1983).

There is no other published data available on the prevalence of varieties and serotypes of clinical isolates of C. neoformans from India. However in the present study, 83% of the clinical isolates were C. var grubii serotype A, 12.5% were C. neoformans var neoformans serotype D and remaining 4.6% of the isolates were Cryptococcus gattii serotype C .Therefore this is probably the third documented report after Padhye et al. (1993) from North India and Asha et al., (1995) from Southern India and the first report of the occurrence of the serotype A and D with the Indian clinical isolates of C. neoformans. Thus, the present study confirms earlier observation that the predominant variety and serotype among the Indian clinical isolates are var neoformans and serotype A (Jain et al., 2005)

The incidence of Cryptococcosis in AIDS patients varies according to geographic regions. The clinical isolates from such patients have been identified predominantly as var neoformans, even in areas where the gattii is endemic (Swinne et al., 1986; Shimizh et al.,1986; Rinalli et al., 1986; Bottone et al., 1987; Staib et al., 1987; St Germain et al., 1988),where as gattii has a propensity for causing disease in immunocompromised host and is gradually increasing (Fisher et al.,1993; Mitchell et al., 1995).The reason for this disparity between isolates from patients with and without AIDS and speculative but likely relate to differences in pathogenicity.

However in the present study, var neoformans was predominantly associated with HIV positive patients than HIV negative patients. Isolation of var gattii from AIDS patient is rarely reported (St Germain et al., 1988; Rozenbaum et al., 1990).This study also suggested that although C. gattii has a relative predilection for healthy hosts, C. gattii does cause the infection in HIV positive
patients and it must be probably due to the incidental exposure to this variety. It is interesting to note that, the higher prevalence of *C. gattii* (5 %) and the occurrence of var *neoformans* serotype D (12.5%) was observed.

All infectious Cryptococci belong to one of the five serotypes viz., *C. n. grubii* (A), *C. n. neoformans* (D) & AD and *C. gattii* (serotype B &C). The serotypes are identified by typical agglutination of capsular glycoproteins with sero-specific monoclonal Ab. Generic Abs available as kits help identify Cryptococci with Latex Agglutination kits (Sanofi). These kits are expensive. Positive reactions in this test for clinical isolates from CSF (table 18) showed were only 38% positive specimens while culture test on SAB showed 43%. However, this was unable to group serotypes as the antigen used was capsular polysaccharide specific.

Being expensive, determination of the serotypes was done using classical biochemical reactions in specific growth media (Shadomy *et al*., 1987).

**Efficacy of the method determining varietal status:**

The differentiation of varietal status of *C. neoformans* was established with colour change on CGB agar and assimilation of D-proline, although negligible colour change on CGB agar was observed with some var *neoformans* isolates. In previous studies (Bennett *et al*., 1978); 62% of serotype B and 45% of serotype C isolates were identified by GCE on modified Staib agar.

Kwon-chung *et al*. (1982) and Shadomy *et al*.,(1987) reported the superiority of CGB agar over GCP agar, although the developers of GCP agar claimed a 100% accuracy in differentiating the varities (Salkin and Hurd,1982). This present study also agrees with their findings, although 64 isolates were used in the present in the study.
Therefore these observations indicate that, the use of CGB agar and D-proline assimilation test are effective for the determination of varietal status of the isolates of *C. neoformans*.

Following recommended media, growth of 61 clinical isolates was tested. Inability of D-proline utilisation by *C. neoformans* or *C. neoformans* var. *grubii* was assessed and 61/64 isolates could not grow around the proline soaked discs. (Table 15, Fig.7).

The change in colour of media containing inhibitors such as L-canavanine (CGB) or cycloheximide (GCP) was used to determine the serotype (Shadomy *et al.*, 1987) as an inexpensive alternative to LA test.

The results presented for clinical & environmental isolates showed that 61/64 clinical isolates belong serotype A/D while 2 of the environmental isolates also typed to serovar A (var. grubii). Table 16 & 17. Three of the clinical isolates and 11 of environmental isolates typed serotype b/c based on the colour change on CGB and CGP agar (Table 16 & 17, Fig. 10 & 11).

To check if these serotypes produced unique antigens in the infected host, sera of sick patients were used to immunoblot cellular antigens separated by SDS PAGE. Cellular antigens of *Cryptococcus grubii* (serotype A), *C. neoformans* (serotype D) and an unrelated yeast spp. (*Candida albicans*) were used in this experiment. Sera of patients used to look for serotype specific or genera specific antigen did not produce any conclusive protein patterns. However antibodies for both serotypes and the unrelated yeast were detected (Fig. 14 & Table 20) (Lamura *et al.*, 2000).

Routine presumptive identification of meningitis and confirmatory tests using laboratory growth and biochemical tests is time consuming. Sometimes
these tests can be misleading. These problems can be addressed by adopting quicker nucleic acids detection techniques using PCR. Primers specific to internal ribosomal sites (5.8s rRNA; Mitchell et al., 1994). Of yeasts were used to amplify total DNA both from cultures cells as well as from neat clinical samples. Results for these are compared in the Table below:

<table>
<thead>
<tr>
<th>Primer Pairs</th>
<th>Positive CSF samples (%)</th>
<th>Amplicon size bp</th>
<th>Table/ Fig. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS1- ITS4</td>
<td>38 (62.2%)</td>
<td>600bp</td>
<td>22/16</td>
</tr>
<tr>
<td>CN4 - CN5</td>
<td>45 (74%)</td>
<td>136</td>
<td>23/17</td>
</tr>
<tr>
<td>ITS1- CN4</td>
<td>57(93.4%)</td>
<td>415</td>
<td>24/18</td>
</tr>
<tr>
<td>CN5- CN6</td>
<td>57(93.4%)</td>
<td>116</td>
<td>25/19</td>
</tr>
</tbody>
</table>

When total DNA of 61 Cryptococcal cultures were used as templates any primer pair could be used to obtain the relevant amplicon in all samples.

In the neat CSF samples, the primer pair spanning yeast specific rRNA site ITS1 and the generic primer CN4 could detect the yeast genes in 93.4% of the samples. Four samples that failed could not also be detected when reamplified with internal primers CN5 and CN6 indicating that was not be because of limiting template DNA alone. Other modifications of neat samples would have to be tried to overcome this problem during routine diagnostics. Paschoal (2004) and Mitchell (1994) also reported about 93 % sensitivity using these sets of primers.

The primers selected here were yeast specific primers or species specific primers of the conserved 5.8s rRNA gene. Hence, serovars or molecular typing of the isolates was not possible s. Several reports including Jain et al. (2005) studied the prevalence of Indian isolates using (GACA)n, M13 and Tn primers for patient
samples. 51/57 isolates in this study belonged to serotype A based on M13 minisatellited profiling.

In an attempt to use novel methods for rapid diagnosis of this fatal disease we examined to application of FTIR methods. The spectral analysis on 15 CSF samples were obtained. These CSF samples were already culture positive on the selective SAB medium.

Apart from conventional clinical methods, there is a need to develop new approaches which are simple, objective and noninvasive for early diagnosis of various human disorders. Among the optical methods available, Fourier transform infrared (FTIR) spectroscopy has shown encouraging trends in the field of medicine. Various bimolecular components of the cell give a characteristic IR spectrum, which is rich in structural and functional aspects. The biochemical fingerprint of cells, tissues and fluids altered in a diseased state can be detected using IR spectroscopy. There are reports of the application of FTIR spectroscopy in the diagnosis of various types of cancer. Yun Xiang et al. (1999) carried out a FTIR study of the human breast, normal and carcinoma tissues and their method of analysis resulted in nearly 100% diagnostic accuracy of carcinoma tissues from normal ones. The grading of breast tumors has also been achieved by FTIR successfully.

In this study, characterization of control and CSF infected with Cryptococcus neoformans samples was carried out FTIR-spectroscopy. FTIR characterization of CSF sample which is one of the biofluids is not yet reported in the literature. Our results showed significant IR spectral variations between control and infected CSF samples. Unique IR band appeared at 1746 cm\(^{-1}\) in the case of all 15 patient CSF samples, which was absent in the control CSF samples. This unique IT band at 1746 cm\(^{-1}\) could be attributed to symmetric stretching
vibrations of carbonyl group in the ester or acid functional group. It indicates the presence of cells having fatty acids/phospholipids in the infected CSF samples. Literature search on composition of human CSF suggested that the lymphocytes are absent in the normal CSF samples (Jones, 2004). But, in the case of *Cryptococcus neoformans* infected CSF samples, due to the cell mediated immune response, lymphocytes are observed in larger numbers present in the CSF samples (Lemmer *et al.*, 2004). Lymphocytes present in the infected CSF samples are responsible for unique 1746 cm⁻¹ IR band observed with infected CSF samples. In addition, IR absorbance in the region between 950-1200 cm⁻¹ for CSF samples belonging to AIDS patients was much higher than the reported controls. It might be due to chronic status of these three AIDS patients resulting in larger number of lymphocytes count in the CSF sample accounting for higher absorbance in the 950-1200 cm⁻¹ region. Similar absorbance changes could also be observed in the higher wave number region between 2800-3000 cm⁻¹. Our results showed clear IR spectral differentiation between control and infected CSF samples.

To throw more light on sensitivity and specificity, hierarchical cluster analysis on FTIR spectra collected on control and *Cryptococcus neoformans* infected CSF samples was performed. Best classification was obtained with spectral window of 1200-1500 cm⁻¹.

Giving rise to sensitivity of 87% and 100% specificity for AIDS patients. In the case of TB patients, 93% sensitivity and 100% specificity was obtained with spectral window of 1200-1500 cm⁻¹. Our preliminary results showed on cluster analysis of FTIR spectra of CSF samples good classification between control and patients. Our student’s T-test results rejected the null hypothesis indicating that two different populations are present in the FTIR dataset.
Laboratory diagnosis of cerebrospinal fluid (CFS) is traditionally based on microscopic examination of India ink preparations and on the detection of Cryptococcal capsular polysaccharide antigen by a latex agglutination on test. Direct microscopic examination is a rapid but quite insensitive test and strongly depends on the operator’s skills. The latex agglutination test is a more sensitive method but may still yield false-positive and false-positive and false-negative results with either serum or CSF (Table 18, Fig.12). Moreover, the simple culture of CSF samples on Sabouraud agar is time-consuming; in fact, at least 4 days is necessary to detect positive cultures of C. neoformans. An enzyme-linked immunosorbent assay kit for the detection of capsular antigen is also available, with sensitivity comparable to those of agglutination tests (Saha et al., 2008).

In this report, we have characterized CSF samples from control and Cryptococcus neoformans infected patients using FTIR spectroscopy. Our studies with limited data showed good classification between control and patients. Viable biomedical technology for rapid, highly sensitive, simple and cost-effective analysis of CSF samples from meningitis patients looks promising in the future. More detailed investigation with larger number of patient subjects and also application of more advanced computational techniques such as Neural Networks will help to achieve the above mentioned goal in the future.

MIC for three antifungal agents of all 64 isolates obtained from patients samples was obtained (Table 19). None of the isolates showed unusual resistance to amphotericin B - the drug of choice during treatment. However, development of resistance to these synthetic drugs can never be underestimated. Hence search for novel antifungal moieties from plants was attempted for the first time for this pathogen.
Swietinia mahagoni, a member of the neem family, Meliaceae, has been reported to produce a number of terpenoids (Govindachari et al. 1999, Hisao et al., 1990, Rosabel et al., 1993) from seeds or bark extracts. Dried leaf powder extracts (methanolic) were found to be bioactive against clinical isolates of the fungus in TLC based bioautography assays (Fig.30 & Table 30). The antifungal compounds eluted with Rf’s of 0.59 in crude extracts, chloroform soluble E1 also contained a similar eluting compound at Rf 0.41 and 0.50 in addition had two compounds with a low Rf 0.05-0.25. Chloroform insoluble (E2) fraction similarly had 2 antifungal zones on TLC. One eluted at Rf 0.40 and the other at Rf 0.10 whether these are different compounds partitioning in non-polar solvents differentially needs to be ascertained by chemical isolation techniques. Since the inhibition zones (Table 31 & Fig. 34) & MIC Table 33 & Fig.36 Tables 32 & Fig.35) of these extracts was potent as compared to Amphotericin B (10ppm) it would be very important to characterize the antifungal nature of the compounds.

To conclude, Cryptococcus the opportunistic, fatal yeast causing meningitis was isolated from CSF samples (61) and extraneural samples (blood-11 & sputum-3) on selective SAB media. In addition to routine laboratory test such as microscopy, culture and biochemical tests attempts to diagnose using modern methods such as PCR and novel spectroscopy tools such as FTIR are the most important contributions of the present study. Identification of new compounds with antifungal activity to these yeasts from a potent medicinal tree species (Swietinia mahagoni) offers a new path for future research.