Summary and Conclusions
7. SUMMARY AND CONCLUSIONS

In the present study on cryptococcosis in AIDS patients and characterisation of Indian isolates of *C. neoformans* from clinical and environmental sources, carried out in the department of Microbiology, Dr.ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai - 600 113, the salient observations and conclusion are as follows:

The prevalence of cryptococcosis in hospitalised AIDS patients at Chennai during the study period (January 1996 - October 1997 were found to be 1.7% pulmonary and 11.4% meningeal cryptococcosis respectively.

For the isolation of *C. neoformans* from the pulmonary specimens of AIDS patients, Staib's agar with biphenyl 0.01% was found to be the most suitable medium.

The most common symptoms observed with meningeal cryptococcosis in AIDS patients were headache (94.4%), fever (72.2%) and Malaise (50%). Stiff neck was associated with 33.3% of cases. Visual complications were observed with 22.2% of the patients. Thus high index of suspicion is required particularly in patients with HIV infection because, most cryptococcal meningitis patients have clinically non-specific presentation. Moreover patients with suspected meningitis should be screened routinely for cryptococci, irrespective of their immune status.
Among

in CSF (sensitivity of 100%) was found to be effective in diagnosis, when compared with the Negative staining method (sensitivity of 77.8%) and time consuming method such as Culture (sensitivity of 100%). The detection of antigen in serum may be useful as a initial screening test for febrile patients, as the serum specimen shown the sensitivity of 88.9% and it does not involve any highly invasive procedure like lumbar puncture. Extraneural culture was positive in 55.6% of cryptococcal meningitis cases (of 18 cases, 50% of blood, 33.3% of urine and 5.6% of sputum).

The In-House standardised Co-A test was found to be a rapid and reliable diagnostic method. It is specific (100%), sensitive (94.4% with CSF and 88.9% with serum) and easy to prepare. Being highly cost-effective, it is recommended as a simple test, within the reach of any routine diagnostic laboratory.

The skin test was positive only in patients with higher CD\textsubscript{4} cell count (mean 270). Thus it can be employed for assessing the immunologic status of the AIDS patients rather than using for diagnostic purpose.

Although amphotericin B is the only antifungal drug of proved value available for the treatment of cryptococcosis, the treatment with amphotericin B for the active cryptococcosis did not provide favourable clinical outcome.

However the in vitro antifungal sensitivity test revealed that the MIC range of 0.125 - 1, 0.125 - 16 and 0.25 - 2 were observed with amphotericin B,
5-flucytosine and ketoconazole respectively. The wider range of MIC with fluconazole (0.5 - 64) might be due to the chronic exposure of fluconazole for the treatment of oral candidiasis in AIDS patients. Hence, the regular monitoring of the drug sensitivity pattern especially with the isolates from AIDS patients would be useful for the treatment purposes.

The analysis of clinical data obtained along with the isolates from various centres revealed HIV infection (77.3%) was the most predominant predisposing factor for cryptococcal infection. The predominant sex and age group associated with cryptococcosis were male (91.7%) and 26-35 years (53.8%) respectively.

The occurrence rate of *C. neoformans* in pigeon droppings was 9.6% and none of the *Eucalyptus* tree specimens yielded the growth of *C. neoformans*. The natural occurrence of *C. neoformans* in avian species should be considered from public health point of view as the pathogen may produce disease in healthy as well as immunocompromised hosts.

The efficacy of different media for the isolation of *C. neoformans* from positive specimens of pigeon droppings were, of 12 positive specimens, 91.7%, 100%, 33.3%, 100% and 75% of the specimens were positive by Staib’s agar, Staib’s agar with biphenyl 0.01%, Staib’s agar with biphenyl 0.1%, Staib’s agar with methyl violet and Sunflower seed agar respectively. Thus Staib’s agar with biphenyl 0.01% and methyl violet are most suitable media for the recovery of *C. neoformans* from contaminated environmental specimens such as pigeon droppings.
Regarding the characterisation of cryptococcal isolates, all the isolates of \textit{C. neoformans} were capsulated, urease positive, growth at 37°C positive, nitrate reduction negative, BCE positive, sugar non-fermentative and pathogenic to mice.

Of 164 Indian isolates, 94.5% of clinical and 100% of environmental isolates were identified as var \textit{neoformans} and remaining 5.5% of clinical isolates were var \textit{gattii}.

The use of CGB agar and assimilation of D-proline (sensitivity of 100%) are the most suitable method for the determination of varietal status. It is suggestive that, clinical laboratories should adopt the use of these tests as part of their standard identification procedures.

Serotyping revealed that, serotype A was predominantly associated with the isolates of clinical (87.2%) and environmental (66.7%) sources. Other serotypes of clinical isolates were, 4.3% of serotype D, 3% of serotype AD, 4.9% of serotype B and 0.6% of serotype C and environmental isolates were, 8.3% of serotype D and 25% of serotype AD. To best of our knowledge, the occurrence of serotype B in the clinical sources is the third report from India, after Padhye \textit{et al.} (1993) and Asha \textit{et al.} (1995). Moreover the occurrence of serotypes C and D in the clinical source is the first report from India.

Virulence study in mice revealed, the virulence of the isolates from different states were varied and the higher virulence was observed with the clinical isolates of Maharashtra and Andhra Pradesh. Among the serotypes,
serotype AD was found to be higher virulent than other serotypes, although serotype A was more prevalent in clinical and environmental sources. Thus, further investigation with the analysis of other virulence factors such as melanin production, mannitol production, proteases, phospholipases etc., are necessary.

The distribution of varieties and serotypes in different states are as follows:

Var gattii were found only in 3 states and the prevalence rates were, 3.8% in Tamilnadu, 7.9% in Karnataka and 4.3% in Maharashtra.

Serotype A was highly prevalent in all the states (88.5% in Tamilnadu, 85.5% in Karnataka, 100% in Andhra Pradesh, 85% in Maharashtra and 100% in West Bengal), serotype D was found in three states (3.8% in Tamilnadu, 5.3% in Karnataka and 4.3% in Maharashtra), serotype AD was found in three states (3.8% in Tamilnadu, 1.3% in Karnataka and 6.4% in Maharashtra), serotype B was found in three states (3.8% in Tamilnadu, 6.6% in Karnataka and 4.3% in Maharashtra). Serotype C (1.3%) was found only in Karnataka. Moreover all the five serotypes were found only in Karnataka state.

The predominant mating type was found to be 'α' type of F. neoformans var neoformans in clinical (86.7%) and environmental (100%) sources.
An attempt was made to compare the RAPD profiles of the isolates from clinical and environmental sources in a limited geographical regions. Five different profiles were observed, of these, 50% of the clinical and 33.3% environmental isolates used for this study belonged to same profile (Profile II). However further studies are warranted with the larger number of isolates.

To conclude, the present research work augment the information on the clinical and epidemiological pattern of cryptococcosis in AIDS patients, isolation techniques, efficacy of diagnostic tests, treatment outcome and the current status of typing pattern of C. neoformans isolates in India.