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Full Length Research Paper

Pathogenic fungal isolates in sputum of HIV positive patients

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Fungal pathogens were isolated and identified from sputa of 100 HIV positive patients admitted at ART centre, K.G.H, Visakhapatnam during the period of 2 months from June 2010 to July 2010. The sputum samples from HIV positive individuals were collected in a sterile container and processed by standard methods. Two consecutive samples at the interval of three days were collected. If two samples yielded the same growth, then only they were considered as positive for fungal pathogens. Patients age ranged from 6 to 55 years in both sexes. Samples were collected from 67 males and 33 females. Out of 100 samples, single fungal isolates were obtained in 54 samples, mixed fungal species in 20 samples and no fungal isolates in 26 samples*. *Candida* **species predominate (42 samples) in single isolates followed by ***Cryptococcus neoformans*** **(4 samples***), Penicillium* **spp., *Aspergillus fumigatus* and *Aspergillus niger* (2 each), *Scedosporium apiospermum, Cunninghamella bertholletiae, Sporothrix schenckii* and *Geotrichum candidicum* were also isolated. The *Penicillium marneffei* isolates (2 samples) were from cases with CD_4 counts below 100.**

Key words: Opportunistic fungal infections, immunocompromised individuals, CD_4 counts, penicillium marneffei.

INTRODUCTION

A predominant source of morbidity and mortality among HIV positive individuals in late stages of HIV infection and low CD_4 count below $500/_{cumm}$, is opportunistic infection caused by agents that rarely infect immuno competent individuals (Jawetz et al., 2007). The occurrence of opportunistic fungal infections has risen progressively in recent years. Invasive fungal infections has been reported in recent years in 26% of chronically and intensively immunosuppressed patients (Topley and Wilson's, 2005). Infections with *Candida albicans* appear when CD_4 count is between $500\text{-}200/_{cumm}$ and may be the first indication of immunodeficiency. Cryptococcal infection occurs when CD_4 count has fallen below $150/_{cumm}$ (NACO Specialist's Training and Reference Module, 2000). Penicillosis is observed in patients with CD_4 count of less than$100/_{cumm}$ (Guidelines for prevention and treatment of opportunistic infections in HIV infected adults and adolescents, 2009). The phagocytic cells and lymphocytes (T&B both) are believed to function together in protecting the host against fungal pathogens but the exact degree to which each is involved is not yet fully known. It has been shown that vegetative hyphal structures of *Aspergillus* and *candida* are ingested and killed by neutrophils (Jagdish, 2009). Skin and mucosal surfaces play an important role in primary defense against pathogens. The muco ciliary action of mucus membrane is the prime clearance mechanism active against inhaled fungal spores. As the HIV positive individuals are prone to get recurrent respiratory infections, the mucosal barrier may be damaged and they are more vulnerable to develop fungal respiratory infections (Topley and Wilson's, 2005).

The fungal infections depend on, exposure to sufficient inoculum size of organism and general resistance of the host. Immune deficiency predispose to progression of infections by established pathogens that is *Penicillium marneffei* and *Histoplasma capsulatum*. *Aspergillus* spp., *Rhizomucor, Absidia* spp., *Cunninghamella* spp., *Apophysomyces* spp. are mould species commonly

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Table 1. Showing age and sex wise distribution of patients in relation to CD_4 counts.

Age (years)	CD_4 counts										Total
	<100		101 - 200		201 - 300		301 - 400		401 - 500		
	M	F	M	F	M	F	M	F	M	F	
0-10	2	0	1	0	1	0	0	0	0	0	4
11-20	0	0	1	1	1	1	0	2	0	1	7
21-30	2	0	9	1	10	8	4	5	2	2	43
31-40	1	0	2	0	1	2	10	0	7	7	30
41-50	1	0	0	0	1	2	5	0	4	1	14
51-60	0	0	0	0	1	0	1	0	0	0	2
Total	6	0	13	2	15	13	20	7	13	11	100

isolated in HIV positive individuals (Harrison's, 2008). In Thailand *P. marneffei* infection is the third most frequent infection in HIV infected persons after tuberculosis and cryptococcosis (Anantanarayan and Paniker's, 2009). Now CDC recognized Manipur State, India, as a new endemic area of *P. marneffei* (Guidelines for prevention and treatment of opportunistic infections in HIV infected adults and adolescents, 2009). With introduction of antifungal agents, the cause of candida infections shifted from *C. albicans to C. glabrata* and other non albicans species, *as C. glabrata* and *C. krusei* develop resistance to fluconazole (Topley and Wilson's, 2005). Fungal infections may disseminate and cause fungaemia, which is a grave condition in immunosuppressed individuals. Thus, prompt diagnosis by standard microbiological methods and treatment are crucial.

MATERIALS AND METHODS

We got approval from Ethics Committee, Andhra Medical College, Visakhapatnam to conduct the study. The copy of the Ethics Committee Approval was enclosed (Annexure 1). Two consecutive sputum samples at an interval of 3 days were collected from HIV the patients and their CD_4 counts were presented in Table 1. All patients complained of cough and fever for more than one week. Sputa samples were collected in a sterile wide mouthed container. Patients were asked to wash their oral cavity with distilled water before collecting sputum in order to avoid contamination of sputum with commensal flora from oral cavity (Koneman color Atlas and Text book of Diagnostic Microbiology, 1997). Specimens were processed by doing Gram's staining for direct smears and KOH mount. Gram's stained smears were examined under oil immersion objective of microscope for the presence of inflammatory cells (pus) cells and fungal elements. Quality of the sputum was assessed by examining Grams staining smears. The specimen was considered as acceptable when number of squamous epithelial cells are less than 10/ LPF (Bailey and Scott's, 2002; Koneman color Atlas and Text book of Diagnostic Microbiology, 1997). The samples that showed fungal elements and pus cells in direct Gram's staining were processed further (Koneman color Atlas and Text book of Diagnostic Microbiology, 1997; Topley and Wilson's, 2005).

Sputum was inoculated on two sets of Sabouraud's dextrose agar (SDA with antibiotic gentamicin alone and SDA with gentamicin and cycloheximide) and incubated at 25°C in BOD for 4 weeks. SDA bottles were examined for growth once in two days during 1st week and twice a week thereafter up to 4 weeks. SDA medium with growth was processed by standard methods (Mackie and McCartney, 2008). Mucoid, yeast like growth was processed by doing Gram staining, capsular staining, germ tube test, urease test and inoculation onto corn meal agar. Gram's staining was done to all the isolates with mucoid and yeast like growth and observed for gram positive budding yeast cells.

Capsular staining with 10% Nigrosin: All the isolates with mucoid and yeast like growth were tested for capsulated budding yeast cells for identification of *Cryptococcus neoformans*.

Germ tube test: All candida isolates were tested for germ tube formation. A colony was inoculated in human serum and incubated at 37°C. After 2 to 4 h wet mount was prepared and observed for germ tubes.

Chlamydospore formation: All candida isolates were tested for the production of chlamydospores in corn meal agar. After inoculation and incubation at 25°C, the plates were examined under microscope for the presence of chlamydospores.

Urease test: Christensens' Urease test was done for *Cryptococcus. Cryptococcus neoformans* was urease positive. Lactophenol cotton blue (LPCB) mount was done for filamentous growth to see arrangement of conidia.

Slide cultures: Slide culture in Mycology is used to study undisturbed morphological details of fungi, particularly relationship between reproductive structures like conidia, conidiophore and hyphae (Jagdish, 2009). When two samples yielded the same fungal isolate, then only the fungal isolate was considered as causative agent.

Identification of *P. marneffei: P. marneffei* is a dimorphic fungus. At 25°C on SDA it grows as a mycelial fungus producing rapidly growing greenish yellow sporulating colony with a red centre and dark green edges with diffusible brick red pigment. At 37°C on SDA it produces smooth glabrous off white yeast like growth with little pigment. Microscopically the fruiting heads sometimes have terminal conidia larger than the ones beneath them called Corda's phenomenon, characteristic of *P. marneffei* (Figure 4).

RESULTS

Out of 100 sputum samples, 54 samples yielded single fungal isolates. Mixed fungal isolates were observed in 20 samples and rest of 26 samples were negative for

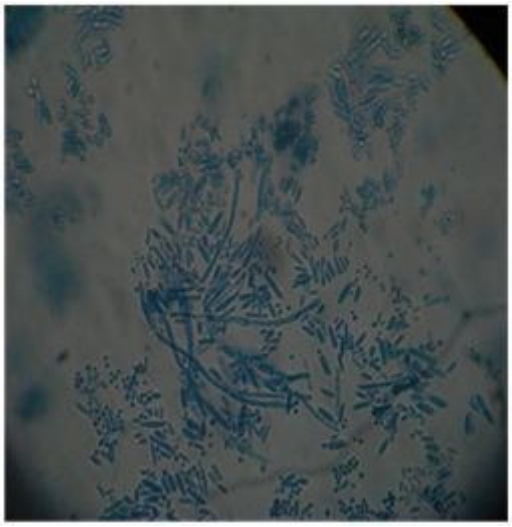

Figure 1. Microphotograph of *Scedosporium apiospermum.*

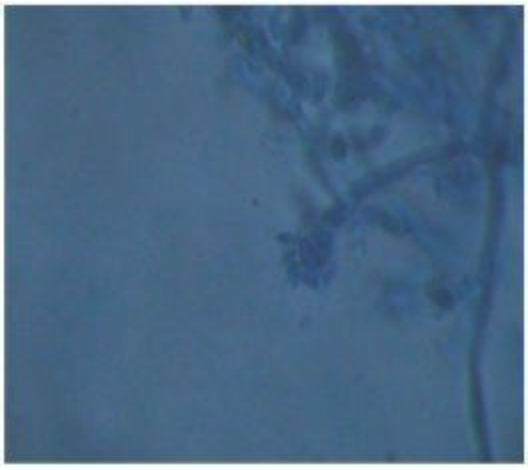

Figure 2. Photomicrograph of *Cunninghamella bertholletiae.*

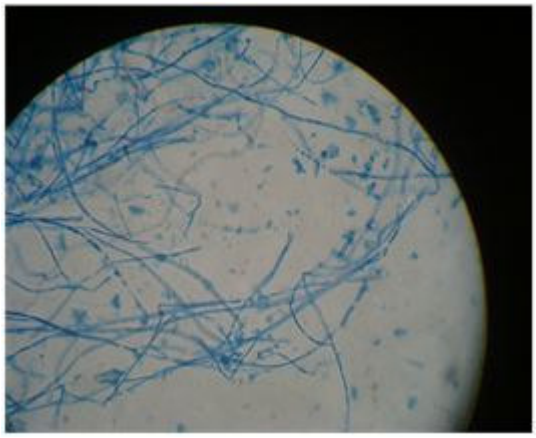

Figure 3. Photomicrograph of *S. schenckii.*

fungal growth. Out of 54 single isolates, *Candida* spp. were predominant isolates. Those yeasts were identified in 42 samples. *C. albicans* in 18, *candida* non *albicans* in 24. Other fungi were isolated mainly *Cryptococcus neoformans* (4 samples), *Penicillium* spp. and *Aspergillus fumigatus* (each one in 2 samples) *Scedosporium apiospermum* (Figure 1), *Cunninghamella bertholletiae* (Figure 2), *Geotrichum candidicum* and *Sporothrix schenckii* (Figure 3) (each in one sample) as shown in Table 2. Among mixed isolates *Candida* spp. were isolated in 13 cases and in 7 cases several moulds were isolated as shown in Table 2. *P marneffei* was isolated in samples in combination with *C. albicans* in one sample and with *A. fumigatus* in another sample. The CD4 counts of *P. marneffei* was both below 100.

Out of total 96 isolates *Candida spp*. was the major group comprising 55 in number (57%). *C. albicans* in 26 samples (27%) and non albicans spp. in 29 (30%) Next common isolate was *Aspergillus* spp.in13 samples that is 13.5% (with *A. fumigatus* and *A. niger* each one in 6 samples, *A. flavus* in one sample) followed by *Penicillium* spp. in 6 (6.25%) including two *P*. mar*neffei* (Figure 4) *isolates. C. neoformans* and *S.schenckii* (Figure 3) each one in 5 samples (5.2%), *Geotrichum candidicum* in 4 (4.16%), *Wangiella dermatitidi*s (Figure 5) and *Scedosporium apiospermum* each one in 2 samples, *Absidia corymbifera* (Figure 6), *Cunninghamella berthollatiae, Nigrospora* spp (Figure 7) and *Prototheca* spp. each one in one sample were isolated.

DISCUSSION

The number of opportunistic fungal infections in general have increased in HIV positive patients and therefore the incidence of fungal infections has also been on the rise. Recent pandemic of AIDS caused by HIV virus has been the precipitating cause for the increased incidence of two common fungal diseases i.e., isesophageal candidiasis and cryptococcosis. Candida is part of normal flora of upper respiratory tract, when immunity is low, it acts as an opportunistic pathogen. Usually HIV positive individuals also have other predisposing factors like neutropenia, lymphopenia, selective T cell defects, altered monocyte macrophage function, frequent use of antibiotics for prophylaxis and treatment of various bacterial infections, lowered immunity due to low CD_4 count (Ananthnarayan, 2009). All these factors contribute to the development of opportunistic fungal infections like pulmonary candidiasis, cryptococcosis, zygomycosis, asperigillosis.

Invasive fungal infections like Canidiasis, Aspergillosis, Mucormycosis, Cryptococcosis, Histoplasmosis and Coccidiodomycosis are known infections whereas infections with *S. schenckii* (Figure 3) and *P. marneffei* are emerging ones in HIV positive patients (Carol, 1998). Other fungi that cause pulmonary infections in HIV/AIDS patients are *Scedosporium apiospermum,* (Figure 1),

Table 2. Fungal pathogens in mixed infection.

No.	Names of the fungal pathogens in mixed infection	No. of samples
1	Non albicans *Candida* spp.+*Aspergillus fumigatus*+*Aspergillus niger*	1
2	*Candida albicans*+*Penicillium* spp.+*Wangiella dermatitidis*	1
3	*Candida albicans* + *Penicillium marneffei*	1
4	*Candida albicans*+*Absidia corymbifera*	1
5	*Candida albicans* + *Sporothrix schenckii*	2
6	Non albicans Candida spp.+*Aspergillus niger*	1
7	*Candida albicans*+*Aspergillus niger*	1
8	Non albicans *Candida* spp.+*Geotrichum candidicum*	1
9	*Candida albicans* +*Cryptococcus neoformans*	1
10	*Candida albicans* +*Nigrospora* spp	1
11	Non albicans Candida spp.+*Wangiella dermatitidis*	1
12	*Scedosporium apiospermum* +*Geotrichum candidicum*	1
13	*Aspergillus fumigatus*+ *Penicillium marneffei*	1
14	*Sporothrix schenckii* + *Penicillium* spp.	1
15	*Aspergillus fumigatus* +*Aspergillus niger*	1
16	*Aspergillus fumigatus*+*Prototheca* spp.	1
17	*Aspergillus niger*+*Sporothrix schenckii*	1
18	*Aspergillus niger* +*Aspergillus flavus*	1

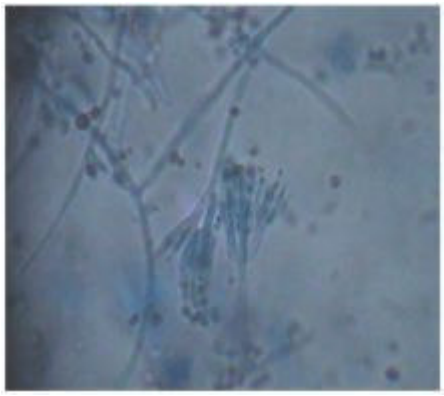

Figure 4. Photomicrograph of *P. marneffei* showing Corda's phenomenon.

Figure 6. Photomicrograph of *A. corymbifera*.

Figure 5. Photomicrograph of *W. dermatitidis.*

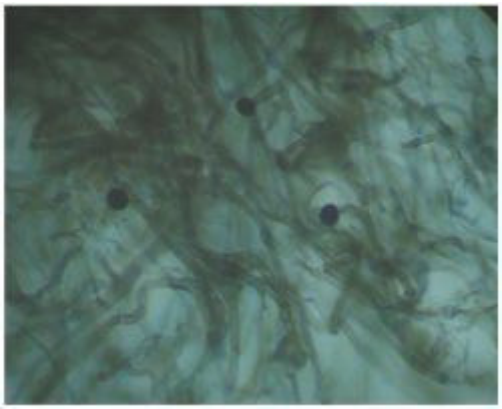

Figure 7. Photomicrograph of *Nigrospora* spp.

Absidia corymbifera (Figure 6), *Wangiella dermatitidis* (Figure 5) (Topley and Wilson's, 2005).

Today's concern about Candidiasis is emergence of fluconazole resistant *C. albicans* in AIDS patients with recurrent attacks of oral thrush and less susceptibility *of C. krusei* and *C. glabrata* to fluconazole. *C. albicans* is generally considered as major pathogen among *Candida* spp (Topley and Wilson's, 2005).

In the present study 74 sputum samples yielded 96 isolates. Out of 96 isolates *Candida* spp. were 55 (*C. albicans* in 26 samples, non albicans in 29 samples) showed the change in the trend of candidial infections towards non albicans spp. As non albicans spp. like *C. krusei* and *C. glabrata* develop resistance to fluconazole, commonly used antimycotic drug for prophylaxis, isolation of more number of non *Albicans* spp. is gaining importance. Sailaja et al. (2004) in Hyderabad, India also isolated more number of non albicans spp. in 18 cases than *C. albicans* in 6 cases (Sailaja et al., 2004). Aruna Agarwal et al. (2005) Punjab isolated *C. albicans* in 20 (62.5%) out of 32 isolates (Usha et al., 2005). Jha et al. (2006) from Khatmandu isolated 20 *C. albicans* and 10 non albicans from 462 samples of lower respiratory tract infections (Jha et al., 2006). Prasobh et al. (2009) Tamilnadu, performed resistotyping of 350 *C. albicans* isolated from sputum samples. All the above studies were geographically from the same region. C.albicans was isolated from sputum samples in all these studies. In the study of opportunistic fungal infections in HIV/AIDS patients by Rakhamanova (1998), the culture positivity for *C. albicans* and *C. neoformans* in pulmonary infections was 4% each (Rakhmanova et al., 1998). Yongabi et al. (2009) isolated 12 strains of *C. albicans* from 98 sputum samples *albicans* spp. predominate in the present study and study by Sailaja et al. (2004) where as *C. albicans* was predominate species in Jha study. Only *C. albicans* was mentioned in other studies. But the importance of *C. albicans* as causative agent in pulmonary infection was observed in all these studies.

Aspergillus spp. were the next commonest fungi isolated in the present study in 13 samples (13.5%). *A. fumigatus* and *A. niger* each from 6 samples and *A. flavus* from one sample were isolated where as Sailaja et al. isolated *A. niger* in one case. Nash et al. (1997) identified 17 cases of AIDS related pulmonary aspergillosis at autopsy (Nash et al., 1997). Mylonakis et al. (1998) after reviewing 342 AIDS cases concluded that invasive pulmonary aspergillosis usually occur among patients with less than 50 CD_4 cells /cumm (Mylonakis et al., 1998).

C. neoformans was isolated from 5 samples (5.2%) in the present study, 2 samples from a study by Sailaja et al. (2004). Isolation was 4% by Rakhamanova (1998) and 2.04% by Yongabi et al. (2009). A retrospective study by Mirvium L Cameroon between 1981 and 1989 revealed 12 cases of cryptococcal pneumonia with culture positivity in 10 cases (Mirium et al., 1990). *C. neoformans* as causative agent of pulmonary fungal infection was established in all these studies. Other organisms isolated were *Penicillum* spp. (including two *P. marneffei*) in six *Geotrichum candidicum* in four, *Wangiella dermatitidis* and *Scedosporium apiospermum* each one in two, *Cunnighamella bertolletiae, A. corymbifera, Nigrospora* spp. and *Prototheca* spp. each one in one sample. The above fungi were isolated for the first time in our geographical area. The emerging fungus *P. marneffei* was isolated from sputum in two samples in this study. The same was reported from four autochthonous cases by Singh et al. (1999) from Manipur, India (Singh et al., 1999). Ranjana et al. (2002) reported disseminated *P. marneffei* infection in 50 cases from the same hospital (Ranjana et al., 2002). CDC recognized Manipur, India, as new endemic area of this fungus and observed that penicillosis was seen in HIV positive patients who have CD_4 counts less than 100/cumm (Guidelines for prevention and treatment of opportunistic infections in HIV infected adults and adolescents, 2009).

Gupta et al. (2007) from New Delhi isolated *P. marneffei* from lymph node and blood cultures of HIV positive patient with multiple lymphadenopathy and massive hepatosplenomegaly. Bhagyabathi et al. (2009), Imphal, India isolated *P. marneffei* from HIV positive individuals whose CD4 counts were less than 100 (21.4%) (Bhagyabati et al., 2009). Bharathi et al. (2010) Thanjavur, India isolated the same in one case of HIV positive patient.

Conclusions

It is concluded that *Candida* species (*C. albicans* in 26 samples and non *albicans* in 29 samples) were the most common species isolated in the present study. Next common species were *Aspergillus* species and *Cryptococcus neoformans*. *Geotrichum candidicum, Wangiella dermatitidis, Scedosporium apiospermum*, *Cunnighamella bertholletiae, Absidia corymbifera, Nigrospora* spp. and *Prototheca* spp. were also isolated though in less number of cases, stressing the need for more number of studies for fungal pathogens in HIV positive patients. Isolation of *P. marneffei* an emerging fungal pathogen in two cases with CD_4 counts less than 100 in the present study. We made an attempt for the first time in Andhra Medical College, Visakhapatnam to isolate fungal pathogens from lower respiratory tract. Still it needs further and extensive study. It is necessary to screen all HIV positive cases with cough for fungal pathogens in addition to bacterial pathogens. By that early diagnosis and early treatment reduces further complications.

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REFERENCES

Anantanarayan, Paniker's (2009). Text Book of Microbiolgy,8th ed. Published by Universities press. Chapter 62 pp. 573 & Chapter 75: pp. 708.

Bailey, Scott (2002). Diagnostic Microbiology, Betty A .Forbes PH.D.,Daniel F Sahn Ph.D, Alice S Weissfeld Ph.D. 11th edition. Chapter 56: pp. 886-896.

Bhagyabati Devi S, Santi Devi Th, Robinson Ningshen Ng, Ranjana Devi Kh, Bhimo Singh Th, Brajachand Singh Ng (2009). Penicillium marneffei, an Emerging AIDS-related pathogen—A RIMS Study. J. Indian Med. Assoc., 107(4): 208-211.

Bharathi B, Siva Sankar S, Swamidoss Danial (2010). Incidence of bacterial and fungal coinfections in some HIV infected Indian population. Indian J. Biotechnol., 3(2): 199.

Carol A Kauffman MD, Michigan (1998). Fungal Infections in immunocompromised Hosts: Focus on Epidemiologic Aspects of Infection. National Foundation for Infectious diseases. 1: 4

Guidelines for prevention and treatment of opportunistic infections in HIV infected adults and adolescents (2009). National Institutes of Health, The Centers for Disease control and Prevention and The HIV Medicine Association of the infectious diseases Society of America. Morbidity, mortality Weekly Report (MMWR), 58(4): 98.

Gupta S, Madhur P, Maskey D, Wig N, Singh S (2007). Immune restoration syndrome with disseminated Penicillium marneffei and Cytomegalo virus co infection in an AIDS patient. AIDS Res. Ther., 4:21 doi: 10. 1186/1742- 6405-4-21.

Jagdish C (2009). Textbook of Medical Mycology. 3 rd ed. Mehta Publishers Chapter 4 PP 45 & 47.Appendix C: pp 523.

Jawetz, Melnick, Adelberg (2007). Medical Microbiology. 24th ed. Published by McGraw Hill Medical Chapter 44. pp.612.

Jha BK, Dey S, Tamang MD, Joshy ME, Shivananda PG, Brahmadatan KU (2006). Charecrerization of Candida species from cases of lower respiratory tract infection. Khatmandu Univ. Med. J., 4(15): 290-294.

Koneman color Atlas and Text book of Diagnostic Microbiology (1997). 5th ed. Publisher Lippincott. Chapter 2, 3 and19: pp.129, 131, 990.

Mackie, McCartney (2008): Practical Medical Microbiology.14th ed.Publisher Churchill Livingstone. Chapter 41, pp. 695-720.

Mirium L Cameroon, John A.Bartlett, Harry A. Gallis, Hetty A. Wasker (1990). Manifestations of pulmonary cryptococcosis in patients with Acquired Immunodeficiency Syndrome". From division of infectous diseases, Dept. of Medicicne, Duke university Medical Centre, Durhan, North Carololina.

Mylonakis E, Barlam TF, Flanigan T, Rich JD (1998). Pulmonary Aspergillosis and Invasive disease in AIDS: Review of 342 cases, Chest., 114: 251-262.

NACO Specialist's Training and Reference Module (2000). Ministry of Health and Family Welfare, Govt. of India. pp. 114 -116.

Nash G, Irvine R, Kerschmann RL, Herndier B (1997). Pulmonary Aspergillosis in Acquired Immunodefficiency Syndrome. Autopsy study of an emerging pulmonary complication of human immuno deficiency virus infection. Hum. Pathol., 28: 1268-5

Prasobh KK, Udhaya V, La kshmi Saraya Y (2009). Resistotyping of Candida albicans isolated from sputum samples patients attending to TB clinics in and around Chidambaram. Recent Res. Sci. Technol., 1: 4.

Rakhmanova A, Gyaurgieva OH, Romanova EL, Gurkalo GM, Prigozhina VK, Viasov NN, Chaika NA (1998). Oppurtunistic fungal infections in HIV/AIDS patients. International conference on AIDS. 1998; 12: 308 (Abstract no.22229)

Ranjana KH, Priyokumar K, Singh TJ (2002). 'Disseminated Penicillum marneffei infection in HIV infected patients in the Manipur state, India. J. Infect. Dis., 45(4): 268-271.

Sailaja VV, LA Pai, Dr. Madhur, V Lakshmi (2004). Lower respiratory tract infections in patients with HIV infection, Ind., J. Med. Microbiol., (22) 1: 28.

Singh PN, Ranjana K, Singh YI (1999). Indigenous disseminated *Penicillum marneffei* infection in the state of Manipur, India. J. Clin. Microbiol., 37: 2699-2702.

Topley, Wilson (2005): Microbiology and Microbial Infections: Bacteriology 10th ed. Published by Hodder Arnold. Chapter 12, pp 293.

Topley, Wilson (2005). Microbiology and Microbial Infections:Medical Mycology 10th ed. Published by Hodder Arnold. Chapters 1,9,30,33,35 and 36, pp. 11,371,375,585-6,661,663,740 and 756.

Usha Arora, Aruna Aggarwal, Renuka Bazaz, Kavita Kumari (2005). Clinico-Microbiological Study in HIV Seropositive patients. J. Indian Acad. Clin. Med. (JIACM), 6(2): 142-145.

Yongabi KA, Mbacham WF, Nubia KK, Singh (2009).Yeast strains isolated from HIV seropositive patients in Cameroon and their sensitivity to extracts of eight medicinal plants. Afr. J. Micro. Res., 3(4): 133-136.

ANNEXURE 1

BIO MEDICAL RESEARCH ETHICS COMMITTEE ANDHRA MEDICAL COLLEGE

VISAKHAPATNAM

LETTER OF CONSENT

This letter of consent is hereby accorded to ..... Dr. M. Bharathi - Asst. Prof. Microbiology *for conducting the research work titled* Study of fungal pathogens in sputum of HIV positive individuals .....after the necessary scientific evaluation and ethical review of the above cited research protocol, by the ETHICS COMMITTEE, ANDHRA MEDICAL COLLEGE, VISAKHAPATNAM.

SECRETARY

ETHICS COMMITTEE Dt. 2/6/10

CHAIR PERSON