

Medical Mycology in India (1957-2007): Contributions by the VPCI Mycoses Group*

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INTRODUCTION

The Mycoses Group of Vallabhbhai Patel Chest Institute (VPCI) comprises the past and the present faculty and research scholars working in the Departments of Medical Mycology, Clinical Research and Pathology. The aim of this paper is to present an overview of the notable contributions made by the Mycoses Group during the last 50 years and to reflect upon the magnitude of the challenging task that lies ahead. The paper is prefaced with a brief historical note on genesis of the Department of Medical Mycology in the Institute.

A small diagnostic Mycology Unit was set up in 1957 at VPCI with an ICMR-sponsored project on bagassosis under Professor Raman Viswanathan, Founder-Director of the Institute as its Principal Investigator, in which Dr H.S. Randhawa joined as Assistant Research Officer (Mycologist). The Unit initially functioned as a part of the then Department of Bacteriology, headed by Dr (Lt. Col.) A.J.H. deMonte, and later re-named as Department of Microbiology. In its formative stage, the Mycology Unit benefited considerably from the unstinted support of Dr H.D. Tandon, a founding faculty member of the Department of Pathology, VPCI, and Dr Hans F. Smetana, an eminent visiting pathologist from the Armed Forces Institute of Pathology, Washington, DC, USA, who served VPCI under a Technical Cooperation Mission. It was magnanimous of Dr Smetana to put the Mycology Unit in contact with a globally renowned mycologist, Dr Chester W. Emmons, Chief, Medical Mycology Section, National Institutes of Health, Bethesda, USA, who generously provided his invaluable expertise and material scientific assistance in the growth and development of Clinical Mycology in VPCI for more than a decade until he retired. The Mycology Unit was also fortunate to receive liberal assistance and guidance from another distinguished expert, Dr Libero Ajello, Director, Mycology Division, Centers for Disease Control, Atlanta, USA. In September 1959, VPCI established a full-fledged Department of Medical

Mycology with recruitment to one post each of Assistant Director, Senior Research Officer and Junior Research Officer, and the requisite technical and supportive staff. Over the years the Department of Medical Mycology has globally emerged as a leading centre for research and training in fungal diseases of the lungs and other internal organs. Several faculty members and former research scholars of the department have received coveted international honours such as the Lucille George ISHAM Award, Vice-Presidency of the International Society for Human and Animal Mycology and editorship or Editorial Board membership of reputed specialty journals such as *Medical Mycology*, *Mycoses* and *Mycopathologia*. A succinct account of the significant scientific contributions made by the Medical Mycology Department through inter-departmental and inter-institutional collaboration at national and international level follows:

DISCOVERY OF NEW FUNGI

A yeast isolated from cerebrospinal fluid (CSF) of a fatal case of meningitis in 1958 and subsequently from sputum of another patient was characterised and reported as a new species designated as *Candida viswanathii* in honour of the visionary Founder-Director of VPCI, the late Professor Raman Viswanathan. Circumstantial evidence of its original isolation from CSF and data on experimental pathogenicity in animals suggested that *C. viswanathii* was a potential pathogen.^{1,2} A taxonomic description of this species has been included in *The Yeasts*, a monograph and in many other reference books of Medical Mycology.^{3,4} Among the other new fungi discovered later by the mycologist faculty and research scholars included three keratinophilic species, viz., *Aphanoascus terreum*, *Chrysosporium indicum* and *Chrysosporium evolceanui*. Besides, a new species of *Penicillium*, *P. indicum*, isolated from sputum of an asthmatic patient and a new variety, *Aspergillus nidulans* var *dentatus*, isolated from a case of nail infection, were reported.⁵⁻⁹

*Dedicated to the late Professor Raman Viswanathan, formerly Editor-in-Chief of the IJCDAS and Founder-Director of VPCI, and his eminent successor, the late Professor A.S. Paintal, FRS, for their keen interest and support to the Department's research programme on pulmonary and systemic mycoses.

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NEW LABORATORY TECHNIQUES

A number of new techniques for rapid isolation and/or identification of systemic pathogenic fungi were developed and introduced. This includes Berthelot colour reaction as a substitute for the conventional Christensen urea agar for rapid testing of urease activity in yeast-like fungi, L-Dopa pigmentation test for rapid identification of cultures of *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis, and simplified Staib's medium for selective isolation and presumptive identification of *C. neoformans* and *C. gattii*. Besides, a modified inositol assimilation test was developed for identification of the genus *Cryptococcus*. For further information pertaining to these laboratory diagnostic techniques, reference may be made to an earlier review article.¹⁰ Information about indigenous in-house preparation of immunodiagnostic reagents and their clinical application has been given under specific mycotic disease headings. Also, we have evaluated anhydrous silica gel for long-term preservation of fungi. This technique was first used in 1962 by Perkins for maintenance of *Neurospora* cultures. The method was later applied successfully by Gentles and Scott to some dermatophytes and yeast-like fungi which remained viable for periods of five or more years. Extending these observations, Khan *et al*¹¹ from our laboratory demonstrated that 32 cultures representing 16 species of fungi and two of thermophilic actinomycetes, namely *Thermoactinomyces vulgaris* and *Micropolyspora faeni*, were maintainable in anhydrous silica gel at -20 °C for as long as 12 to 13 years. The method is highly advantageous in that it is far more convenient and less expensive than lyophilisation, thus warranting more extensive trials and a wider application.

NOCARDIOSIS AND HYPERSENSITIVITY PNEUMONITIS DUE TO THERMOPHILIC ACTINOMYCETES

Nocardiosis is a bacterial disease, usually characterised by primary pulmonary lesions which may be subclinical or pneumonic, transitory or chronic with haematogenous dissemination to various organs including the central nervous system. It is caused by several species of *Nocardia* which are aerobic and occur in soil as saprobes. Species of *Nocardia* produce thin, branched and septate filaments morphologically resembling hyphae. They were initially classified among fungi purely because of their morphological resemblance. Besides, nocardiosis clinically mimics systemic mycoses. In view of these similarities of the aetiological agents to fungi, nocardiosis has been dealt with in many earlier textbooks of Medical Mycology and it came to be included in the research programme of the Mycoses Group of VPCI.

Pulmonary Nocardiosis: An Epidemiologic Study

In a comprehensive environmental survey, employing the selective paraffin bait technique, it was demonstrated that *Nocardia asteroides*, *Nocardia brasiliensis* and *Nocardia caviae*, three of the multiple aetiological agents of actinomycetoma and pulmonary nocardiosis, were widely distributed in soil all-over India¹². Subsequently, the paraffin bait technique was applied to the selective isolation of *Nocardia asteroides* from sputum which harbours a variety of contaminant bacteria and fungi that interfere with its isolation.^{13, 14} The introduction of this technique in clinical surveys of nocardiosis in patients with bronchopulmonary diseases conducted in Delhi and Amritsar revealed an over-all prevalence of 1.7% and 1.4%, respectively.^{15, 16} Of the various bronchopulmonary diseases investigated, the clinical category of pulmonary tuberculosis negative for acid-fast bacilli had a higher prevalence of 4.6 % in Delhi. Significantly, introduction of the paraffin baiting technique in the studies led to *ante-mortem* diagnosis of the largest series of bronchopulmonary nocardiosis cases reported from India, indicating that it needed to be reckoned with in the differential diagnosis of any obscure bronchopulmonary disease. It may be pointed out that the work on application of paraffin bait technique for the laboratory diagnosis of nocardiosis has been cited in several textbooks of Medical Microbiology and Infectious Diseases. It is noteworthy that prior to our clinical application of paraffin bait technique, most of the nocardiosis cases reported worldwide were diagnosed only *post-mortem*.

Pulmonary Nocardoma (Nocardial Ball)

A rare case of ball formation due to a *Nocardia* species occurring in bronchiectatic cavity of a 40-year-old housewife was reported by Kurup *et al*.¹⁷ The patient gave history of cough with expectoration for three years and recurrent haemoptysis of six months duration at the time of admission to VPCI in August 1963. Following a bout of severe haemoptysis during her investigation and observation she was transferred to Silver Jubilee Tuberculosis Hospital, Delhi, later re-named Rajan Babu Tuberculosis Hospital, where she underwent thoracotomy. The resected right lower lobe revealed a greyish white mass, measuring 1.4 cm × 1 cm, which proved to be a nocardial ball upon direct microscopy, histopathologic and culture examinations. The patient's postoperative recovery was uneventful and she was hale and hearty during one year of follow up. Previously six cases of nocardial ball in pulmonary cavities had been reported in the literature. We propose that such cases of nocardial ball in the lungs may be termed as 'Nocardomas'.

Pathogenicity of *Nocardia* species for Laboratory Animals

The pathogenicity of multiple isolates of *N. asteroides*, *N. brasiliensis* and *N. caviae* was tested extensively in white mice, guinea pigs, rabbits and chorio-allantoic membrane of the developing chick embryo.¹⁸ All of the 14 test strains belonging to the three species originating from soil, human and animal sources were found to be pathogenic. *Nocardia caviae* strains were invariably more virulent than those of *N. asteroides* and *N. brasiliensis*. Also, *N. caviae* was equally virulent for white mice, guinea pigs and rabbits, whereas *N. asteroides* and *N. brasiliensis* were less virulent for guinea pigs and rabbits than for white mice. Histologically, the lesions caused in the various organs were in the form of abscesses, showing granules or filaments and cocco-bacillary bodies due to *N. caviae* and only filaments or coccobacillary bodies due to *N. asteroides*. In contrast, *N. brasiliensis* consistently produced granules in the lesions. It was further noted that chorio-allantoic membrane of the developing chick embryo yielded quicker and more consistent results in pathogenicity tests than with the various laboratory animals. The low cost of the eggs and the ease with which they can be handled are additional points in support of using them in the pathogenicity tests on *Nocardia* species. In subsequent studies, a murine model of nocardiosis was developed in normal and cortisone treated mice with the afore-mentioned three *Nocardia* species, employing the intravenous route of infection with a pre-determined LD₅₀ inoculum dose.¹⁹ It was observed that the three *Nocardia* species caused a higher and more rapid mortality, as also more extensive lesions, in the cortisone treated than in the normal mice. The LD₅₀ of *N. asteroides* and *N. caviae* was seven- and eight-fold lower than their respective inoculum doses for the normal mice. Furthermore, LD₅₀ of *N. brasiliensis* for cortisone treated mice was twenty-six and thirty-fold higher than that of the preceding two species.

Hypersensitivity Pneumonitis and Thermophilic Actinomycetes

Of the varied forms of extrinsic allergic alveolitis, farmer's lung disease (FLD) has been most frequently reported. It is an immuno-inflammatory disease, manifested by cough, malaise, breathlessness and fever developing in susceptible individuals following repeated exposure to thermophilic actinomycetes colonizing decaying vegetable matter such as hay or straw undergoing self-heating. Investigations on this problem were initiated at VPCI in the early 1980's.

Environmental survey. A survey of clinically important thermophilic actinomycetes, covering 341 samples of soil and vegetable substrates collected from a number of sites in Delhi, U.P., Punjab and Jammu and

Kashmir was conducted.²⁰ *Thermoactinomyces vulgaris* was the commonest species isolated, occurring in 56% of the samples, followed by *Saccharomonospora viridis* (29%), *Thermoactinomyces thalpophilus* (27%), *Saccharopolyspora rectivirgula* (*Faenia rectivirgula*/*Micropolyspora faeni*) in 21% and *Thermoactinomyces sacchari* in 14 percent. The commonest substrate for *S. rectivirgula* was hay, yielding 44% of its isolates. This widespread environmental distribution of thermophilic actinomycetes suggested that exposure of humans and animals to these microbes would be frequent in north-western India, warranting investigation of their role in hypersensitivity pneumonitis.

Aeromicrobiologic and sero-epidemiologic study. The study led to an investigation of aerial prevalence of the same actinomycetes and of the occurrence of precipitating antibodies against them in sera of workers employed in two cane-sugar mills, i.e. Ramala Co-operative Sugar Mills, Baraut and Upper Doab Sugar Mills, Shamli, in Uttar Pradesh, India.²¹ The predominant aerial species found in both the mills was *T. sacchari* (prevalence 50% to 55%), followed in descending order by *T. vulgaris*, *T. thalpophilus*, *S. viridis* and lastly *S. rectivirgula* with the lowest prevalence of 0.7 percent to 3.9 percent. Precipitating antibodies against thermophilic actinomycetes were demonstrable in 34 (22%) of 153 workers. Of these, *T. sacchari* alone accounted for 20 of the positive precipitin reactors, followed by *S. rectivirgula* in 10. Interestingly, the mean absorbance values for immunoglobulin (IgG) antibody activity against *T. sacchari* and *S. rectivirgula* were found to be elevated significantly in the symptomatic workers than in the asymptomatic group. The study showed widespread occurrence of thermophilic actinomycetes in cane sugar mills and that *T. sacchari* and *S. rectivirgula* were the main species involved in sensitisation of the workers.

Farmer's lung disease. An exploratory study on the prevalence of farmer's lung disease (FLD) was conducted in collaboration with the Departments of Clinical Research and Cardio-Respiratory Physiology.²² The test subjects were 197 workers engaged in farming in Delhi area and having respiratory complaints of variable duration. Of these, 60 (30%) workers related their symptoms to exposure to wheat straw/thresher's dust or other vegetable substrata in their working environment. An overall prevalence of 13.2% precipitating antibodies against clinically important thermophilic actinomycetes was recorded. Of this, *S. rectivirgula* alone accounted for 85% of the positive reactors whereas *T. vulgaris* and *T. thalpophilus* had only 1.5% and 0.5% precipitin positive subjects, respectively. Two workers concomitantly demonstrated *S. rectivirgula* and *T. vulgaris*-specific serum precipitins. Based upon a suggestive clinical history, roentgenography, pulmonary

function studies and demonstration of serum precipitins against *S. rectivirgula*, FLD was diagnosed in four workers. These observations indicated that a comprehensive, country-wide survey of the prevalence of FLD in India was warranted in view of its large farming population.

BRONCHOPULMONARY ASPERGILLOSIS

Aspergillosis comprises a wide clinical spectrum of infections caused by *Aspergillus fumigatus* and by other species of *Aspergillus*. It includes aspergilloma, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, *Aspergillus* sinusitis and invasive aspergillosis. A definitive diagnosis of aspergillosis requires demonstration of the fungus in tissues/appropriate clinical specimens and isolation of the aetiological agent in culture. The highlights of work done on aspergillosis are as follows:

Aspergilli of Human Respiratory Tract

A comprehensive survey of *Aspergillus* species associated with the human respiratory tract was carried out during 1961-64 by Sandhu and Sandhu.²³ They isolated and characterised 29 species and one new variety of *Aspergillus* from 1,238 clinical specimens collected from 812 patients with various bronchopulmonary diseases registered in the Clinical Research Centre of VPCI. During the same period, four cases of aspergilloma were diagnosed in patients referred for mycological investigation from Rajan Babu Tuberculosis Hospital, Delhi.²⁴ Two of these had *A. niger* as the aetiological agent whereas the third had *A. fumigatus*. The fourth case was diagnosed by histopathologic examination alone and its aetiological species remained undetermined. It seems pertinent to mention here that the first case of aspergilloma from India had also been reported previously from VPCI by Gupta and Viswanathan.²⁵

Aspergillus fumigatus in vitro Inhibition by *Candida albicans* and a New Culture Medium

Our laboratory was the first to report on the *in vitro* inhibition of *Aspergillus fumigatus* by *Candida albicans*, a well known commensal of the human respiratory tract and its coloniser in bronchopulmonary diseases.²⁶ This work was corroborated by Becker *et al* from Germany.²⁷ Subsequently, we developed peptone glucose fluconazole agar as a selective medium for rapid and enhanced isolation of *A. fumigatus* from respiratory tract of bronchopulmonary aspergillosis patients colonised by *Candida albicans*.²⁸ The availability of this selective medium has facilitated a rapid laboratory diagnosis of aspergillosis in patients with *C. albicans* colonisation of respiratory tract which otherwise is likely to be delayed, if not missed altogether.

Allergic Bronchopulmonary Aspergillosis

Work on the indigenous preparation and standardisation of immunodiagnostic reagents for skin testing and serologic diagnosis carried out by Sandhu and co-workers in our laboratories in the late 1960's led to the recognition of allergic bronchopulmonary aspergillosis (ABPA) as an important clinical entity which had been previously masquerading in India as pulmonary tuberculosis, bronchial asthma or some unknown respiratory disorder.²⁹ This was followed by publication of a comprehensive study on 46 cases of ABPA with special reference to laboratory diagnostic aspects by Khan *et al*.³⁰ In another noteworthy study on the natural occurrence of *Aspergillus fumigatus* in cane-sugar mills and its correlation with the occurrence of ABPA in the mill workers, it was reported that persistent exposure to high environmental concentration of the aetiological agent would not necessarily lead to any marked sensitisation or development of ABPA.^{31, 32} In other words, constitutional predisposition of the host appeared to be a more important factor than the extent of exposure to the fungus in the development of ABPA. These studies stimulated greater awareness and interest in ABPA which led to its recognition as one of the commonest bronchopulmonary mycoses in India.³³ It is recognised that *A. fumigatus* is the principle aetiological agent of ABPA. However, other species of *Aspergillus* such as *A. terreus* may play a contributory role or they may also act as the primary aetiological agent. Khan *et al*³⁴ have reported an interesting case of ABPA in which *A. terreus* and *A. fumigatus* acted as successive aetiological agents. As far as we are aware, no other ABPA case of this type has been reported in the literature.

Allergic *Aspergillus* Sinusitis/Allergic Fungal Sinusitis or SAM Syndrome

In 1983, Katzenstein *et al*³⁵ demonstrated that *Aspergillus* species could cause mucoid impaction in the paranasal sinuses akin to that of ABPA. They termed this newly recognised clinico-pathologic entity as allergic *Aspergillus* sinusitis (AAS). Later studies showed that apart from *Aspergillus* species, dematiaceous fungi were also aetiological agents of this disease. Consequently, the name AAS was replaced by allergic fungal sinusitis (AFS).³⁶ The occurrence of AAS in India was first recognised by Shah *et al* from VPCI who described two cases.^{37, 38} One of the subjects was a 26-year-old male business executive, resident of New Delhi, who complained of progressive nasal symptoms of bouts of sneezing, purulent discharge, occasional blockage, passage of brownish plugs, breathlessness, frontal headache, etc. The patient showed serum precipitating antibodies as well as strong type I and type III cutaneous hypersensitivity against *A. fumigatus* and *A. flavus*. Cultures of pathological material from maxillary sinuses yielded heavy growth of *A. fumigatus*. The

diagnosis of AAS was further supported by elevated total and *A. fumigatus* specific IgE levels, histopathologic observations similar to those of mucoid impaction in ABPA, and dramatic relief of symptoms following therapy with prednisolone. The second case was a 24-year-old male student who was also a resident of New Delhi. He had nasal symptoms, occasional wheezing and mucopurulent expectoration with brownish plugs. His detailed clinical and laboratory evaluation led to the diagnosis of concomitant AAS and ABPA, a rarely reported association in those days. Subsequently, with enhanced awareness of the disease numerous concomitant cases of this type came to be diagnosed by VPCI investigators and also from other centers in India.^{39, 40} Notably, the predominant aetiologic agent in cases of AFS in our country is *A. flavus*. Taking cognisance of the cases of concomitant occurrence of AAS and ABPA reported by Shah *et al* and investigators outside India, Venarske and deShazo⁴¹ contended that the presence of concomitant AFS and allergic bronchopulmonary mycosis in the same patient represents an expression of the same process of fungal hypersensitivity in the upper and lower airways. Thus, they have, coined the term SAM syndrome, an acronym for sinobronchial allergic mycosis and proposed the various diagnostic criteria which include chronic sinusitis involving multiple sinuses, asthma, immediate cutaneous reactivity to fungal allergens, peripheral eosinophilia and radiographic evidence of bronchiectasis.⁴¹

Invasive Pulmonary Aspergillosis (IPA)

Pulmonary involvement is the commonest manifestation of invasive aspergillosis. Patients with prolonged and profound neutropenia (< 100 neutrophils/ μ l) are at high risk for invasive aspergillosis. Other patients at high risk for invasive aspergillosis include patients undergoing organ and bone marrow transplantation and those receiving corticosteroids or other newer immunosuppressive therapies, including tumour necrosis factor- α (TNF- α) antagonists such as infliximab and others. The clinical presentation of IPA is variable involving progressive dry cough, dyspnoea, pleuritic chest pain, fever despite coverage with broad spectrum antibiotics, and pulmonary infiltrates. Diagnosis of IPA is reliant on a very high degree of clinical suspicion and documentation of tissue invasion in biopsy specimens or recovery of the fungus from otherwise sterile sites.⁴²

A rare case of invasive pulmonary aspergillosis in a one-month-old male child caused by *A. flavus* has been reported by Singh *et al*.⁴³ The case was diagnosed by repeated demonstration of hyaline septate hyphae in KOH wet mounts of bronchoalveolar lavage, histopathologic examination of a tissue mass in right main bronchus and repeated isolation of *A. flavus* in culture. His main predisposing factors were combined variable immunodeficiency/transient hypoglobu-

linemia of infancy and repeated administration of broad spectrum antibiotics for recurrent bacterial infections. The neonate was treated successfully by combined therapy of intravenous amphotericin B and oral itraconazole aided by partial removal of the infected tissue mass by wire basket and bronchoscopic suction. It was probably the first case of its type to survive the infection due to timely diagnosis and appropriate antifungal therapy. We believe that this solitary case may not be reflective of the actual occurrence of IPA in the patient population with chest diseases seeking consultation at VPCI.

Induction of an Avirulent Mutant in *A. fumigatus*

Mutant isolates deficient for p-aminobenzoic acid (PABA) or ammonium nitrogen were experimentally induced in a wild-type isolate of *A. fumigatus*, originating from lung biopsy of a case of aspergilloma.⁴⁴ The mutants developed following treatment of conidial suspension of the wild type *A. fumigatus* isolate with a mutagenic agent, N-methyl-N'-N-nitrosoguanidine (Aldrich Chemical Co. Inc., Milwaukee, Wis., USA). Notably both of the p-aminobenzoic acid requiring mutant isolates proved to be avirulent for normal as well as cortisone-treated white mice whereas the control wild-type strain was pathogenic. However, the mutants maintained their virulent character for those mice which had been administered PABA either intramuscularly or orally in drinking water. Immunisation of the mice by repeated intramuscular inoculation with viable conidial suspension of the PABA-requiring mutant strain served to protect the animals against intravenous challenge by a 10^6 spore suspension of the wild type *A. fumigatus*. Furthermore, immunogenicity of the PABA- requiring mutants compared favourably with the wild type strain of the fungus in agar-gel double diffusion test, using clinical or hyperimmune sera and in skin tests on patients with ABPA. It seems pertinent to mention here that development of avirulent mutants is important in that it provides fungal strains that are entirely safe to handle in the production of immunodiagnostic reagents as well as for possible development of vaccines against fungal infection.

HISTOPLASMOSIS: AN EPIDEMIOLOGIC STUDY

Histoplasmosis is an endemic systemic mycosis caused by the small-celled yeast form of the fungus *Histoplasma capsulatum* variety *capsulatum* with a global distribution, or by the large celled yeast form of *Histoplasma capsulatum* variety *duboisii* with geographic distribution restricted to the African continent. The two forms of the disease are clinically and histologically distinct entities and have been termed as histoplasmosis capsulati (classical or small-form histoplasmosis, Darling's

disease) and histoplasmosis duboisii (African or large-form histoplasmosis).⁴⁵

An exploratory epidemiologic study on histoplasmosis was carried out in Delhi area during 1957-59 by skin testing with histoplasmin small segments of population residing in rural or riverine area and also the out-patients attending the Clinical Research Centre of VPCI and a Tuberculosis Clinic in old Delhi.⁴⁶ The prevalence of 12.3% histoplasmin sensitivity found in the riverine area was the highest ever reported from any part of India. However, no active case of histoplasmosis documented by culture or histologic examination was found among the histoplasmin positive reactors either in this pilot study by Viswanathan *et al*⁴⁶ or subsequently by Basu Mallick who investigated a larger population sample of 8062 and found 6.8% prevalence of histoplasmin sensitivity.⁴⁷ The first autochthonous case of healed pulmonary histoplasmosis diagnosed at VPCI by histopathology was reported by Chakravarty *et al*.⁴⁸ Subsequently, a well documented autochthonous case of disseminated histoplasmosis involving a rare extension to epididymis was reported by Randhawa *et al*.⁴⁹ The patient was a 55-year-old male industrialist, resident of Saharanpur, Uttar Pradesh, India. He was diagnosed by histopathologic examination of an epididymal biopsy and culture of *Histoplasma capsulatum* from his semen. He was successfully treated with surgical excision of epididymis, followed by therapy with amphotericin B (dose 2.3g over 11 weeks). An updated review of work done on histoplasmosis in India (Randhawa, 2007, unpublished) revealed that since 1954 about 75 authentic cases of the disease have been reported from different parts of the country. However, the disease has not been documented in any domestic or wild animals, nor has the aetiologic fungus been isolated from environmental sources, barring a solitary isolation from soil containing bat guano collected from a 350-year old palatial building in Sirampore near Calcutta.⁵⁰ In order to explore the possible role of local bats in the environmental dissemination of *H. capsulatum*, Randhawa *et al* cultured the visceral organs of 849 bats representing four insectivorous and one fungivorous species captured from Delhi/New Delhi metropolitan area but the results were negative.⁵¹

BLASTOMYCOSIS

Blastomycosis caused by the thermal dimorphic fungus *Blastomyces dermatitidis* is another endemic systemic mycosis. It has been only sporadically reported from India. It is apparent from the publications reviewed hereunder that the evidence about endemicity of the disease in our country and information pertaining to its other aspects has largely emanated from the Mycoses Group of VPCI.

Epidemiologic Aspect

Epidemiologic investigations carried out by Khan *et al*⁵² and Randhawa *et al*^{53, 54} in the 1980's provided strong evidence for the endemic occurrence of blastomycosis in India. The evidence was based upon authentic isolations of *Blastomyces dermatitidis* from visceral organs of local insectivorous bats belonging to *Rhinopoma hardwickei hardwickei*, and from the bronchial aspirate of a benign case of pulmonary blastomycosis in a house-wife, resident of Badaun, Uttar Pradesh, India, who had never traveled abroad to a country known for endemic occurrence of the disease.

In vitro Mould-Yeast Conversion

An important criterion for definitive identification of *B. dermatitidis* is verification of its dimorphic character by mold-yeast conversion through its cultivation at 37 °C on pharmamedia, a cotton seed based proteinaceous product, marketed by the Buckeye Cellulose Corporation, USA. After extensive trials our laboratory devised several agar media based upon aqueous extract of any of the local varieties of cotton seed (*Gossypium hirsutum* or *G. arboreum*) or the commonly available garden pea seed (*Pisum sativum*) which serve as inexpensive and excellent indigenous substitutes for the imported pharmamedia.^{55, 56}

Evaluation of Primary Culture Media

Smith and Goodman⁵⁷ reported in 1975 that *C. albicans*, a commensal of the human respiratory tract, interfered with the isolation of *B. dermatitidis* from clinical specimens harbouring *C. albicans*. A comparative evaluation of various culture media for isolation of *B. dermatitidis* from sputum colonised by the antagonistic *C. albicans* was therefore undertaken. The results highlighted the inadequacy of routine peptone glucose agar media fortified with antibiotics for primary isolation of *B. dermatitidis*. It was shown that BHI-egg albumin agar, followed by the yeast extract phosphate agar, were the media of choice for primary isolation of *B. dermatitidis* from sputum harbouring *C. albicans*.⁵⁸

Interactions with Pathogenic Fungi

Investigation of *in vitro* interactions between *B. dermatitidis* and other pathogenic fungi revealed that *Pseudoallescheria boydii* and *Sporothrix schenckii* were markedly antagonistic to *B. dermatitidis* whereas *C. neoformans* actually lysed it.⁵⁹ These observations supported the concept that *B. dermatitidis* is a poor competitor *vis-à-vis* other fungi in nature and that is why it has been seldom reported from environmental sources.

Murine Model of Systemic Blastomycosis

A non-lethal experimental murine model of systemic

blastomycosis was developed, employing the intravenous route of infection with approx. 10^2 yeast form cells with each of the five *B. dermatitidis* strains of diverse origin. Groups of 20 white mice were infected with each of the test *B. dermatitidis* strains.⁶⁰ Five mice from each of the groups were sacrificed at 2, 4, 6 and 8 weeks post-infection, and the following parameters were studied: (1) organ distribution of lesions, (2) organ load of the infecting *B. dermatitidis* strain, (3) delayed type of hypersensitivity response, and (4) antibody response. The lungs proved to be the target organs of infection regardless of the test *B. dermatitidis* strain. Lesions in liver and spleen were caused by only the most virulent *B. dermatitidis* strain, S-3922. All of the *B. dermatitidis* test strains elicited cellular and humoral immune response which peaked at four weeks post-infection. The most virulent strain, S-3922, elicited the maximum footpad swelling (delayed type hypersensitivity) and the highest antibody titers whereas the least virulent strain, IP 973, elicited the minimum footpad swelling and the lowest antibody response. One of the significant findings was the frequent development of cutaneous lesions in mice infected with the African strain, IP-973. Going by the available literature, cutaneous lesions had not been previously reported in experimental studies on murine blastomycosis although they are known to frequently occur in clinical cases of disseminated blastomycosis. As strain IP-973 had the highest extra-cellular phospholipase-A activity, it was postulated that this attribute might be related to its ability to incite cutaneous lesions in the experimentally infected mice.

CRYPTOCOCCOSIS

Cryptococcosis is a major cosmopolitan systemic mycosis, with predilection for the central nervous system, caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. Epidemiologically, the two aetiological agents differ from each other mainly with respect to geographic distribution, preference for natural habitat, pathobiology and host infectivity (immunocompetent versus immunocompromised) and genetics. The primary focus of infection is almost always in the lung, which is usually subclinical and transient. However, the infection in other cases may be chronic, subacute or acute and fulminant, especially in debilitated patients when it is fatal if not diagnosed early and treated with antifungal therapy.^{61, 62} In VPCI, we have mostly come across cryptococcosis of the subclinical pulmonary type which resolves spontaneously without any therapy. A synopsis of our work on cryptococcosis follows.

***Cryptococcus neoformans* as Commensal or Transient Resident in Human Respiratory Tract**

About five decades ago it was believed that the isolation

of *C. neoformans* from sputum carried the same importance as that of acid-fast bacillus in pulmonary tuberculosis. Subsequently, some investigators raised the question of commensalisms or saprobic colonisation of respiratory tract by *C. neoformans*. Their studies and those by Randhawa and co-workers in VPCI showed that *C. neoformans* could be sporadically isolated as a transient resident or harmless coloniser from oropharyngeal washings and skin of healthy human population as also from sputum and bronchial aspirate of patients with bronchopulmonary diseases without any evidence of cryptococcosis.⁶³⁻⁶⁵ Nevertheless, a comprehensive clinical and mycoserologic evaluation was strongly advocated in all such patients to rule out the possibility of localised or systemic cryptococcosis, even though definitive signs of the disease might be lacking.

Natural Habitats of *C. neoformans* and *C. gattii*

Attention to old pigeon excreta as a natural habitat of *C. neoformans* was first drawn in India in 1966 by Sethi *et al.*^{66, 67} This association was confirmed by Khan *et al.*⁶⁸ and extended to other avian species by Randhawa *et al.*⁶⁹ and Pal *et al.*⁷⁰ On the other hand, information on the natural occurrence of *C. gattii* was lacking until Chakrabarti *et al.* reported its isolation from the flowers of *Eucalyptus camaldulensis* in Ferozpur, Punjab.⁷¹ It was noteworthy, however, that their results were negative with 623 additional samples of plant debris of *E. camaldulensis*, *E. tereticornis* and *E. citrodora*. In a more comprehensive study based upon investigations of a much larger sample of plant debris of *E. camaldulensis*, *E. tereticornis*, *Syzygium cumini*, *Ficus religiosa*, *Butea monosperma*, *Tamarindus indica* and other trees in north-western India, Randhawa *et al.* observed that the most important host tree for *C. gattii* as well as for *C. neoformans* in India was *S. cumini* and not any *Eucalyptus* species as reported from Australia.⁷²⁻⁷⁴ This conclusion was supported by the data on high prevalence, fungal population density and perennial colonisation of decayed wood in trunk hollows of the tree species investigated. Furthermore, these results reinforced an emerging concept that the primary ecologic niche of *C. gattii* as well as *C. neoformans* is not specific to wood or other plant debris of a particular tree species but instead is a more generalised phenomenon.

***Cryptococcus laurentii*: Melanin⁺ Variant and Its Pathogenicity**

Of considerable interest was a report by Mussa *et al.* of repeated isolations of a unique, melanin-forming (Mel⁺) variant of *Cryptococcus laurentii* from the hollowed part of an old decaying timber beam of salwood (*Shorea robusta*) found lying near the boundary wall of the Engineering Department, University of Delhi.⁷⁵ The persistent occurrence of the Mel⁺ variant in the decayed

beam demonstrated over a period of two and a half years and its considerable population density (mean 1×10^4 cfu/g of wood debris) indicated that decayed wood inside the hollowed part of the beam was its natural habitat. It was further noteworthy that the Mel⁺ variant incited macroscopic lesions on the liver and spleen of cortisone-treated (immunosuppressed) white mice infected intraperitoneally with its culture suspension. These observations suggested that the Mel⁺ variant of *C. laurentii* had a pathogenic potential and it may be advisable to be vigilant about its possible occurrence in clinical specimens and a potential aetiological role. Interestingly, the *C. laurentii* variant was also isolated on two occasions from the nest of two rose-breasted cockatoos (*Kakatoe leucorhynchos*) in an aviary of the National Zoological Park, New Delhi. Keeping in view the fact that the variant shares with *C. gattii* characteristics of phenoloxidase activity, a positive CGB reaction and inhibition of urease activity by EDTA, it would be advisable to bear in mind the possibility of its misidentification due to this phenotypic resemblance.

Swabbing versus Conventional Sedimentation Technique for Environmental Surveillance of *C. neoformans* and *C. gattii*

An in-house swabbing versus the conventional sedimentation technique was investigated for isolation of *C. neoformans* and *C. gattii* from decayed wood inside trunk hollows of living trees.⁷⁶ The data on significantly higher frequency of isolation, greater population density and low false positive results obtained with the swabbing technique demonstrated its superiority over the conventional sedimentation technique in ecological studies on *C. neoformans* and *C. gattii*. Employing this technique, it was shown that over 80% of the *S. cumini* trees in Delhi harboured the *C. neoformans* species complex in decayed wood inside their trunk hollows. The potential health hazard posed by such environmental foci of infection to the humans and animals inhabiting in their vicinity cannot be over-emphasised. Considering that the swabbing technique is more efficacious, simple, inexpensive, less time-consuming and safer to the sample collector, it warrants a wider application in studies on the environmental distribution of pathogenic cryptococci.

Antifungal Susceptibility

Khan *et al.*⁷⁷ reported the *in vitro* antifungal susceptibility testing of *C. neoformans* (serotype A, n=117) and *C. gattii* (serotype B, n=65) isolated from environmental sources in north-western India. A comparison of the geometric means of MICs (mg/L) revealed that *C. gattii* was less susceptible than *C. neoformans* to amphotericin B, fluconazole, itraconazole, ketoconazole and voriconazole. The results further showed that the occurrence of primary resistance in environmental

isolates of *C. neoformans* and *C. gattii* is rare. Investigations on the antifungal susceptibility of clinical isolates of *C. neoformans* and *C. gattii* are currently in progress. It seems pertinent to add that antifungal susceptibility testing has recently gained further importance, especially for patients who have been receiving prophylactic antifungal chemotherapy.

CANDIDIASIS

Candidiasis is a mycotic disease with an extremely wide spectrum of clinical manifestations caused by *Candida albicans* or by any of the many other species of *Candida*. The infection may be acute or chronic, superficial or deep-seated on any organ of the body. Pulmonary candidiasis is, however, more of an autopsy finding than a clinical entity. On the basis of repeatedly positive sputum cultures, Chakravarty (1967) reported a case of 'primary pulmonary candidiasis' in a 50-year-old male patient which subsequently proved to be paragonimiasis (lung fluke) upon histopathologic examination following his lobectomy.^{78, 79} This case underscores the importance of great care in interpreting the aetiological significance of positive cultures and direct microscopy of respiratory secretions for *C. albicans* which occurs as a frequent commensal or coloniser of the respiratory tract of patients receiving prolonged antibacterial antibiotics or corticosteroids for varied bronchopulmonary disorders. To the best of our knowledge, no authentic case of primary pulmonary candidiasis has been reported so far from India.

Myco-taxonomic and Antifungal Susceptibility Study

Early studies in the Medical Mycology Laboratory were concerned with isolation and identification of various species of *Candida* and other yeast genera such as *Cryptococcus* and *Trichosporon* occurring in the respiratory tract of patients with bronchopulmonary diseases or in clinical specimens originating from other parts of the body.^{80, 81} The predominant species found was *Candida albicans*, the principal aetiological agent of candidiasis, followed by *Candida tropicalis*, *Candida pseudotropicalis*, *Candida krusei*, *Candida guilliermondii*, *Candida claussenii*, *Candida rugosa*, *Candida mycoderma*, *Candida scottii*, *Candida parapsilosis*, followed by *Cryptococcus diffluens*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Hansenula anomala* and *Trichosporon cutaneum*. A particularly striking observation was the finding of a new species, *Candida viswanathii*, isolated from a CSF sample collected from a fatal case of meningitis. Also investigated was *in vitro* susceptibility to nystatin of 125 yeast isolates belonging to the genera *Candida*, *Hansenula*, *Rhodotulula* and *Trichosporon*.⁸² Barring a solitary resistant isolate of *T. cutaneum*, all of the test yeast isolates were found to be susceptible with their

MIC's in the range of 4-20 µg/mL. Apart from the aforementioned investigations, given below is a review of our other recent studies.

Pathogenicity of Ordinarily Harmless *Candida* spp and Other Yeasts

In view of an increasing number of reports incriminating saprobic or harmless fungi causing opportunistic systemic mycoses in immunocompromised hosts, an experimental study was conducted to investigate the pathogenic potential of yeast-like fungi such as *Candida requinyii*, *Candida famata*, *Pichia etchellsii*, *Sterigmatomyces aphidis*, etc. for white mice immunosuppressed with cortisone acetate administration.⁸³ The test species proved to be non-pathogenic to normal white mice challenged intravenously with an infective dose of up to 10⁷ cells. In strong contrast, the cortisone treated mice suffered mortalities ranging from 25% to 100% depending upon the infecting yeast species and inoculum dose during an observation period of three weeks. Based upon the data on mortality, macroscopic and histopathologic lesions on visceral organs, *C. requinyii* was rated as the most virulent species, followed by *S. aphidis*, *C. famata* and *Trichosporon cutaneum*. *Pichia etchellsii* was found to be the only test species that failed to incite any macroscopic or histopathologic lesions in the infected mice. These observations were in conformity with the rising trend of opportunistic systemic mycoses in immunosuppressed patients caused by fungi which had been widely regarded as harmless saprobes or contaminants in clinical specimens.

Candidemia Outbreak

Chowdhary *et al*⁸⁴ have reported an outbreak of candidemia due to *Candida tropicalis*, involving 16 neonates in Kalawati Saran Children's Hospital, New Delhi. *Candida tropicalis* was recovered from blood in all the 16 neonates whereas urine cultures were positive in 14 of them. Environmental sampling yielded *C. tropicalis* from blankets and mattresses used for the neonates. Arbitrarily primed polymerase chain reaction (PCR) revealed that the clinical and environmental isolates had the same genotype. Fourteen of the 16 neonates completely recovered from fungemia with amphotericin B therapy. The report highlighted the emergence of *non-albicans Candida* species as aetiologic agents of candidemia in neonates in a pediatric hospital. To the best of our knowledge, this was the first instance of an outbreak of candidemia due to *Candida tropicalis* in which clonal origin of the aetiologic agent was documented.

Molecular Techniques as Epidemiologic Tools

Molecular typing methods are used to ascertain the degree of relatedness among multiple strains of a

microbial pathogen in order to determine the extent to which serial isolates of a given pathogen from the same patient or isolates from different patients in one or more geographic areas are related to each other. The results obtained are vital for elucidating the epidemiology of a given infectious disease. Two important molecular typing tools, *i.e.* southern hybridisation with a complex probe Ca3 and multilocus sequence typing (MLST) have been employed to subtype isolates of *C. albicans* which can cause severe invasive infections in immunocompromised patients. The complex probe Ca3 has been extensively investigated for its usefulness in analysing serial isolates from recurrent *Candida* infection. The novel tool MLST is a DNA sequence based subtyping method shown to have high degree of intra species discriminatory power. However, its utility for studying the molecular epidemiology of sequential isolates from recurrent disease has not been established. This study evaluated the concordance of the two methods for their utility in clustering sequential oral isolates of *C. albicans* from 10 HIV positive patients with recurrent oropharyngeal candidiasis from India and USA.⁸⁵ In four of the 10 patients, serial isolates were indistinguishable by Ca3 but highly related by MLST, suggesting that MLST is capable of detecting minor genetic variation, or microevolution, even when it is difficult to see such differences using the Ca3 probe. Multilocus sequence typing is inherently more precise and less subjective than Ca3 fingerprinting and produces unambiguous data that are easily assembled into a database to facilitate data exchange and global epidemiological studies of this important opportunistic pathogen.

PULMONARY ZYGOMYCOSIS

Zygomycosis comprises a group of frequently fatal infections caused by a number of fungi belonging to the class Zygomycetes. Its wide clinical spectrum includes pulmonary zygomycosis which occurs mostly in patients with leukemia or lymphoma with severe neutropenia and corticosteroid therapy. The risk factors associated with respiratory zygomycosis also include diabetic ketoacidosis, granulocytopenia, deferoxamine therapy, neonatal prematurity and HIV infection. The disease has varied clinical manifestations, such as bronchopneumonia, segmental or lobar consolidation, cavitation, fungal ball formation, pleural effusion, vascular invasion, thrombosis, infarction, haemoptysis, fistulas, etc. Generally, the infection progresses rapidly but it can be subacute or chronic. Subacute pulmonary infection may go on for weeks or months, occurring in diabetics with pulmonary infiltrates. Open lung biopsy may be necessary to clinch the diagnosis.

A solitary case of pulmonary zygomycosis caused by *Rhizopus oryzae* in a 61-year-old, diabetic male farmer has been reported from VPCI by Diwakar *et al*.⁸⁶ The

patient had fever, productive cough, anorexia and generalised weakness. Computerised enhanced computed tomographic (CECT) scan of the chest revealed a cavitating lesion in the apical segment of the right lower lobe. His sputum examination was negative for AFB. Mantoux test was also negative. A computed tomographic (CT) guided fine needle aspiration biopsy (FNAB) had been performed in a private laboratory where it was erroneously diagnosed as invasive aspergillosis based upon histological examination. The diagnosis of zygomycosis in VPCI was based upon review of the H&E stained biopsy sections in the Medical Mycology Department revealing broad, aseptate, branching hyphae with focal bulbous dilatations. It was confirmed by repeated isolation of *Rhizopus oryzae* from a series of six consecutively collected sputum samples as also from his bronchial aspirate and bronchoalveolar lavage (BAL). The patient was treated successfully by surgical resection of his pulmonary cavity done under cover of amphotericin-B administration. An updated review of literature has revealed that 461 cases of zygomycosis have so far been reported from India. Of these, 269 cases had rhino-orbito-cerebral zygomycosis, followed by cutaneous/subcutaneous disease (66 cases), renal and gastrointestinal disease (34), disseminated (32) and pulmonary (28). The risk factors identified in the Indian cases were diabetes mellitus, cancer and post-transplant immunosuppression. Approximately 70% of the reported cases, including 25 of the 28 pulmonary cases had been diagnosed at Postgraduate Institute of Medical Education and Research, Chandigarh. This is attributed to better expertise, infrastructural facilities for mycological diagnosis and enhanced awareness of the interdisciplinary faculty working jointly on this disease. We strongly believe that the actual incidence and prevalence of pulmonary zygomycosis in India would be much higher than indicated by the published reports. Therefore, a high index of clinical suspicion and vigorous laboratory investigations are warranted for early diagnosis of zygomycosis and to reduce the morbidity and mortality associated with it.

MISCELLANEOUS MYCOSES

The miscellaneous mycoses comprise a number of diseases in the immunocompromised patients caused by heterogeneous fungi, previously regarded as harmless, or by the emergence of new fungi assuming a pathogenic role. This section briefly deals with disseminated trichosporonosis, pulmonary sporotrichosis, ustilagomycosis of brain and chromoblastomycosis as illustrative examples of miscellaneous mycoses.

Disseminated Trichosporonosis

Invasive infections by rare and new opportunistic

fungal pathogens are currently posing a serious problem in the treatment of immunocompromised hosts. Two fatal cases of disseminated trichosporonosis are reviewed here. The first was described in 1998 by Mussa *et al.*⁸⁷ The patient was a 9-year-old girl afflicted with chronic granulomatous disease. Initially, she had pneumonitis which later disseminated to lumbar region and costo-vertebral junction. Her diagnosis of disseminated trichosporonosis was based upon demonstration by direct microscopy of yeast cells and arthroconidia in aspirated material from lumbar lesion and fine needle aspirate from the lung, supported by isolation of *Trichosporon inkin* in pure culture. Following the diagnosis, the patient's guardian got her admitted to a general hospital where she succumbed to the infection soon after initiation of antifungal therapy with ketoconazole. This case was noteworthy in that *Trichosporon inkin* was documented for the first time as an aetiological agent of disseminated trichosporonosis.

The second fatal case of opportunistic trichosporonosis was reported in 2004 by Chowdhary *et al.*⁸⁸ Its aetiological agent was *Trichosporon asahii*, a species emerging with increasing frequency as aetiological agent of disseminated trichosporonosis. The patient was a 41-year-old male, presenting with acute myeloblastic leukemia, cough and fever who had received cytotoxic drug therapy, broad spectrum antibiotics and was neutropenic. He developed manifestations of pneumonia and fungemia which did not respond to antibacterial therapy. Repeated demonstration of budding yeast cells and arthroconidia in sputum specimens and isolation of *T. asahii* in cultures from sputum as well as blood provided unequivocal evidence of disseminated trichosporonosis. The patient's condition deteriorated progressively and he died despite treatment with amphotericin B for four weeks.

Pulmonary Sporotrichosis

Pulmonary sporotrichosis is a rare disease caused by inhalation of *Sporothrix schenckii*, a dimorphic aetiological agent of one of the important subcutaneous mycoses. The clinical pattern of the disease is very similar to that of tuberculosis. Approximately one-third of the patients are alcoholic, one-third have another concomitant disease, such as tuberculosis, diabetes mellitus, sarcoidosis or steroid use, and the remaining one-third are apparently normal. The symptoms usually include productive cough, low-grade fever, or weight loss. The chest radiograph generally reveals cavitory lesions, often with parenchymal infiltrate. Occasionally, pleural effusion and hilar lymphadenopathy may occur. The disease may also disseminate hematogenously, causing isolated osteo-articular, central nervous system or ocular lesions in the normal host, or widespread disease in the immunosuppressed host.⁸⁹ In India, there is a solitary case report of a fatal case of pulmonary

sporotrichosis, caused by *S. schenckii* var *luriei*, and diagnosed *post-mortem*.⁹⁰ Such cases are likely to be missed for want of a pathognomonic clinical syndrome and due to inadequate awareness or lack of mycological diagnostic facilities. Our laboratory has come across three cases of lymphocutaneous sporotrichosis occurring in patients who were residents of Ranikhet and Teri Garhwal areas of Uttaranchal.^{91, 92} As these patients gave no history of travel to a region known to be endemic for sporotrichosis, they indicated that Uttaranchal was also endemic for sporotrichosis in addition to West Bengal, Assam and Himachal Pradesh which were already known for endemic occurrence of sporotrichosis. For an update on sporotrichosis in India, reference may be made to a review article by Randhawa *et al*⁹³ who reported the first case in a Delhi resident who had not travelled to any endemic area for sporotrichosis.

Ustilagomycosis

A fatal case of ustilagomycosis of brain caused by *Ustilago maydis* with tumour like growth rupturing through cribriform plate and protruding into the nose was reported in 1959 by Randhawa *et al*,⁹⁴ in a 14-year-old girl who complained of headaches, loss of vision and smell. The case was diagnosed by histopathologic examination of biopsy from brain lesion and isolation in culture of *Ustilago maydis*, a plant pathogenic fungus, from a pathological discharge mixed with blood coming from the lesion through her nose. The patient left the hospital against medical advice and died soon after without any antifungal therapy. No predisposing factors were identified in the patient. The case illustrates the growing number of mycoses due to fungi which were previously seldom implicated as aetiologic agents.

Chromoblastomycosis: *Rhytidhysterion* sp. as a New Aetiologic Agent

A case of chromoblastomycosis caused by a non-sporulating species of *Rhytidhysterion* (Order Patellariales, Ascomycota) identified by molecular methods has been reported.⁹⁵ The patient was a post-renal transplant recipient, male aged 50 years and resident of Delhi. The diagnosis was made by direct microscopy, histopathology and isolation of multiple colonies in pure culture of the dematiaceous mould from a biopsy of one of the lesions occurring on his left foot and extending to the shin and thigh. The lesions comprised necrotic granulation tissue with indurated and thickened margins. After two weeks of therapy with itraconazole, 100 mg twice daily, the lesions partially retracted. However, the patient developed complications of acute abdominal pain with respiratory distress, necessitating admission to a tertiary-care hospital where he died due to multiple organ failure within 48 hours. All attempts to induce sporulation in the fungal isolate on various mycological culture media

were unsuccessful. Thereafter, sequencing of the 18S rRNA gene of the isolate and its comparison with GenBank database revealed that it belongs to the genus *Rhytidhysterion*. As far as we are aware, this is the first case of human infection caused by a fungus of the Order Patellariales. The case underlines the relevance of molecular techniques as valuable tools for identification of non-sporulating opportunistic pathogenic fungi.

FUTURE PERSPECTIVES

Pulmonary and systemic mycoses pose an ever increasing threat to public health due to the growing number of immunocompromised patients resulting from a variety of causes such as the AIDS and use of immunosuppressive therapy in patients with organ transplantation. There are wide gaps in our knowledge of these diseases in India. For instance, we know very little about the country-wide prevalence or incidence of histoplasmosis and blastomycosis. Their zones of endemicity remain to be determined, and virtually no information is available on their environmental foci of infection or animal hosts. Likewise, we know very little about the natural habitat of *Penicillium marneffeii*, another dimorphic fungus which is the aetiologic agent of penicilliosis marneffeii, occurring in AIDS patients in north-eastern India. In this context, application of molecular techniques such as PCR-based assays to detect dimorphic pathogens from environmental samples need to be undertaken. Also among the non-culture methods, the usage of immunodiagnosis such as detection of fungal antigen and antibody by using techniques such as ELISA, immunodiffusion, western blotting etc and non immunological approach such as determination of fungal metabolites (D-arabinitol, 1-3 - β -D-glucan) in patients with invasive fungal infections will be highly useful. The problem of antifungal resistance is likely to acquire more serious dimensions due to more frequent and prolonged administration of antifungal drugs either for therapeutic or prophylactic use. Future studies should focus on development and introduction of new techniques, classical as well as molecular, for facilitating rapid diagnosis, a prerequisite for early administration of specific antifungal therapy. Among the promising rapid molecular techniques are PCR, DNA probes, and sequencing of ITS regions. Establishment of facilities for molecular mycological techniques is of paramount importance because the conventional diagnostic procedures are generally more time consuming. For more reliable taxonomic studies it would be imperative to undertake nucleotide sequencing of ITS and SSU regions because classical methods have serious limitations, especially for fungi which do not sporulate or have no known teleomorphic state. Besides, molecular methods are better suited to investigate phylogenetic relationships among various pathogenic fungi.

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