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Occurrence and Significance of *Cryptococcus neoformans* in the Respiratory Tract of Patients with Bronchopulmonary Disorders

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Cryptococcus neoformans was cultured from 13 (3%) of 469 clinical specimens examined from the respiratory tract of patients with bronchopulmonary diseases. These isolations came from 5 (2%) of 207 patients; 11 isolates were from sputum and 1 each were from bronchoscopic aspirate and empyema pus. The fungus was not cultured from the oropharyngeal washings of 101 apparently healthy volunteers. Of the 5 patients, 3 had pulmonary tuberculosis, including one with pyopneumothorax and 2 with allergic bronchopulmonary aspergillosis as the underlying disease. In the tuberculosis patient with pyopneumothorax and *C. neoformans* in empyema pus, the fungus was presumably a tissue invader, whereas its role could not be unequivocally ascertained in the remaining 4 patients from whom it was isolated from sputum or bronchial aspirate on at least two consecutive occasions. The question of *C. neoformans* being a transient resident, commensal, or incitant of benign minimal lesions in the tracheobronchial tree is discussed. A comprehensive laboratory and clinical follow-up is warranted in patients from whose sputum or bronchial aspirate *C. neoformans* may be cultured even though definitive signs of cryptococcosis may be lacking.

It is now recognized that pulmonary infections due to *Cryptococcus neoformans* may be of a transitory and mild character which may be missed in the absence of symptoms and radiological shadows. Until recently, most cases of pulmonary cryptococcosis were diagnosed postmortem. In a review of 101 cases of pulmonary cryptococcosis, Campbell (1) pointed out that the diagnosis was made during surgery or autopsy in at least 71 patients. It was also believed that the isolation of *C. neoformans* from sputum had the same importance as that of acid-fast bacillus in pulmonary tuberculosis (9). During the preceding decade, however, several investigators have reported on the question of saprobic colonization of the human tracheobronchial tree by *C. neoformans* (4, 7, 13, 15). These reports prompted us to culture respiratory tract specimens for *C. neoformans* in patients with bronchopulmonary disorders; the results are presented in this paper.

MATERIALS AND METHODS

Four hundred and sixty-nine specimens such as sputum, bronchial aspirate, empyema pus, and lung biopsy were collected aseptically from 207 patients with diverse respiratory disorders. Cerebrospinal fluid, blood, and urine of several of the patients, who eventually were shown to have *C. neoformans* in their respiratory tract, were also cultured. The specimens were from inpatients or outpatients at the Clinical Research Centre, V.P. Chest Institute, University of Delhi, the Rajen Babu Tuberculosis Hospital, and occasionally from other hospitals in Delhi. Fifty-nine of the patients had pulmonary tuberculosis, 28 had pyopneumothorax, 22 each had bronchial asthma and chronic bronchitis, 17 had bronchopneumonia, 11 had interstitial fibrosis, and 48 had miscellaneous chest diseases. After the patient had rinsed his mouth with sterile distilled water, sputum was collected, brought to the laboratory, and processed promptly. Pus from empyema patients was collected from the lesion directly into a sterile glass bottle through a rubber tubing. Cerebrospinal fluid was aspirated through lumbar puncture, and blood samples were collected in McCartney bottles containing heparin. Oropharyngeal washings were examined from 101 apparently healthy volunteers, selected at random and varying in age from 6 to 67 years. Sixty of the volunteers (89 males, 12 females) came from rural areas of greater Delhi and the rest were from Delhi and New Delhi. Occupationally, they were categorized as follows: office workers, 32; school or college students, 31; agriculturists, 14; housewives, 6; dairy farmers and dogcatchers, 3 each; and others, 12.

Sputum samples and lung biopsies were homogenized before direct microscopy and culture. The samples were streaked onto Sabouraud glucose agar (Emmons modification) supplemented with chloramphenicol, and onto Staib *Guizotia abyssinica* seed agar, also known as bird seed agar and thistle seed agar, prepared as described by Randhawa et al. (10).

The cultures were incubated at 37°C for 3 weeks before discarding them as negative for *C. neoformans*. Identification of *C. neoformans* isolates was based on the methods described by van der Walt (14) and by verification of their pathogenicity in white mice using the intraperitoneal route of inoculation.

RESULTS

The cultural data are shown in Table 1. Of 469 specimens examined from 207 patients, 13 (3%) yielded *C. neoformans*. The positive specimens came from 5 patients (2%). Eleven isolates were from sputum and 1 each were from bronchial aspirate and empyema pus; 12 of these grew on Staib medium and only 1 grew on Sabouraud agar. *C. neoformans* was not cultured from the oropharyngeal washings of any of the 101 apparently healthy human volunteers. All 13 isolates were brownish at 25°C within 3 to 7 days on Staib medium. They failed to develop pseudomycelium or true mycelium in Dalmau cultures on potato glucose agar; glucose, sucrose, raffinose, maltose, galactose, and lactose were not fermented. Sucrose, maltose, raffinose, galactose, and glucose were assimilated. Lactose and potassium nitrate were not utilized. Urea hydrolysis and growth at 37°C were positive. There was no mortality among the mice inoculated with any of these cultures, but the yeast was isolated from the lungs, liver, spleen, and kidney when sacrificed after 2 weeks. Also, histopathological lesions showing organisms characteristic of *C. neoformans* were demonstrable in one or more of these organs, with each of the isolates.

Of the five positive patients, three had tuberculosis, including one with pyopneumothorax, and two had bronchial asthma. The patient with pyopneumothorax and *C. neoformans* in empyema pus presumably had cryptococcosis superimposed upon pulmonary tuberculosis, but unfortunately he was not available for further investigation. The mycological follow-up of the remaining four patients ranged from 15 to 539 days (Table 2). *C. neoformans* was isolated from sputum in patients 1 and 2 on four occasions each and twice in patient 3. Two sputum specimens were cultured in patient 2 on 21 October and 22 October 1974, but only one was positive on both days. Patient 4 yielded positive cultures from sputum on 9 November 1974 and from bronchial aspirate on 14 January 1975. The number of *C. neoformans* colonies in primary cultures seldom exceeded one or two in any patient. Cultures of cerebrospinal fluid (not done in patient 2), urine, and blood gave negative results. Isolations of *Aspergillus fumigatus* from sputum and demonstration of dual-skin hypersensitivity (type I and type III) and of serum precipitins against this species

TABLE 2. *Mycological follow-up of patients yielding C. neoformans*

Patient no.	Follow-up period (days)	Culture for *C. neoformans*[a] (no. positive/no. of samples examined)			Dates of sputum culture	
		Sputum	Bronchial aspirate[b]	Urine	Positive	Negative
1	171	4/8	0/1	0/7	9/3/74	11/27/74
					10/1/74	11/28/74
					11/30/74	11/29/74
					12/2/74	2/21/75
2	36	4/6	ND[c]	0/3	9/16/74	10/21/74
					9/17/74	10/22/74
					10/21/74	
					10/22/74	
3	15	2/6	ND	0/3	11/7/74	11/12/74
					11/16/74	11/14/74
						11/18/74
						11/22/74
4	539	1/8	1/1	0/7	11/9/74	11/25/74
						11/26/74
						11/27/74
						11/28/74
						12/2/74
						1/1/75
						4/28/76

[a] Solitary specimens of blood cultured in all four patients and of cerebrospinal fluid in patients 1, 3, and 4 yielded negative results.

[b] Cultured on 7 December 1974 in patient 1 and 14 January 1975 in patient 4.

[c] ND, Not done.

TABLE 1. *Isolations of Cryptococcus neoformans from the respiratory tract*

Healthy volunteers	Patients	Clinical specimens				
		Sputum	Empyema pus	Bronchial aspirate	Others	Total no. isolates
0/101[a]	5/207 (2%)	11/429	1/22	1/9	0/9[b]	13/469 (3%)

[a] The numerator denotes the number yielding *C. neoformans* and the denominator is the number examined.

[b] Seven specimens of oropharyngeal washings and two of lung biopsy.

revealed that patients 1 and 2, representing the bronchial asthma group, where cases of allergic bronchopulmonary aspergillosis, the details of which are given elsewhere (Z. U. Khan, Ph.D. thesis, University of Delhi, Delhi, 1976). Three patients contacted 18 months post-study stated that they were doing well.

To evaluate the significance of *C. neoformans* in the respiratory tracts of the aforementioned patients, 103 samples of pigeon excreta were examined from the environments of patients 1 and 4. The fungus was frequently isolated, using Staib medium, from a heap of mortar-like, old pigeon excreta found in a neighboring house of patient 1 where the pigeons roosted, in pigeon excreta which had accumulated inside a Charity Bird Hospital located near the residence and working place of patient 4, and also from the air at several indoor sites in the same bird hospital.

DISCUSSION

Notwithstanding the use of Staib *Guizotia abyssinica* seed agar, a selective culture medium, *C. neoformans* was cultured from only 2% of the patients investigated, suggesting that the species is a rare inhabitant of the respiratory tract in patients with bronchopulmonary disorders. This is broadly in agreement with Howard (7) who reported three isolates of *C. neoformans* from 561 sputum samples. Previously, Reiss and Szilagyi (11) had reported the fungus from throats of 6 of 92 patients with malignancies; additionally, one of their positive patients yielded the fungus from a nasal swab.

The study focuses attention on the significance of *C. neoformans* in sputum or bronchial aspirate, which may not be readily interpretable. In our series, patients 1 and 2 had allergic bronchopulmonary aspergillosis as an underlying disease and gave history of treatment with broad spectrum antibiotics and corticosteroids; patients 3 and 4 had pulmonary tuberculosis including diabetes in the latter and they had been treated with antituberculous drugs. In the absence of radiological and any other definitive evidence suggestive of cryptococcosis, the significance of positive cultures in these patients was open to three interpretations: (i) *C. neoformans* was causing a benign, minimal focus of infection in the respiratory tract that was not detected due to lack of a characteristic clinical syndrome and failure to carry out certain recently introduced serological tests (2); (ii) it was a saprobic colonizer or commensal of the tracheobronchial tree; (iii) it was a transient inhabitant of the respiratory tract after inhalation from the environment. The last possibility (iii) was supported by demonstration of saprobic reservoirs of *C. neoformans* near the residence and/or working place of patients 1 and 4; it is also noteworthy that the fungus was isolated from air inside a bird hospital in the residential area of patient 4. Moreover, *Guizotia abyssinica* seed agar, a selective medium, is likely to pick up *C. neoformans* much more frequently both from clinical materials and natural substrata even though the fungus was not carried in any significant number.

It is pertinent to refer to earlier reports concerning benign pulmonary cryptococcosis or saprobic colonization of the tracheobronchial tree by this species (3–8, 12, 13, 15, 16). These reports included a noteworthy series of 32 cases described by Tynes et al. (13), 18 of which were classified as a "saprophytic colonization" by *C. neoformans* who were not treated with amphotericin B even though the pathogen was isolated over 10 months to 3 years. Likewise, the report of Warr et al. (15) included several patients with sputum positive for *C. neoformans*, who were established cases of carcinoma, tuberculosis, or chronic bronchitis and who were either doing well without any antifungal therapy or died of unrelated illness. We agree with Howard (7) who stated that the distinction between saprobic colonization or commensalism and minimal infections of the tracheobronchial tree by *C. neoformans* is a problem with some semantic overtones. Finally, it is quite apparent that more extensive investigations on the prevalence of *C. neoformans* in the human respiratory tract are required to obtain a better insight into the epidemiology of cryptococcosis. For the present, it seems prudent to continue taking seriously any isolation of *C. neoformans* from the respiratory tract unless adequate clinical and laboratory follow-up precludes the possibility of localized or disseminated cryptococcosis.

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