Occurrence and Significance of *Cryptococcus neoformans* in the Oropharynx and on the Skin of a Healthy Human Population

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Cryptococcus neoformans was cultured on one occasion from the oropharyngeal washings in 1 of 820 and from the interdigital areas of the feet in 6 of 723 healthy persons. Among the positive persons were two school children, two laboratory workers, one gardener, one plumber, and one printing press worker. Repeat cultures were negative in all seven positive persons; serological tests for cryptococcal antigen/antibody were negative in three. The results demonstrate that *C. neoformans* may occur as a transient inhabitant of the oropharynx or skin of healthy people.

The saprobic colonization of the human respiratory tract by *Cryptococcus neoformans* has been discussed in a number of recent publications (3, 4, 9, 12-14). However, these reports were based entirely or largely on investigations of patients with varied disorders. Except for investigations by McGinnis et al. (6, 7), no comprehensive study seems to have been reported on the association of *C. neoformans* with a normal human population. This paper deals with a survey of *C. neoformans* in the oropharynx and on the skin of apparently healthy human volunteers.

MATERIALS AND METHODS

Oropharyngeal washings were cultured for *C. neoformans* from 820 apparently healthy persons, whose particulars of occupation, sex, and age are summarized in Table 1. Skin swabs from the interdigital areas of the feet were cultured simultaneously in 723 persons of the same population. The test subjects belonged to the northern areas of Delhi and greater Delhi. Six hundred and fifteen of them were children, aged 10 to 17 years, examined from three schools, whereas the remaining 205 subjects were adults working in the campus of the University of Delhi and its neighborhood. Each person was provided with about 10 ml of sterile physiological saline in a screw-capped, 1-ounce (about 30-ml) McCartney bottle. After rinsing of the oropharyngeal cavity, the saline was collected in the same bottle. The interdigital areas of the feet were sampled with a sterile, cotton-tipped swab slightly moistened with physiological saline. About 1 ml of each oropharyngeal washing was cultured by spreading it on Staib *Guizotia abyssinica* seed agar, prepared as described by Randhawa et al. (10) except that streptomycin and penicillin were replaced by chloramphenicol (50 µg/ml). Skin swabs were smeared di-

rectly on plates of the same medium. The inoculated plates were incubated at $25 \pm 2^\circ\text{C}$, with care being taken not to handle *C. neoformans* cultures in the same incubator or in the laboratory while this work was in progress. The plates were periodically observed for up to 3 weeks. The yeastlike colonies exhibiting a brown color were tentatively designated *C. neoformans*, which was confirmed by verification of pathogenicity in white mice and by a detailed study of morphological and biochemical characteristics, following the criteria given by Phaff and Fell (8). The pathogenicity test was done by intracerebral inoculation of each isolate in two Swiss mice. Each mouse received about 0.02 ml of a suspension of the organism (approximately 10^6 cells/ml), prepared from 2- to 3-day-old cultures on Sabouraud agar at 25°C .

RESULTS

C. neoformans was cultured from the oropharyngeal washings in 1 of 820 persons and from the interdigital areas of the feet in 6 of 723 persons (Table 2). All of the seven positive persons were males; two were 12-year-old school children from Narela (Delhi), and five were adults working in various departments of the University of Delhi. The positive adults included one 28-year-old gardener, one 34-year-old printing press worker, one 45-year-old plumber, and two laboratory workers, 21 and 25 years old, from the Department of Botany. Of the persons yielding *C. neoformans* from the interdigital spaces, two customarily wore leather shoes with socks, three wore open-toed sandals, and one wore canvas shoes without socks. Six persons, including the one with a positive oropharyngeal washing, yielded solitary colonies of *C. neoformans*; the seventh yielded 20 colonies from the

TABLE 1. *Particulars of occupation, sex, and age of 820 healthy volunteers investigated as carriers of C. neoformans*

Occupation	No. of males	No. of females	Age range (yr)				
			10-20	21-30	31-40	41-50	51-60
Students	320	295	615	0	0	0	0
Gardeners	50	0	4	15	21	9	1
Printing press workers	50	0	7	15	20	6	2
Office workers	32	0	3	16	10	1	2
Plumbers	29	0	1	12	5	11	0
Laboratory workers	15	4	1	10	4	3	1
Others	6	19	3	9	7	6	0
Total	502	318	634	77	67	36	6

TABLE 2. *Occurrence of C. neoformans in a healthy population*

Subjects	No. positive/no. examined	
	Oropharyngeal washings	Interdigital areas of feet
School children	0/615	2/520
Gardeners	0/50	1/50
Printing press workers	0/50	1/50
Office workers	0/32	0/32
Plumbers	1/29	0/29
Laboratory workers	0/19	2/17 ^a
Others	0/25	0/25
Total	1/820	6/723

^a The positive laboratory workers were not handling any yeastlike fungi.

interdigital areas of his feet, although he too had no discernible lesions. A chest roentgenogram of the person yielding *C. neoformans* from his oropharyngeal washing was found to be normal. Repeat cultures of oropharyngeal washings or skin swabs from the feet of the positive persons were negative for *C. neoformans*. Sera were available for detection of cryptococcal antigen/antibody in only three of the positive persons; two had *C. neoformans* in the interdigital areas of their feet, and one had the organism in the oropharyngeal cavity. The serological tests were carried out through the courtesy of L. Kaufman, Center for Disease Control, Atlanta, Ga., and the results were negative.

All seven isolates of *C. neoformans* exhibited the characteristic brown pigment on *G. abyssinica* seed agar, grew at 37°C, hydrolyzed urea, failed to ferment glucose, sucrose, raffinose, galactose, maltose, and lactose, and assimilated glucose, galactose, sucrose, maltose, trehalose, melezitose, xylose, L-arabinose, D-arabinose, rhamnose, ribitol, dulcitol, mannitol, glucitol, α -methyl-D-glucoside, salicin, and inositol. Lactose, melibiose, inulin, and glycerol were not assimilated. The assimilation of sorbose, cello-

biose, raffinose, D-ribose, ethanol, erythritol, lactate, succinate, and citrate was variable. Potassium nitrate was not utilized as sole source of nitrogen. All of the mice inoculated intracerebrally with the aforementioned strains of *C. neoformans* died in 4 to 16 days and revealed masses of encapsulated cryptococci in mash preparations from the brain, examined with or without the use of a nigrosin stain.

DISCUSSION

It is apparent from these results that *C. neoformans* may occur as a transient inhabitant of the oropharynx or skin of healthy humans and that its mere isolation from clinical material may not necessarily imply an etiological role. In earlier studies based on the investigation of smaller population samples, the fungus could not be isolated from the oropharynx of healthy individuals (4, 9). The higher recovery of *C. neoformans* from the interdigital areas of the feet than from the oropharynx as reported herein may be attributed to the fact that the former anatomical sites not only are more directly exposed to the environment but also are likely to better retain any microbes that may be deposited there. In this context, it is interesting to note that a markedly fungicidal effect of human salivary secretions on *C. neoformans* has been reported (5).

Our isolations of *C. neoformans* from clinically normal feet corroborate a preliminary study by McGinnis et al. (6, 7), who isolated this fungus from the interdigital spaces of the feet in 5 of 27 male volunteers showing no signs of infection. It should be pointed out that the validity of earlier reports (1, 2) on a yeast isolated from healthy individuals and tentatively identified as similar to *C. neoformans* has been questioned on the grounds of inadequate identification criteria (4). Likewise, it is difficult to evaluate critically the report of Ravits (11) on the isolations of "many species of cryptococci" from the skin of normal subjects.

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