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Prevalence of *Cryptococcus neoformans* var. *neoformans* (Serotype D) and *Cryptococcus neoformans* var. *grubii* (Serotype A) Isolates in New York City

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Analysis of 40 New York City *Cryptococcus neoformans* isolates revealed that 39 were typeable, of which 85 and 12.5% were *Cryptococcus neoformans* var. *grubii* (serotype A) and *Cryptococcus neoformans* var. *neoformans* (serotype D), respectively. The prevalence of serotype D isolates in New York City appears to be significantly higher than indicated by previous studies of North American isolates.

Cryptococcus neoformans is an encapsulated yeast that can cause life-threatening meningitis in immunocompromised patients (23). Based on biochemical, morphological, and genetic characteristics, *C. neoformans* was originally divided into two varieties: *Cryptococcus neoformans* var. *gattii* (serotypes B and C) and *Cryptococcus neoformans* var. *neoformans* (serotypes A and D) (17, 18, 20). These two varieties can be distinguished by biochemical tests (21, 27). Recently a proposal was made to further subdivide the *C. neoformans* var. *neoformans* strains into two varieties: *C. neoformans* var. *neoformans* (serotype D) and *Cryptococcus neoformans* var. *grubii* (serotype A) (13). The serotype classification is based on antigenic differences detected with rabbit adsorbed sera (11, 12, 15). *C. neoformans* var. *gattii* usually infects patients with normal immune status living in tropical and subtropical areas (9, 10). In contrast, *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* are distributed throughout the world (1) and are usually the causative agent of cryptococcosis in patients afflicted with AIDS or immunocompromised in some other way (3).

There is increasing evidence that there are clinical differences between serotype A and serotype D infections (7, 8). For example, serotype D infections are more likely to result in skin involvement and afflict older patients (8). The prevalence of serotype D among clinical isolates has ranged from 0 to 100% depending on the region of the world in which samples were taken (Table 1). The majority of North American isolates pooled from various geographic locations have been reported to be serotype A isolates (Table 1). New York City has been a major site of cryptococcal infections in the United States, and in the early 1990s the prevalence of cryptococcal infection in that city alone was more than 1,000 cases per year (6). The majority of clinical isolates in New York City belong to serotype A or serotype D. Serotype B strains have occasionally been isolated in New York City (2). A previous study of a small number of isolates revealed no serotype D isolates in New York State (1). To our knowledge the prevalence of serotype A and serotype D in New York City has not been determined. In this study we characterized various New York City clinical isolates of *C. neoformans* by two methods and compared the results to those in literature studies.

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The lab stock contained 40 clinical strains that were obtained from cryptococcal meningitis patients between 1991 and 1999 in two New York City hospitals, Jacobi Medical Center (J strains) and Montefiore Medical Center (M strains) (Table 2). Strain 24067 was obtained from the American Type Culture Collection (Manassas, Va.), and MY2061 was obtained from Merck & Co., Inc. (Whitehouse Station, N.J.). These two strains were used as controls for serotype A and serotype D, respectively. Serotyping was done by factor sera agglutination with the Crypto-Check kit (Iatron Inc., Tokyo, Japan) and indirect immunofluorescence with monoclonal antibody (MAb) 13F1 (4; W. Cleare, M. E. Brandt, and A. Casadevall, Letter, J. Clin. Microbiol. **37:**3080, 1999). All samples were prepared simultaneously to avoid variation in growth conditions. The isolates were grown on Sabouraud dextrose (SAB) broth (Difco Laboratories, Detroit, Mich.) agar for 48 h at 30°C. A single colony from each isolate was used to inoculate 10 ml of SAB broth. The SAB broth cultures were incubated with continuous shaking for 72 h to reach stationary phase. Stationary-phase cultures were washed in phosphate-buffered saline (PBS) (0.137 M NaCl, 0.003 M sodium phosphate [pH 7.4]) three times and fixed in 2.5% formaldehyde overnight. For indirect-immunofluorescence serotyping, 10^6 formaldehyde-killed cells were immobilized on polylysine-coated slides (Sigma, St. Louis, Mo.) and incubated for 2 h at room temperature with 10 μg of either MAb 12A1 or 13F1 per ml. The cells were washed with PBS and incubated with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin M (Southern Biotechnology, Birmingham, Ala.) for 1 h at room temperature in the dark. Cells were washed in PBS and mounted on the polylysine slides using a solution of 50% glycerol–0.1 M *n*-propyl gallate (Sigma) in PBS. The slides were viewed with a fluorescein isothiocyanate filter-equipped Zeiss (Thornwood, N.Y.) Axiophot microscope, and serotypes were determined based on binding patterns as described previously (4; Cleare et al., letter). MAb 13F1 produces a punctate pattern on serotype D strains and an annular pattern on serotype A and AD isolates (4; Cleare et al., letter). MAb 12A1 produces an annular pattern on serotype A, D, and AD strains and was used as a control for distinguishing punctate and annular patterns (4; Cleare et al., letter). Without knowledge of the immunofluo-

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TABLE 1. Prevalence of *C. neoformans* var. *grubii* (serotype A) and *C. neoformans* var. *neoformans* (serotype D) in various regions

Location	No. of isolates studied	% Serotype[a]:			Yr of study	Reference(s)
		A	D	AD		
Brazil	19	100	0	0	1992	26
Canada	78	79.5	6.5	6.4	1984	19
France	413	79.5	20.5	0	1996	7, 8
Germany	21	62	23.8	14.3	1981	22
India	15	87	0	13	1993	24
Italy	7	0	100	0	1984	19
Italy	207	24.6	71	3.4	1997	29
Italy	97	100	0	0	1997	5
Taiwan	22	90	0	0	1994	14
Japan	52	95	0	0	1994	16
Thailand	169	93	1.8	1.8	1996	28
Thailand	139	95	0	0	1997	25
United States	203	74.6	4.8	4.0	1984	19
Venezuela	27	63	3.7	0	1989	30

[a] The sum of percentages for is <100 because those studies also report serotypes B and C.

rescence results, serotypes were confirmed using an agglutination assay kit (Iatron Inc.). The agglutination patterns were analyzed as follows: serotype A strains agglutinated with both factors 1 and 7, and serotype D strains agglutinated with both factors 1 and 8, as per the manufacturer's instructions.

Table 2 shows the distribution of serotype A and serotype D in the 40 clinical New York City isolates. All isolates agglutinated rapidly with the Iatron Crypto-Check sera. Both control strains yielded the expected results: MY2061 was classified as *C. neoformans* var. *grubii* (serotype A) and strain 24067 was classified as *C. neoformans* var. *neoformans* (serotype D). Of the 40 samples, 33 were serotype A and 5 were serotype D. One strain, J50, did not agglutinate with either serum 7 or 8 and was not typeable using this method. Strain J3 agglutinated with both Iatron sera 7 and 8 and is therefore serotype AD. To confirm the agglutination results, indirect immunofluorescence analysis was performed using both the 12A1 and the 13F1 MAbs (Table 2). It is important to use both 12A1 and 13F1 antibodies on each strain because capsular differences between strains cause slight variations in binding. All strains grouped as serotype A by the Crypto-Check method produced annular fluorescence when stained with MAb 13F1. Strains grouped as serotype D produced punctate fluorescence when stained with MAb 13F1. The J3 strain produced annular immunofluorescence with MAb 13F1, consistent with the prior observation that MAb 13F1 produces annular binding on AD strains (4; Cleare et al., letter). Strain J50 could not be serotyped by the Crypto-Check method and produced annular immunofluorescence with MAb 13F1, suggesting that it may be an atypical serotype A isolate or an AD isolate.

In summary, of the 39 typeable strains, 85% were *C. neoformans* var. *grubii* (serotype A), 12.5% were *C. neoformans* var. *neoformans* (serotype D), and 2.5% were serotype AD. The percentage of serotype D strains in New York City was twice that reported in prior studies of North American isolates (1, 19). The occurrence of regional variability is illustrated by reports from northern (29) and southern Italy (5) in 1997 which revealed that the prevalence of serotype D isolates was 71 and 0%, respectively. The factors responsible for geographical variation in the prevalence of serotype A and serotype D are not understood. The relatively high prevalence of serotype D strains in New York City, combined with the variability in prior studies (Table 1), suggests a need for detailed regional surveys to ascertain the distribution of serotypes in various parts of the world.

TABLE 2. New York City isolate serotyping results

Isolate	Crypto-Check	Pattern with MAb	
		13F1	12A1
24067	D	Punctate	Annular
MY2061	A	Annular	Annular
J1	A	Annular	Annular
J2	A	Annular	Annular
J3	AD	Annular	Annular
J4	A	Annular	Annular
J6	D	Punctate	Annular
J8	A	Annular	Annular
J9	D	Punctate	Annular
J10	A	Annular	Annular
J11a	A	Annular	Annular
J12	A	Annular	Annular
J17	D	Punctate	Annular
J20	A	Annular	Annular
J23	A	Annular	Annular
J28	A	Annular	Annular
J33	A	Annular	Annular
J36	A	Annular	Annular
J39	D	Punctate	Annular
J40	A	Annular	Annular
J41	A	Annular	Annular
J42	A	Annular	Annular
J43	A	Annular	Annular
J44	A	Annular	Annular
J45	A	Annular	Annular
J47	A	Annular	Annular
J48	A	Annular	Annular
J50	?	Annular	Annular
J51	A	Annular	Annular
J52	A	Annular	Annular
J53	A	Annular	Annular
J54a	A	Annular	Annular
M3	A	Annular	Annular
M5	A	Annular	Annular
M6	A	Annular	Annular
M7	A	Annular	Annular
M8	A	Annular	Annular
M9	A	Annular	Annular
M10	A	Annular	Annular
M11	A	Annular	Annular
M14	A	Annular	Annular
M16	D	Punctate	Annular

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