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). 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, 106 formaldehyde-fixed 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% glyceral–0.1 M n-propyl gallate (Sigma) in PBS. The slides were viewed with a fluorescein isothiocyanate filter-equipped Zeiss (Thornwood, N.Y.) Axioptot 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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In summary, of the 39 typeable strains, 85% were \textit{C. neoformans} var. \textit{grubii} (serotype A) and strain 24067 was classified as \textit{C. neoformans} var. \textit{neofor}-
mans (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 MAb’s (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 immunofluorescence 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 results, serotypes were confirmed using an agglutina-
tion 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 \textit{C. neoformans} var. \textit{grubii} (serotype A) and strain 24067 was classified as \textit{C. neoformans} var. \textit{neofor}-
mans (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 MAb’s (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. 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, consistent with the prior observation that MAb 13F1 produces annular binding on AD strains (4; Cleare et al., letter).

In summary, of the 39 typeable strains, 85% were \textit{C. neoformans} var. \textit{grubii} (serotype A), 12.5% were \textit{C. neoformans} var. \textit{neofor}-
mans (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.

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