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Serotype B/C *Cryptococcus neoformans* Isolated from Patients in Nonendemic Areas

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Of 90 clinical isolates of *Cryptococcus neoformans* studied, 3 were determined to be serotype B/C. The patients from whom these B/C isolates were obtained were identified as never having lived in or visited the areas associated with B/C serotypes. This finding suggests a broader geographic distribution of this serotype group than previously believed. The glycine-cycloheximide-phenol red medium described by Salkin and Hurd (J. Clin. Microbiol. **15**:169–171, 1982) was shown to be more accurate in differentiating A/D and B/C serotype pairs of *C. neoformans* than the creatinine-dextrose-bromthymol blue medium described by Kwon-Chung et al. (Int. J. Syst. Bacteriol. **28**:616–620, 1978).

Antigenic heterogeneity in *Cryptococcus neoformans* is well known, and four serotypes (A, B, C, and D) are recognized (8). Serotypes A and D are associated with *C. neoformans* var. *neoformans*, and serotypes B and C are associated with *C. neoformans* var. *gattii* (3).

Historically, B/C serotypes of *C. neoformans* generally have been limited to clinical isolates from southern California and Southeast Asia (1). One report suggests that southeastern Oklahoma also may be an area associated with B/C serotypes (4). The geographic distribution of the serotypes of *C. neoformans* is of epidemiological interest in light of a report that the virulence and chronicity associated with B/C serotypes may differ from that seen with A/D serotypes (D. K. Henderson, J. E. Edwards, Jr., W. E. Dismukes, and J. E. Bennett, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, F11, p. 315).

Ninety clinical isolates of *C. neoformans* obtained from a multicenter chemotherapeutic trial representing 13 states were tested for serotype by culturing on creatinine-dextrose-bromthymol blue (CDB) agar as described by Kwon-Chung et al. (2) and glycine-cycloheximide-phenol red (GCP) agar as described by Salkin and Hurd (5). Known serotypes obtained from John E. Bennett, National Institute of Allergy and Infectious Disease, and Harold G. Muchmore, Veterans Administration Medical Center, Oklahoma City, Oklahoma, were used as controls. Differentiation of serotype pairs with CDB medium is based upon the ability of B/C serotypes to assimilate creatinine rapidly. In contrast, differentiation with GCP is dependent upon the ability of B/C serotypes to assimilate glycine as the sole source of carbon and their greater resistance to cycloheximide. Differentiation of serotype pairs on both media is based on the presence or absence of growth and subsequent changes in pH leading to color changes in the inoculated agar.

Each medium was prepared according to published procedures and dispensed into petri plates (50 by 9 mm) with tight lids (Falcon 1006, Cockeysville, Md.). The GCP medium was prepared with the suggested optimal concentrations of glycine (1%) and cycloheximide (1.6 μg/ml). Yeast isolates were subcultured from stock slants to fresh modified Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) plates. After 48 h of incubation at 27°C, portions of each culture were suspended in sterile saline to a turbidity equal to that of no. 1 McFarland standard, and 0.1 ml was streaked onto plates of CDB and GCP media. Cultures were incubated at 27°C and checked daily for yeast growth and color change of the medium. CDB plates were read at 48 h, whereas GCP plates were incubated for up to 5 days.

The 90 clinical and 10 control isolates initially were tested on CDB medium. Of the 90 clinical isolates, 12 (13.3%) changed the color of the medium from golden yellow to blue-green within 48 h, indicating rapid assimilation of creatinine which is characteristic of the B/C serotype group (Table 1). The same 90 isolates subsequently were tested on GCP medium. Only three (3.3%) produced a color change (yellow to red) charac-

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teristic of the B/C serotype group (Table 1). These 3 isolates were among the 12 which appeared to be serotype B/C on the CDB medium. These tests were performed three times with separate lots of media. The results were identical in each experiment, and control isolates responded properly in each test. The 12 isolates that appeared to be B/C serotypes according to their reactions on CDB medium were sent to J. E. Bennett at the National Institutes of Health for determination of serotype by agglutination procedures, using specific antisera. Results confirmed that the three isolates determined to be serotype B/C with both the CDB and GCP media were, in fact, serotype B. The remaining nine isolates were serotype A or D, thus contradicting the results obtained on the CDB medium for these organisms.

The clinical records of the three patients infected with *C. neoformans* B/C serotype were examined for birthplace and travel history. The states in which the patients were residing when the diagnosis of cryptococcosis was made included Alabama, Louisiana, and Tennessee. None of the patients lived in, visited, or was born in the B/C-associated areas of Southern California or Southeast Asia.

There has been an increasing interest in the geographic distribution as well as clinical significance of disease of the A/D and B/C serotype groups of *C. neoformans*. Early studies indicated that B/C serotypes were limited in their geographic distribution (1, 2), but a recent report by Muchmore et al. (4) suggested that the strict geographic distribution once proposed for B/C serotypes is not as limited. Kwon-Chung et al. (3) recently reported that the 12 isolates reported by Muchmore et al. (4), all of which produced blue color on CDB medium, actually consisted of 1 serotype C and 11 serotype A strains when serotyped with specific antisera at the National Institutes of Health. Our report supports the concept of broader distribution of B/C serotypes in that three additional patients diagnosed as having cryptococcis due to *C. neoformans* B/C serotypes were identified, each of which had no history of domicile in or travel to serotype B/C-associated areas.

The ease and accuracy of determining serotype groups are important in epidemiological studies. The CDB medium developed by Kwon-Chung et al. (2) has been described as a special medium which can easily distinguish *C. neoformans* serotype groups by their rate of metabolism of creatinine (7). Schmeding studied 97 strains of *C. neoformans* and noted that creatinine was utilized by 21 of the 97 strains. Only 13 of the 21 isolates which assimilated creatinine were serotype B/C, indicating that creatinine metabolism is not an accurate means of determining serotype group (K. A. Schmeding, Ph.D. thesis, George Washington University, Washington, D.C., 1980). A subsequent report by Schmeding et al. (6) identified 37 isolates of *C. neoformans* (a selected group from the 97 reported in the dissertation) which were studied for sexual compatibility between serotypes of *Filobasidiella neoformans*. Among these isolates were the 13 B/C serotypes which assimilated creatinine. None of the A/D serotypes selected for study in this report assimilated creatinine. The CDB medium used in the study reported here detected B/C serotypes with a specificity of only 25%; therefore, the relative value of CDB medium in determining serotype groups of *C. neoformans* should be reassessed.

The newly developed serotype medium described by Salkin and Hurd (5) which utilizes glycine assimilation and susceptibility to cycloheximide predicted the proper serotype groups of the 90 clinical isolates tested in this study with a specificity of 100%. In addition, the GCP medium in easier to prepare than the CDB medium, thus making it a more suitable medium for the differentiation of serotype pairs of *C. neoformans*.

TABLE 1. Serotype groups of *C. neoformans* as determined by CDB and GCP media

Medium	No. of isolates[a] (%) of following serotype:	
	A/D	B/C
CDB	78 (86.7)	12 (13.3)
GCP	87 (96.7)	3 (3.3)

[a] Control isolates (five A/D and five B/C) were tested correctly on each medium.

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