

Disseminated Infection Caused by Urease-Negative *Cryptococcus neoformans*

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We report a case of fungemia and disseminated disease caused by a urease-negative strain of *Cryptococcus neoformans* in a patient with the acquired immune deficiency syndrome. Except for failure to hydrolyze urea, the microbiological characteristics of the isolate were typical of *C. neoformans*. Laboratory specialists should be aware of the occurrence of atypical strains of *C. neoformans*, particularly those recovered from patients with the acquired immune deficiency syndrome.

Cryptococcus neoformans typically produces a chronic or subacute pulmonary, systemic, or meningeal infection and is recovered most frequently from patients with immunosuppressive disorders, including the acquired immune deficiency syndrome (8). Although detection of cryptococcal antigen by the latex agglutination test frequently enables early institution of antimicrobial therapy in patients with suspected cryptococcal infection, isolation and rapid identification of the organism are important in disease confirmation and management. Techniques for early differentiation of *C. neoformans* from other genera have frequently relied on the consistent ability of *C. neoformans* to assimilate inositol and hydrolyze urea (1). We report recovery of a non-urease-producing isolate of *C. neoformans* from a patient with the acquired immune deficiency syndrome and disseminated cryptococcal disease.

A 41-year-old homosexual male was admitted to our hospital with complaints of headache and incoordination of 6 weeks duration. On admission, the patient was febrile to 101°F (38.3°C); neurological examination was significant for nominal aphasia and evidence of cerebellar dysfunction. Serology for human immunodeficiency virus was positive. Cerebrospinal fluid obtained by lumbar puncture on the following day revealed 7 erythrocytes per mm³ and 2 leukocytes per mm³ (with a differential count of 56% polymorphonuclear leukocytes, 32% lymphocytes, 10% monocytes, and 2% non-leukocytes); the glucose and protein concentrations were 59 and 68 mg/100 ml, respectively. The cerebrospinal fluid was inoculated onto Sabouraud-brain heart infusion agar (SABHI) and inhibitory mold agar (both from BBL Microbiology Systems, Cockeysville, Md.) and incubated according to published recommendations (5); media appropriate for recovery of bacteria and mycobacteria were also inoculated. Cryptococcal antigen was detected in the serum of the patient at 1,024 dilutions and in diluted spinal fluid by a latex agglutination test (Meridian Diagnostic Inc., Cincinnati, Ohio).

Two sets of blood cultures (each consisting of a BACTEC 6B and a BACTEC 7D bottle [Johnston Laboratories, Inc., Towson, Md.]) were obtained at the time of admission, and specimens of urine and sputum were obtained for routine culture. Inhibitory mold agar was inoculated with urine (5), and brain heart infusion-cycloheximide-gentamicin agar with

blood (BBL) plus SABHI were inoculated with sputum (5) for recovery of fungi. Computerized axial tomography of the brain revealed several nonenhancing, hypodense lesions in the cerebrum and cerebellum that were thought to be consistent with cryptococcal infection.

Treatment of suspected disseminated cryptococcal infection with intravenous amphotericin B and oral flucytosine was begun on hospital day 1 and was continued throughout hospitalization. On day 7, microscopic examination of a wet-mount preparation from one of the aerobic blood cultures revealed blastoconidia; 7 days later, similar organisms were detected in the aerobic bottle of the other set. Cultures of cerebrospinal fluid obtained by lumbar puncture and by a ventriculostomy were negative for fungi after 7 days of incubation. Despite antifungal therapy, the neurological condition of the patient deteriorated, and he died on hospital day 19. Autopsy findings included diffuse infiltration of the lungs, spleen, and prostate with slightly to heavily encapsulated budding yeastlike organisms that resembled *C. neoformans*. There was extensive necrosis of the cerebrum, thalamus, midbrain, and cerebellum; cysts and tachyzoites consistent with *Toxoplasma gondii* were observed in large numbers in the necrotic areas, but there was no evidence of cryptococcal infection or the brain or meninges.

The blood isolate formed mucoid, light tan colonies on Sabouraud glucose agar after 3 days of incubation at 25°C. On cornmeal-Tween agar inoculated by the Dalmau method (2), the isolates produced globose blastoconidia. Pseudomycelium was absent. Inoculation of CN medium (Flow Laboratories, Inc., McLean, Va.) produced black pigmented colonies after 48 h. Carbohydrate assimilation tests by auxanography (2) were consistent with identification of the isolate as *C. neoformans*. Despite good growth on Christensen urea agar (Scott Laboratories, Inc., Fiskeville, R.I.) incubated at 25°C, the organism failed to hydrolyze urea after 5 days.

Because of the negative test for urease, the isolate was referred to the Department of Medical Microbiology at the University of California, Los Angeles, School of Medicine for additional testing; urea was not hydrolyzed after growth on Christensen urea agar (BBL) which had been incubated at 25°C for 1 month or after heavy inoculation of urea R broth (Difco Laboratories, Detroit, Mich.) which had been incubated for 1 month at 25 and 37°C. The rapid urease test with a urea base (BBL) (7), urea R broth (Difco) (4), and urea

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broth (Rapid; Remel, Lenexa, Kans.) also failed to demonstrate hydrolysis of urea. The isolate was also referred to the Division of Mycotic Diseases at the Centers for Disease Control, Atlanta, Ga.; except for failure to hydrolyze urea on Christensen urea agar (BBL), the isolate was found to have the morphological and physiological characteristics of *C. neoformans*. Results of biochemical tests performed at the Centers for Disease Control included the following: (i) assimilation of glucose, cellobiose, dulcitol, galactose, inositol, raffinose, sucrose, trehalose, xylose, mannitol, and rhamnose; (ii) failure to assimilate lactose, melibiose, glycerol, or nitrate; and (iii) failure to ferment erythritol, glycerol, mannitol, and rhamnose. In addition, the isolate was found to produce brown colonies on birdseed agar, to grow at 37°C, to be susceptible to cycloheximide at a concentration of 0.5 mg/ml, to stain with a fluorescent-antibody reagent for *C. neoformans* in a direct fluorescent test, and to belong to serogroup A by the method of Kaplan et al. (3). Also, the isolate was identified as *C. neoformans* with the API 20C yeast identification system.

Production of urease by cryptococci is remarkably consistent and has been suggested as a screening tool for this genus. Urease production has been utilized in many clinical laboratories along with other rapid tests for presumptive identification of *C. neoformans*. Failure to produce urease has been used in many laboratories to determine that isolates from respiratory secretions and gastric washings do not belong to the genus *Cryptococcus* (6). Although 1 of the 286 strains of *C. neoformans* reported by Zimmer and Roberts failed to give a positive urease reaction by a rapid 15-min screening method (7), to our knowledge, this is the first report of an isolate of *C. neoformans* that failed to produce urease when evaluated by standard testing methods.

The identity of this isolate was initially suspected from its morphology on cornmeal-Tween agar, and although it did not hydrolyze urea, the results of other confirmatory tests (detection of phenyloxidase production and carbohydrate assimilation by the auxanographic method) were highly suggestive of *C. neoformans*. The organism was identified as *C. neoformans* with the API 20C system; however, the Uni-Yeast-Tek (Flow Laboratories) carbohydrate assimilation test plate system, when used without supplementary

tests, identified this isolate as *Torulopsis candida*. This erroneous test result highlights the importance of correlating results in carbohydrate assimilation and other biochemical tests with the morphology of the isolate and indicates the potential for misidentification of some isolates when limited assimilation studies alone are used.

This isolate of *C. neoformans* is deposited in the American Type Culture Collection, Rockville, Md. (ATCC 64538).

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