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CASE REPORT

Candida glabrata Oropharyngeal Candidiasis in Patients Receiving Radiation Treatment for Head and Neck Cancer

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***Candida glabrata* colonization is common in patients receiving radiation treatment for head and neck cancer, but to our knowledge has never been described as the infecting organism with oropharyngeal candidiasis (OPC). This study presents the first three patients described with *C. glabrata* OPC in this patient population. Patient 1 developed *C. glabrata* OPC and required fluconazole, 800 mg/day, for clinical resolution. Antifungal susceptibility testing revealed a MIC of fluconazole of >64 μg/ml. Elapsed time from initial culturing to treatment decision was 7 days. Patients 2 and 3 developed *C. glabrata* OPC. They were patients in a study evaluating OPC infections, and cultures were taken immediately. CHROMagar *Candida* plates with 0, 8, and 16 μg of fluconazole/ml were employed for these cultures. Lavender colonies, consistent with *C. glabrata*, grew on the 0- and 8-μg plates but not on the 16-μg plate from patient 2 and grew on all three plates from patient 3. Based on these data, a fluconazole dose of 200 mg/day was chosen for patient 2 and a dose of 400 mg/day was chosen for patient 3, with clinical resolution in both. Elapsed time from initial culturing to treatment decision was 2 days. *C. glabrata* does cause OPC in head and neck radiation treatment patients, and the use of fluconazole-impregnated chromogenic agar may significantly reduce treatment decision time compared to that with conventional culturing and antifungal susceptibility testing.**

CASE REPORTS

Patient 1. Patient 1 was a 47-year-old white male diagnosed with squamous cell carcinoma of the base of the tongue, stage T2N2M0. He was treated with a combination of 7,200 cGy of radiation over 8 weeks and concomitant cisplatin and 5-fluorouracil chemotherapy during weeks 1 and 5. During the fifth week of his treatment he developed oropharyngeal candidiasis (OPC) and was placed on fluconazole, 100 mg/day, with initial resolution. After 2 weeks the candidiasis recurred, and the fluconazole dose was raised to 200 mg/day. The OPC did not resolve, and so the fluconazole dose was raised to 400 mg/day, and a swab culture of lesions was taken. The OPC continued. The culture was plated on blood agar, and identification included germ tube evaluation and API-20C confirmation (bioMerieux, Marcy-l'Etoile, France). The culture grew *Candida glabrata*, and antifungal susceptibility testing was performed according to NCCLS-recommended techniques (1). The 48-h MIC of fluconazole was 64 μg/ml. The patient's fluconazole dose was raised to 800 mg/day with clinical resolution of OPC. The total elapsed time for culture and antifungal susceptibility results was 7 days.

Patients 2 and 3. Patients 2 and 3 were participating in a clinical study where they had cultures taken every week during their radiation treatment. With these patients, cultures employed an oral swab and a swish sample of 10 ml of normal saline instilled in the mouth for 10 s and then collected in a sterile container. These samples were plated on blood agar, RPMI medium, and CHROMagar Candida (CHROMagar Company, Paris, France) chromogenic medium. Colony color on chromogenic medium was recorded. Three chromogenic plates were used with 0.0-, 8.0-, and 16.0-μg/ml fluconazole concentrations to screen for yeasts with decreased susceptibility. The CHROMagar Candida medium was prepared from dry powder according to the manufacturer's directions and cooled to 45°C. Fluconazole intravenous solution (2,000 μg/ml; Pfizer-Roerig, New York, N.Y.) was added to the medium (with sufficient stirring) to yield final concentrations of 8 or 16 μg of fluconazole per ml in agar. Twenty-milliliter volumes of the modified medium were dispensed into 100-mm-diameter sterile plates and cooled at room temperature to harden them prior to use. Briefly, 100 μl of a 1:10 dilution (in phosphate-buffered saline) of the swish sample was spread on the surface of each of the three plates, and the plates were incubated at 30°C for 48 h prior to screening yeasts for reduced susceptibility to fluconazole (Fig. 1). Yeasts were identified by standard techniques including analysis of germ tube formation, growth at 37 and 42°C, and identification by API-20C. For all cultures three to five yeast colonies from primary plates were picked and stored on Sabouraud dextrose slants for antifungal susceptibility testing. Broth microdilution antifungal susceptibility testing to fluconazole was performed by the Fungus Testing Laboratory,

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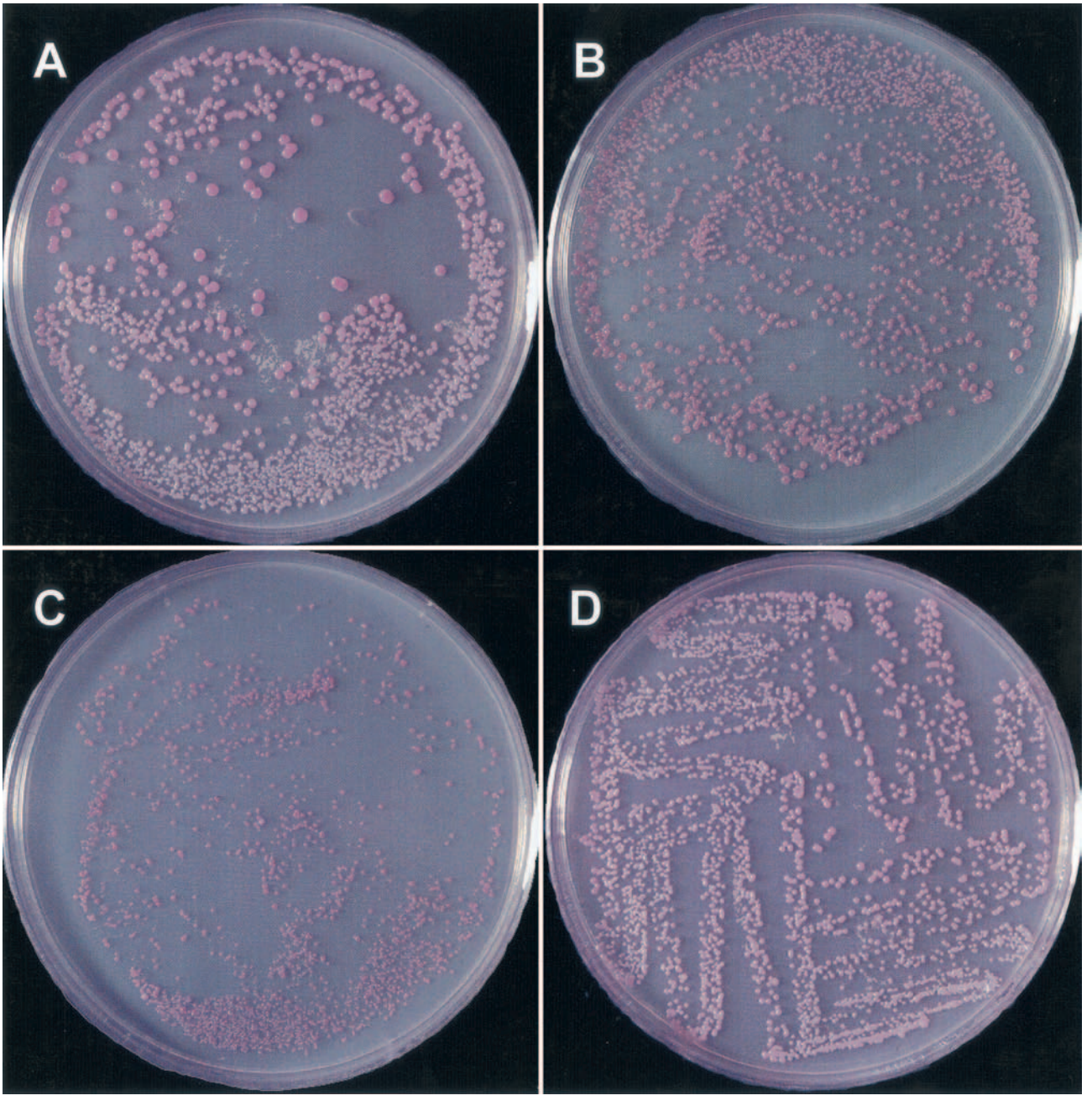

FIG. 1. CHROMagar Candida plates of swish and swab cultures from patient 3. Significant *C. glabrata* colonies are present on the 0.0- (A), 8.0- (B), and 16.0-μg/ml (C) fluconazole plates. This allowed the choice of a 400-mg/day fluconazole dose. The swab culture (D) taken directly from the clinical lesion shows that *C. glabrata* was the only organism involved.

The University of Texas Health Science Center at San Antonio, according to NCCLS-recommended techniques (1).

Patient 2 was a 52-year-old white male diagnosed with squamous cell carcinoma of the floor of the mouth, stage T4N2bM0. He was treated with 5,910 cGy of radiation over a 9-week period. At weeks 2 to 5 serial cultures were positive for *Candida krusei* and/or *C. glabrata*, but he exhibited no clinical disease. During week 6 he presented with white plaques on his oral mucosa which were KOH positive. The swab culture, taken directly from the clinical lesion, and the swish culture were plated on CHROMagar Candida to help with identification. The predominant growths on his swab plate were lavender colonies consistent with *C. glabrata*. There were a few beige-to-pink colonies consistent with *C. krusei*. *C. glabrata* grew only on the swish plates. The predominant organism was confirmed as *C. glabrata* by API-20C. On the CHROMagar Candida plates impregnated with fluconazole, substantial colony growth occurred on the 0.0- and 8.0-μg plates but not on the 16.0-μg plate. This patient was placed on 200-mg/day fluconazole with clinical resolution of OPC. Total elapsed time from culture to CHROMagar Candida results was 2 days. The 48-h MIC of fluconazole ranged from 2 to 32 μg/ml.

Patient 3 was a 40-year-old white male with squamous cell carcinoma of the base of the tongue, stage T4N3M0. He was treated with 7,020 cGy of radiation over an 8-week period and concomitant cisplatin with VP 16 chemotherapy every 3 weeks. At the beginning of radiation therapy he presented with KOH-positive plaques culture positive for *Candida albicans*. He was placed on fluconazole, 100 mg/day. During his second visit he was clinically free of disease but was colonized with *C. glabrata*. During his third visit he presented with white plaques on the tongue which were KOH positive. His fluconazole dose was raised to 200 mg/day. Cultures from this visit grew only *C. glabrata*. The CHROMagar Candida plates from the swish culture grew substantial lavender colonies on the plates with 0.0-, 8.0-, and 16.0-μg/ml fluconazole concentrations, suggesting decreased susceptibility of these isolates compared to those from patient 2. The swab culture, taken directly from the clinical lesion, was plated on CHROMagar Candida and showed growth of *C. glabrata* only (Fig. 1). All cultures were confirmed as *C. glabrata* by API-20C. At his fourth visit the patient still had clinical disease, and his dose was raised to 400 mg of fluconazole/day with clinical resolution of OPC by visit 5. Total elapsed time from culture to CHROMagar Candida results was 2 days. The 48-h MICs of fluconazole ranged from 8 to 16 μg/ml.

Discussion. OPC with *C. glabrata* as the infecting organism has not been previously described for patients receiving radiation treatment for head and neck cancer (2, 3, 5). These three patients add to the picture of *C. glabrata* emerging as a pathogen in the oral cavity (6). For patients 1 and 3 *C. glabrata* was the sole organism isolated. Patient 2 could be said to have a mixed infection, as a few colonies of *C. krusei* grew on the swab culture. However, *C. glabrata* predominated on the swab culture and was present alone on the swish culture. Also this patient responded to a dose of fluconazole consistent with an organism whose susceptibility is felt to be generally dose dependent such as *C. glabrata* and not inherently resistant such as *C. krusei*. Two of our three patients (patients 1 and 3) were receiving concomitant chemotherapy and had been exposed previously to fluconazole. In fact they both developed OPC while receiving doses of 100 mg of this medication/day. This is consistent with emergence of fluconazole-resistant yeasts causing OPC in human immunodeficiency virus-infected patients, since degree of immunosuppression and exposure to fluconazole have been identified previously as risk factors (7). In most clinical cases OPC caused by *C. glabrata* will be suspected only when a patient does not respond to initial therapy. Culture at this point may be indicated. CHROMagar Candida is very helpful in distinguishing *C. glabrata*, as colonies produce a characteristic lavender color on this medium.

All three of our patients were successfully treated with increased doses of fluconazole. We were also able to rapidly predict the correct dose for two patients with a CHROMagar Candida screen with increasing doses of fluconazole (Fig. 1). We developed this technique to rapidly screen for yeasts with decreased susceptibility. (4). Conventional culture and antifungal susceptibility testing took 7 days to complete for patient 1. For patients 2 and 3 the CHROMagar Candida screen gave results in 48 h that accurately predicted the appropriate fluconazole dose. Recently Rex et al. (8) proposed that the area under the curve (AUC)/MIC ratio was the pharmacokinetic parameter which best predicted clinical success with fluconazole. Since the AUC for fluconazole is approximated by the fluconazole dose (in milligrams), that ratio could predict the appropriate fluconazole dose for OPC once the MIC is known. Rex et al. showed that a fluconazole dose/MIC ratio of 25 or higher for a 48-h MIC would predictably give an effective fluconazole dose for OPC. Therefore, a patient would require 800 mg for a MIC of 32, 400 mg for a MIC of 16, 200 mg for a MIC of 8, and 100 mg for a MIC of 4 (8).

We used a modification of this to determine MIC based on significant growth at 0.0-, 8.0-, and 16.0-μg/ml fluconazole concentrations in CHROMagar Candida. Patient 2 responded to a fluconazole dose of 200 mg/day, and his samples had significant growth on the 0.0- and 8.0-μg CHROMagar plates. From these data we predicted that significant growth at only 0.0 μg predicted a dose of 100 mg; at 0.0 and 8.0 μg predicted a dose of 200 mg; and at 0.0, 8.0, and 16.0 μg predicted a dose of 400 mg. This was confirmed with patient 3. These results actually show effective fluconazole doses at MICs 1 dilution higher than that in the AUC/MIC ratio described above for the work of Rex et al. (8). This may be explained in that the data for this calculation were primarily obtained from human immunodeficiency virus-infected patients whose immune status is more compromised than that of patients receiving head and neck radiation treatment. Head and neck cancer patients may be better able to clear clinical disease at lower doses of fluconazole.

Correlation of this screen with traditional MICs yielded variable results. MICs for patient 2 were consistent with the screen, although there was a wide range of results. However, MICs for patient 3 were lower than those in our screen. We plan to continue our preliminary evaluation of this screen by employing it with more patients with *C. glabrata* OPC and expanding it to include patients with OPC caused by other *Candida* species. We will also compare MIC results with this technique to those with NCCLS-approved techniques for multiple isolates of *C. glabrata* in our present collection.

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