JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1996, p. 2205–2209 Vol. 34, No. 9
0095-1137/96/$04.00+0

Techniques for Investigation of an Apparent Outbreak of Infections with *Candida glabrata*

S. ARIF,[1] T. BARKHAM,[2] E. G. POWER,[2] AND S. A. HOWELL[1]*

Department of Microbial Diseases, St. John's Institute of Dermatology,[1] and Department of Microbiology,[2] St. Thomas' Hospital, United Medical and Dental Schools University of London, London SE1 7EH, United Kingdom

Received 11 March 1996/Returned for modification 10 April 1996/Accepted 17 June 1996

A cluster of *Candida glabrata* isolates recovered from seven patients in an intensive care unit over a 10-week period were compared with a collection of isolates from six epidemiologically distinct outpatients and a reference strain by several DNA typing methods. Restriction enzyme analysis with *Hin*fI distinguished 13 strains from the 14 sources and was the method of choice. Pulsed-field gel electrophoresis and random amplification of polymorphic DNA both detected nine types from the 14 sources; however, the results of these two methods did not always correlate. These methods demonstrated that five of the seven patients had distinguishable strains and that cross-infection was unlikely.

Candida glabrata is being recovered with increasing frequency from clinical specimens; for example, Horowitz et al. (5) reported a significant increase in instances of isolation of this yeast species in cases of vaginal candidosis over the last 30 years. This increase may be due in part to the selection of resistant organisms, including *C. glabrata*, by current antifungal agents, especially the azoles (4), though a recent study in a cancer center over 5 years found no increase in the instances of isolation of *Candida* species other than *Candida albicans*, despite the increasing use of antifungal agents (10).

Few outbreaks caused by *C. glabrata* have been reported. A cluster of *C. glabrata* infections that took place in an adult leukemia unit and that was evaluated by restriction enzyme analysis (REA) was deemed to constitute an outbreak attributable to a common strain (11). Further investigation by pulsed-field gel electrophoresis (PFGE) revealed that the outbreak was not of the magnitude originally claimed (9). Thus, it may be necessary to employ several different typing methods to obtain optimal strain delineation.

In this paper, we report on the isolation of *C. glabrata* from a number of patients in an intensive care unit (ICU) within a short period of time and the techniques used to investigate the possibility that this appearance represented an outbreak.

MATERIALS AND METHODS

Epidemiology. *C. glabrata* was isolated from seven patients in the ICU over a period of 10 weeks. This species had not been isolated in the preceeding 9 weeks and was not isolated in the subsequent 7 weeks, and on average, only two isolates a month were recovered from the ICU throughout the following 9 months. Each of the seven patients was present in the ICU with at least one of the other six patients, and none of the patients developed proven fungemia. Patients 1 and 7 did not receive any systemic antifungal agent treatment, patients 3, 4, and 6 received between 1 and 14 days of treatment with fluconazole, patient 2 had received 21 days of treatment with fluconazole for a previous *C. albicans* infection but died from an underlying illness, and patient 5 was treated with fluconazole and then with amphotericin B because of the persistent presence of *C. glabrata* in the patient's urine. Specific control methods beyond basic handwashing and rational prescription of antibiotics were not instituted, and the rate of instances of isolation of *C. glabrata* fell to an average of two per month.

Fungal isolates. Single isolates of *C. glabrata* were available from six patients in the ICU (patients 1 to 4 and 6 and 7), and seven isolates were collected from another patient (patient 5). The isolates were collected during October to early December 1994, with the exception of isolate 30444 (patient 7), which was recovered 1 year previously when the patient was in the same unit. As controls, 11 isolates from six epidemiologically unrelated patients were selected from collections made during 1983 to 1985, and a reference culture isolate, ATCC 90030, was also included. Species identification was initially performed with CHROMagar differential culture medium (14) and then was confirmed with the API 20C AUX system (Biomerieux, Basingstoke, Hants, United Kingdom). Cultures were maintained on Oxoid Sabouraud dextrose agar (Unipath, Basingstoke, Hants, United Kingdom). Details of the isolates are listed in Table 1.

For analysis, cells were grown in YEPD broth (0.3% yeast extract, 1% mycological peptone, 2% glucose [Oxoid]); a loopful of cells was inoculated into 5 ml of broth and incubated at 37°C in an orbital incubator at 140 rpm.

DNA extraction. DNA was extracted from 1.5 ml of overnight culture by an adaptation of the methods of Scherer and Stevens (17). After the cells were pelleted by centrifugation, they were washed and then resuspended in 1 ml of 1 M sorbitol–50 mM potassium phosphate (KH_2PO_4) buffer (pH 7.5). Each extract had 50 μl of Zymolyase 20T (ICN Biomedicals, High Wycombe, United Kingdom) at 8 mg/ml and 3 μl of β-mercaptoethanol added, and this mixture was incubated at 37°C for 120 min. The spheroplasts were collected by centrifugation and resuspended in 0.5 ml of GES reagent (5 M guanidium thiocyanate–100 mM EDTA–0.5% Sarkosyl) and kept at room temperature for 20 min (15). Following the addition of 100 μl of 5 M potassium acetate and incubation on ice for 20 min, 0.5 ml of chloroform-pentanol (24:1, vol/vol) was added, the mixture was mixed, and the phases were separated by centrifugation. The upper aqueous layer was removed, and DNA was precipitated by the addition of 0.5 ml of ethanol. The DNA was pelleted, rinsed with 70% ethanol, and resuspended in 100 μl of TE buffer (10 mM Tris, 1 mM EDTA). The DNA was reprecipitated, and the final pellet was dissolved in 50 μl of TE buffer.

Restriction digests and Southern blots. Restriction digests were performed according to the manufacturers' instructions, with 25 μl of DNA solution being used and incubation taking place at 37°C for 2.5 h with either *Eco*RI or *Msp*I or for 4 h with *Hin*fI. The DNA fragments were separated electrophoretically in a 0.8% agarose gel in TBE buffer (0.089 M Trizma base, 2.5 mM EDTA, 32 mM boric acid) at 30 V for 18 h. The DNA was stained with ethidium bromide, and the results were recorded photographically.

The gels were Southern blotted onto flash prime nylon membranes (Stratagene, Cambridge, United Kingdom) by the method of Maniatis et al. (13), and the DNA was fixed by UV irradiation.

DNA hybridization. Hybridizations with a poly(dG-dT) probe (Pharmacia, St. Albans, Herts, United Kingdom) were conducted at 42°C. The blots were prehybridized for 4 h in a mixture of 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, and 8% fat-free UHT milk. Hybridizations were performed for 18 h in a mixture of 40% formamide, 5× SSC, 1× Denhardt's solution, 5% dextran sulfate, 8% fat-free UHT milk, and heat-denatured, biotin-labelled poly(dG-dT) probe. The membranes were washed five times for 30 min each time in 2× SSC, and hybridization was detected with the BRL BluGENE kit (Gibco BRL, Paisley, Scotland) according to the manufacturer's instructions.

PFGE. Cells were prepared for pulsed-field gel electrophoresis (PFGE) by removing half a loopful of cells from an overnight culture grown on Sabouraud dextrose agar and washing the cells in 200 μl of LET buffer (10 mM Tris, 0.5 M EDTA, 2% sodium hydroxide). The cells were pelleted and suspended in 200 μl of LET buffer containing 5 mg of Zymolyase 20T per ml. A 250-μl aliquot of 1% low-melting-temperature agarose was added, and the solution was pipetted into molds. The agarose plugs were then placed into 1 ml of LET buffer containing 60 μl of β-mercaptoethanol and incubated at 37°C for 24 h. The LET buffer was

* Corresponding author. Phone: 0171 928 9292, ext. 1373. Fax: 0171 928 1428.

TABLE 1. Details of the *C. glabrata* isolates and the different patterns detected by REA with *Hin*fI, by karyotyping by PFGE, or by RAPD

Patient no.	Isolate no.	Isolation date (day/mo/yr)	Sampling site	Source	Pattern no.		
					*Hin*fI	PFGE	RAPD
1	12837	21/09/94	Skin	ICU	1	1	1
2	5309	07/10/94	Sputum	ICU	2	2	2
3	5412	13/10/94	Sputum	ICU	3	3	3
4	45618	18/10/94	Urine	ICU	3	3	3
5	48468	27/10/94	Urine	ICU	4	4	4
5	11353	28/10/94	Skin	ICU	4	4	4
5	11354	28/10/94	Skin	ICU	4	4	4
5	11909	07/11/94	Skin	ICU	4[a]	4	4
5	51070-1	07/11/94	Urine	ICU	4	4	4
5	51070-2	07/11/94	Urine	ICU	4[a]	4	4
5	52604	10/11/94	Urine	ICU	4	4	4
6	4166	15/11/94	Sputum	ICU	5	5	5
7	30444	28/11/93	Blood	ICU	6	4	ND[b]
8	95	03/10/83	Urine	Outpatient	7	4	6
8	96	06/10/83	Vagina	Outpatient	7[a]	4	6
9	110	01/02/84	Urine	Outpatient	8	6	3
10	132	16/02/84	Vagina	Outpatient	9	4	7
11	145	12/03/84	Vagina	Outpatient	10	7	8
12	293	23/07/84	Urine	Outpatient	11	6	3
12	296	30/07/84	Vagina	Outpatient	11[a]	6	3
12	297	30/07/84	Urine	Outpatient	11	6	3
12	299	02/08/84	Urine	Outpatient	11	6	3
13	431	30/08/84	Stool	Outpatient	12	8	9
13	432	13/09/84	Stool	Outpatient	12[a]	8	9
14	ATCC 90030			Type strain	13	9	2

[a] Possible subtype (the pattern differed by only one band from those of other isolates from that patient).
[b] ND, not determined.

then replaced with 1 ml of NDS buffer (10 mM Tris, 0.5 M EDTA, 2% sodium hydroxide, 1% lauroylsarcosine) containing 2 mg of proteinase K (Boehringer Mannheim, Lewes, East Sussex, United Kingdom) per ml and incubated at 50°C for 24 h. The NDS buffer was then replaced with 0.5 M EDTA, and the plugs were stored at 4°C.

Karyotyping of the yeast isolates was performed by PFGE with a Pharmacia-LKB Pulsaphor system with a hexagonal electrode array. The electrophoretic separations were performed in a 1% SeaKem agarose gel in 0.5× TBE at 12°C, with a pulse time of 120 s at 110 V for 22 h and then a time of 180 s at 110 V for 22 h. The gels were stained with ethidium bromide, and the results were photographed.

RAPD. A 10-mer primer, 5′-TGGACCCTGC-3′ (3), was used in DNA amplification reactions as described by Howell et al. (6). Briefly, 200 μM (each) deoxynucleotides (Sigma), 1 μM primer, 100 ng of extracted genomic DNA, 2 U of Dynazyme (Flowgen, Staffordshire, United Kingdom), 5 μl of 10× reaction buffer, and 1.5 mM magnesium chloride were added to a 50-μl reaction volume, and the volume was made up to 50 μl with molecular biology-grade water. The cycling parameters were 94°C for 5 s, 36°C for 30 s, and 72°C for 1 min for 30 cycles on a Hybaid OmniGene using tube control. A 10-μl aliquot of the random amplification of polymorphic DNA (RAPD) assay mixture was electrophoresed on a 2% agarose gel in TBE buffer at 70 V for 3 h; the gels were then stained with ethidium bromide and photographed.

Susceptibility tests. Antifungal agent susceptibility tests were done by an in-house broth microdilution method (13a), with twofold serial dilutions of fluconazole in water (0.06 to 128 μg/ml) and itraconazole in dimethyl sulfoxide (0.06 to 32 μg/ml) being used. The inoculum was prepared from an overnight culture grown on Sabouraud dextrose agar and appropriately diluted in yeast nitrogen base broth (Unipath). The trays were incubated at 35°C for 48 h, and the MIC was determined as the drug concentration that reduced growth by 80% compared with growth of the drug-free control. The breakpoint for resistance to fluconazole was a concentration of 8 to 32 μg/ml, and that for resistance to itraconazole was a concentration of 2 to 8 μg/ml.

The susceptibilities of the ICU isolates were confirmed by the Bristol Mycology Reference Laboratory by a broth microdilution assay (7).

RESULTS

The ICU isolates, the American Type Culture Collection strain, and the isolates from outpatients 8, 10, 11, and 13 produced the same API 20C AUX profile, 2000040. Those from outpatients 9 and 12 additionally utilized glycine and produced a profile code of 6000040. There was no apparent association between the API profile and any of the DNA typing schemes.

Few differences in the restriction patterns obtained with *Eco*RI were seen, with ICU isolates 5412 (patient 3) and 45618 (patient 4) providing one pattern and with all of the remaining isolates, including the outpatient strains, giving a second pattern. *Msp*I provided better discrimination and produced six patterns from the seven ICU patients' isolates, again with isolates 5412 and 45618 being indistinguishable. However, both of these restriction enzymes produce a large number of bands and a high background which hinders interpretation; therefore, a third enzyme, *Hin*fI, was used, and this enzyme revealed a series of very distinct bands in the region from 23 kb to approximately 4.4 kb. These bands indicated that isolates 5412 and 45618 could not be distinguished and that the remaining five ICU patients and six outpatients each carried a separate and individual strain. In cases in which multiple isolates were available from the same patients, it was found that single band differences could be seen within the patterns, which typically comprised six or seven bands (Fig. 1). Between patients, however, the differences amounted to several bands (Fig. 2). A difference of one band was not considered sufficient reason to designate an isolate as a separate strain; therefore, patients 5, 8, 12, and 13 were found to harbor two subtypes of single strains (subtypes indicated in Table 1). With *Hin*fI, a total of 13 patterns were identified from isolates from 13 patients and one reference strain.

Southern hybridizations of the poly(dG-dT) probe to DNA digested with either *Eco*RI or *Msp*I revealed between four and seven bright bands plus some that were fainter (Fig. 3). Four different patterns among the samples from the ICU were detected with both enzymes. A further three patterns were de-

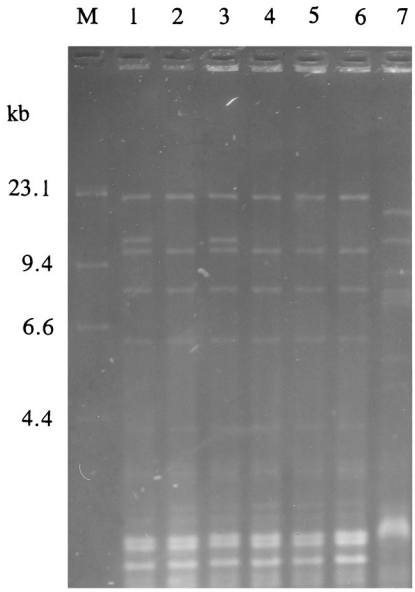

FIG. 1. *Hin*fI digests of multiple isolates from patient 5 and one from patient 7. Lanes: M, lambda *Hin*dIII-digested marker; 1, 11909; 2, 51070-1; 3, 51070-2; 4, 11353; 5, 11354; 6, 48468; and 7, 30444 (patient 7).

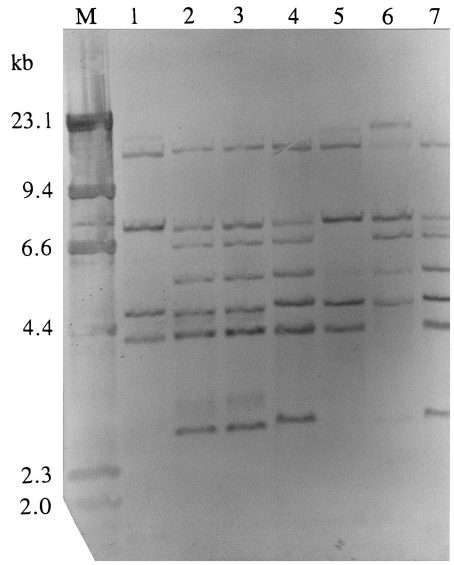

FIG. 3. Poly(dG-dT) fingerprints of *Eco*RI digests of the ICU isolates. Lanes: M, lambda *Hin*dIII digest marker; 1, 5309 (patient 2); 2, 5412 (patient 3); 3, 45618 (patient 4); 4, 4166 (patient 6); 5, 48468 (patient 5); 6, 12837 (patient 1); and 7, 30444 (patient 7).

tected among samples from the control outpatients when *Eco*RI was used.

Comparisons of chromosome bands that had sizes of less than 1.6 Mb and that were separated by PFGE distinguished isolates from five patients (1, 2, 3, 5, and 6) from the ICU and showed that the multiple isolates from patient 5 were indistinguishable from each other and that from patient 7. Isolates 5412 (patient 3) and 45618 (patient 4) appeared to be very similar. Among 12 outpatient isolates examined, representing six patients and one reference culture, five karyotype patterns were detected, one of which resembled the pattern from patient 5 (data summarized in Table 1). A total of nine patterns from isolates from 13 patients and the reference strain were identified.

RAPD typing results were similar to those obtained by PFGE. Strains with a difference of two or more bands were considered distinct. Multiple isolates from patient 5 were indistinguishable, and isolates 5412 (patient 3) and 45618 (patient 4) produced identical fingerprints. All other isolates from the ICU had unique RAPD fingerprints. Six distinct patterns were obtained from the 12 control isolates (six patients and one type culture). One pattern resembled the fingerprint obtained for patients 3 and 4, and the pattern for the reference strain (ATCC 90030) was indistinguishable from that for the isolate from patient 2 (Fig. 4 and Table 1). A total of nine

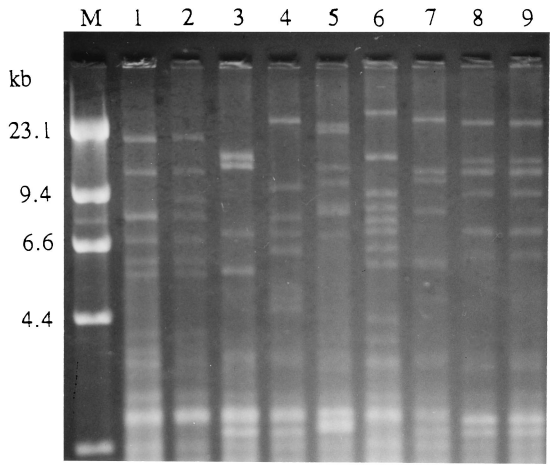

FIG. 2. *Hin*fI digests of strains from patients from the ICU and from outpatients. Lanes: M, lambda *Hin*dIII digest marker; 1, 95 (patient 8); 2, 96 (patient 8); 3, 110 (patient 9); 4, 132 (patient 10); 5, 145 (patient 11); 6, 4166 (patient 6); 7, 5309 (patient 2); 8, 5412 (patient 3); and 9, 45618 (patient 4).

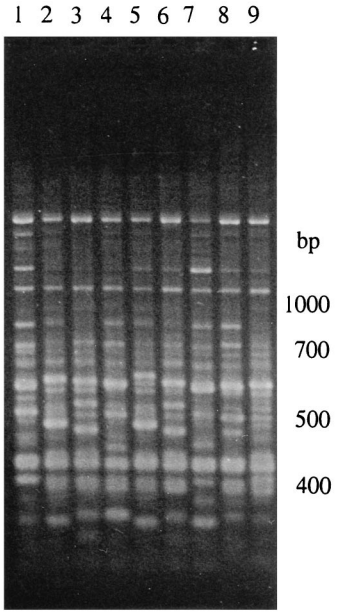

FIG. 4. RAPD fingerprints of some of the ICU and outpatient isolates. Lanes: 1, 48468 (patient 5); 2, 4166 (patient 6); 3, 5412 (patient 3); 4, 12837 (patient 1); 5, 95 (patient 8); 6, 110 (patient 9); 7, 132 (patient 10); 8, 145 (patient 11); and 9, 431 (patient 13).

fingerprints for isolates from 13 patients and the reference strain were identified.

Antifungal agent sensitivity tests showed that the ICU isolates and the outpatient isolates from 1983 to 1985 could be regarded as resistant to fluconazole.

DISCUSSION

Following a sudden and short-lived increase in the recovery of *C. glabrata* isolates from the skin and urine of patients in an ICU, the isolates were examined by DNA typing methods to establish if an outbreak had occurred and which methods would be the most suitable to examine strains of this species.

For REA, the enzyme *Hin*fI proved to be the most discriminatory enzyme for the separation of these *C. glabrata* isolates. These results suggested that patients 3 and 4 (isolates 5412 and 45618 were recovered only 5 days apart) may have shared a single strain but that the remaining five patients in the ICU were infected with distinct strains. The epidemiologically unrelated outpatient isolates acted as controls for the comparisons with the ICU isolates, and each of these outpatients carried a separate, distinguishable strain. The enzyme *Eco*RI failed to discriminate among isolates, while *Msp*I distinguished six of the seven patient isolates. Similar findings were reported by Reagan et al. (16), who detected two patterns among six isolates with either *Bst*I or *Eco*RI, and Lee et al. (11), who could not distinguish among 25 isolates with *Eco*RI but who detected five types with *Xba*I. However, Vazquez et al. (19) differentiated 34 isolates into 14 groups with *Eco*RI and further subdivided 3 of these groups with *Msp*I.

Hybridization of genomic blots with suitable probes provides one approach to improving the discrimination achieved by REA. The simple repeat sequence poly(dG-dT) has been used as a probe to differentiate strains of *C. albicans* (20), and in this study of *C. glabrata*, it was used to detect seven patterns from 13 patients. Sullivan et al. (18) tested a selection of simple repeat oligonucleotide probes for the differentiation of 12 *C. glabrata* isolates and described $(G\text{-}T)_8$ as being the most informative, providing four patterns.

Karyotyping is another method that has been useful in the identification of strains of *C. glabrata*. Kaufmann and Merz (8) examined 33 isolates and detected 22 types with between 8 and 12 bands. Similarly, Vazquez et al. (19) detected 16 types from 34 isolates, and Khattak et al. (9) found 10 types from 30 isolates. Asakura et al. (1) examined 21 isolates of *C. glabrata* and detected 6 to 12 bands in the size range of 0.42 to 2.4 Mb, with one or two bands being highly variable in size. These variable bands hybridized to a ribosomal DNA (rDNA) probe that hybridizes to *C. albicans*. The bands below the rDNA chromosomes were similar, although there were a few bands in each karyotype that enabled the isolates to be distinguished. The chromosomes bearing the rDNA were reported to exhibit clonal variation, and the authors concluded that karyotyping offered a precise identification method if the variable bands were ignored. As hybridization with an rDNA probe confirmed the nature of the heaviest bands in this study (data not shown), bands larger than 1.6 Mb were not included in the analysis of the karyotypes of the isolates in our study. This cutoff enabled five strains from the ICU patients to be recognized, with the multiple isolates from patient 5 being identical.

The last DNA typing method examined used RAPD, as this technique has proven useful for the typing of strains of *C. albicans* (2, 6) and *C. glabrata* (3, 12) and has been considered to be highly discriminatory, reproducible, rapid, and relatively simple. Although primer A3 used in this study was not as discriminatory as *Hin*fI in REA, other primers may improve discrimination. Bostock et al. (2) described two primers for RAPD analysis, of which one was more discriminatory and one less discriminatory compared with those for REA.

In conclusion, the typing technique found to be most suitable for the differentiation of isolates of *C. glabrata* was REA with *Hin*fI, which detected 13 strains from 14 sources. PFGE and RAPD were not as discriminatory as REA, although both detected nine types from the 14 sources; however, these two typing schemes did not correlate in several instances.

These methods demonstrated that what had initially appeared to be an outbreak of *C. glabrata* in an ICU because of the unusual number of isolates and time clustering of their isolation was not due to cross-infection, as five of the seven patients had distinguishable strains. The two patients with indistinguishable isolates could represent an example of cross-infection or simply reflect the existence of a common type. Normal infection control procedures were instituted, and there have been few isolates of this yeast species recovered since this episode.

ACKNOWLEDGMENTS

We are grateful to M. Cunningham of St. John's Institute of Dermatology for supplying the outpatient isolates and to J. May of the Department of Microbiology at St. Thomas' Hospital for help with the antifungal testing.

The St. Thomas' Hospital Special Trustees are thanked for partial funding of this work (E.G.P. and T.B.). We are grateful to Smith and Nephew Ltd. for their support of S. Arif.

REFERENCES

1. **Asakura, K., S.-I. Iwaguchi, M. Homma, T. Sukai, K. Higashide, and K. Tanaka.** 1991. Electrophoretic karyotypes of clinically isolated yeasts of *Candida albicans* and *C. glabrata*. J. Gen. Microbiol. **137:**2531–2538.
2. **Bostock, A., M. N. Khattak, R. Matthews, and J. Burnie.** 1993. Comparison of PCR fingerprinting, by random amplification of polymorphic DNA, with other molecular typing methods for *Candida albicans*. J. Gen. Microbiol. **139:**2179–2184.
3. **Bougnoux, M. E., F. Robert, S. Beria, B. Cassinat, M. H. Nicolas, and J. Dupouy-Camt.** 1994. Use of randomly amplified polymorphic DNA markers to distinguish isolates of Candida. J. Mycol. Med. **4:**3–8.
4. **Ellis, M. E., S. M. H. Qadri, D. Spence, M. A. Halim, P. Ernst, H. Clink, F. Baillie, and E. B. De Vol.** 1994. The effect of fluconazole as prophylaxis for neutropenic patients on the isolation of *Candida* spp. from surveillance cultures. J. Antimicrob. Chemother. **33:**1223–1228.
5. **Horowitz, H. J., D. Giaquinta, and S. Ito.** 1992. Evolving pathogens in vulvovaginal candidiasis: implications for patient care. J. Clin. Pharmacol. **32:**248–255.
6. **Howell, S. A., R. M. Anthony, and E. Power.** 1996. Application of RAPD and restriction enzyme analysis to the study of the oral carriage of *Candida albicans*. Lett. Appl. Microbiol. **22:**125–128.
7. **Johnson, E. M., D. W. Warnock, J. Luker, S. R. Porter, and C. Scully.** 1995. Emergence of azole drug resistance in *Candida* species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. J. Antimicrob. Chemother. **35:**103–114.
8. **Kaufmann, C. S., and W. G. Merz.** 1989. Electrophoretic karyotypes of *Torulopsis glabrata*. J. Clin. Microbiol. **27:**2165–2168.
9. **Khattak, M. N., J. P. Burnie, R. C. Matthews, and B. A. Oppenheim.** 1992. Clamped homogeneous electric field gel electrophoresis typing of *Torulopsis glabrata* isolates causing nosocomial infections. J. Clin. Microbiol. **30:**2211–2215.
10. **Kunova, A., J. Trupl, S. Spanik, L. Drgona, J. Sufliarsky, J. Lacka, V. Studena, E. Hlavacova, M. Studena, E. Kukuckova, T. Kollar, P. Pichna, E. Oravcova, and V. Krcmery.** 1995. *Candida glabrata*, *Candida krusei*, non-albicans *Candida* spp., and other fungal organisms in a sixty-bed national cancer center in 1989–1993: no association with the use of fluconazole. Chemotherapy (Basel) **41:**39–44.
11. **Lee, W., J. P. Burnie, R. C. Matthews, B. O. Oppenheim, and N. N. Damani.** 1991. Hospital outbreaks with yeasts. J. Hosp. Infect. **18**(Suppl. A)**:**237–249.
12. **Lehman, P. F., D. Lin, and B. A. Lasker.** 1992. Genotypic identification and characterization of species and strains within the genus *Candida* by using random amplified polymorphic DNA. J. Clin. Microbiol. **30:**3249–3254.
13. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 9.31–9.46. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

13a. **May, J.** Personal communication.

14. **Odds, F. C., and R. Bernaerts.** 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. **32:**1923–1929.
15. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–156.
16. **Reagan, D. R., M. A. Pfaller, R. J. Hollis, and R. P. Wenzel.** 1990. Characterization of the sequence of colonization and nosocomial candidemia using fingerprinting and a DNA probe. J. Clin. Microbiol. **28:**2733–2738.
17. **Scherer, S., and D. A. Stevens.** 1987. Application of DNA typing methods to epidemiology and taxonomy of *Candida* species. J. Clin. Microbiol. **25:**675–679.
18. **Sullivan, D., D. Bennett, M. Henman, P. Harwood, S. Flint, F. Mulcahy, D. Shanley, and D. Coleman.** 1993. Oligonucleotide fingerprinting of isolates of *Candida* species other than *C. albicans* and of atypical *Candida* species from human immunodeficiency virus-positive and AIDS patients. J. Clin. Microbiol. **31:**2124–2133.
19. **Vazquez, J. A., A. Beckley, S. Donabedian, J. D. Sobel, and M. J. Zervos.** 1993. Comparison of restriction enzyme analysis versus pulsed-field gradient gel electrophoresis as a typing system for *Torulopsis glabrata* and *Candida* species other than *C. albicans*. J. Clin. Microbiol. **31:**2021–2030.
20. **Wilkinson, B. M., L. Morris, D. J. Adams, E. G. V. Evans, C. J. N. Lacy, and R. M. Walmsley.** 1992. A new, sensitive polynucleotide probe for distinguishing *Candida albicans* strains and its use with a computer assisted archiving and pattern comparison system. J. Med. Vet. Mycol. **30:**123–131.