

Epidemiologic Study by DNA Typing of a *Candida albicans* Outbreak in Heroin Addicts

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The epidemiology of an outbreak of *Candida endophthalmitis* in heroin addicts was studied by DNA typing. Although one biotype was prevalent, the 13 isolates of *Candida albicans* from seven of the patients were placed into six separate groups by DNA type. Thus, the outbreak of candidiasis was not, as had been concluded from biotyping, due to a *C. albicans* strain of common origin.

Candida albicans is a ubiquitous commensal yeast of humans that is associated with various body sites such as mucosal surfaces. Most disease episodes are related to factors which compromise host resistance, usually resulting in an opportunistic endogenous infection. However, outbreaks of candidiasis thought to be acquired from a common source have been reported (3, 7, 10, 12, 13). Of interest epidemiologically are recent outbreaks of systemic candidiasis in heroin addicts that have presented clinically with *Candida endophthalmitis* (7, 9–12). In one study of an outbreak in 23 addicts in Glasgow, Scotland, *C. albicans* was cultured from injection paraphernalia, preserved lemon juice, which was used as the heroin solvent, and patients (9–12). Strain differentiation of these isolates was done by the biotyping scheme of Odds and Abbott (4, 5), which is a system consisting of the growth of *C. albicans* on nine agar plate media. The nine tests are scored in groups of three, and the scores are used to generate the three-digit number that represents the biotype of the organism (4, 5). For example, an organism with a biotype of 453 differs by growth on only one medium from one with a biotype of 457 (see references 4 and 5 for further details). Biotyping of the *C. albicans* strains isolated from the Glasgow outbreak resulted in a predominant biotype, 153, being recovered from the different sources (10). The biotype similarity of some patient isolates and isolates recovered from the preserved lemon juice indicated that the lemon juice was a possible source of the outbreak in the addicts (10–12).

To study further the epidemiology of this outbreak, we have compared the isolates for identity and similarity by DNA restriction fragment length polymorphism (RFLP) typing (8, 13). The *C. albicans* isolates from the Glasgow outbreak (10, 12) are described in Table 1. Because there was a possibility that transmission of the yeast was occurring between the addicts' commensal flora and their injection paraphernalia, samples were taken from superficial sites and donated injection materials for comparison (10, 12). The criteria for diagnosis of *Candida endophthalmitis* have been described elsewhere (9). These isolates were given the letter designation G (Glasgow) preceding the isolate number. The 29 *C. albicans* isolates of known DNA type have been

described elsewhere and are referred to by DNA type designation (8, 13). All isolates were maintained by growth and storage on slants of Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) prior to culture for DNA extraction.

The method of DNA extraction used in this study has been described previously in detail and was used with no modifications (8). Restriction endonuclease digestion of *C. albicans* DNA with *EcoRI* (60 U/μl; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was done by using a modification of a previously described method (8). In brief, digestions were done for 90 min at 37°C with 10 μl of DNA in TE (10 mM Tris hydrochloride, 1 mM disodium EDTA [pH 7.5]) plus 10 μl of double-strength restriction buffer (100 mM Tris hydrochloride, 20 mM MgCl₂, 200 mM NaCl [pH 7.5]) containing 40 U of *EcoRI*. Digestion was stopped by heating to 70°C for 5 min. Digested samples were electrophoresed through 4-mm-thick 0.8% agarose gels with a Tris acetate-EDTA buffer (8) at 1 to 2 V/cm overnight. Both the gel and the running buffer contained 0.5 μg of ethidium bromide per ml. DNA was visualized by 300-nm transillumination and photographed through an orange filter with Polaroid 667 or 665 film. The DNA type of each unknown isolate was determined by adjacent lane comparison of the RFLP pattern with those with known patterns (8, 13).

The DNA typing scheme described by Scherer and Stevens (8) and Stevens et al. (13) based on RFLP in DNA bands encoding rRNAs (i.e., rDNA) was used as the reference scheme in these studies. In this system a dimorphic rDNA band separates isolates into two major groups: IA (3.7-kb band) or IB (4.2-kb band) (13). Further subtypes of each major group are determined from the RFLP patterns of rDNA bands at 6 to 7 kb and 2.5 to 3.0 kb and the presence or absence of the remaining, less intense bands and areas of low-intensity staining (8).

Initial screening placed 12 of the 13 G isolates (G2, G4, and G6 to G15) into the IA DNA type group because of the presence of the 3.7-kb band (Fig. 1). Isolate G5 was determined to belong to the DNA type IB group (Fig. 1). Upon comparison of RFLP profiles of the G isolates for identity or nonidentity, six separate patterns containing one or more G isolates were apparent. Isolates G2 and G4, which were obtained from separate patients (Table 1), were the same by RFLP analysis (Fig. 1). Multiple isolates of *C. albicans* were

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TABLE 1. Description of *C. albicans* isolates from heroin addicts from an outbreak in Glasgow

Patient no. ^a	Source ^a	Isolate no. ^a	Biotype ^a	DNA type
7	Lemon juice	G2	153	IA23
9	Scalp	G4	2 ¹ / ₅ 3	IA23
15	Throat swab	G5	153	IB10
18	Mouthwash	G6	153	IA1
18	Mouth swab	G7	1 ¹ / ₅ 3	IA1
19	Mouth swab	G8	15 ³ / ₇	IA22
19	Mouthwash	G9	15 ³ / ₇	IA22
22	Vitreous	G10	214	IA21
22	Vitreous	G11	155	IA21
22	Vitreous	G12	155	IA21
23	Mouthwash	G13	455	IA2
23	Feces	G14	455	IA2
23	Mouthwash	G15	455	IA2

^a Data were compiled from reference 10. Isolates were obtained from patient specimens or injection paraphernalia donated by the patient.

available from four patients (Table 1). Each patient was found to have a different single infecting strain based on the RFLP pattern (Fig. 1).

Further assignment of each G isolate DNA into a subtype was done for archival purposes by comparison of the RFLP pattern with that of a reference DNA subtype (IA1 to IA20 or IB1 to IB9 subtypes) (13). Isolates G6 and G7 subtyped as IA1, and isolates G13 to G15 subtyped as IA2. The remaining G isolates did not match any of the reference strains and were designated as new group IA or IB DNA subtypes of *C. albicans*. The DNA subtype of each G isolate is summarized in Table 1. Inclusion of these results with those reported previously (13) gives a total of 104 isolates of *C. albicans* from various populations and geographic origins that have been DNA typed. These 104 isolates have been placed into 33 subtypes, IA1 to IA23 and IB1 to IB10. IA2 predominates as the most common subtype (38.8%, or 40 of 104 isolates),

regardless of isolate origin. From the populations examined thus far, other subtypes have been found much less frequently (8% or less for each subtype) (see reference 13).

Most methods used to examine the epidemiology of *C. albicans* rely on phenotypic expression of one or more characteristics such as biotyping, antigen expression, or isoenzyme profile as the basis for determination of strain relatedness (1, 2, 4-6). The usefulness of these methods can be hampered by various factors, including insufficient strain discrimination and phenotypic variability (6). More recently, RFLP patterns of highly conserved rDNA regions have been used to study strain relatedness among *C. albicans* isolates (3, 8, 13). Because of the stability of these patterns and relative ease of performance, this method has been proposed as a useful and rapid adjunct to strain discrimination (3, 8, 13). Additional methods of DNA comparison such as electrophoretic karyotype, RFLP analysis of mitochondrial DNA, and Southern blot probing with cloned fragments of DNA have also been used and show promise as tools for the study of *C. albicans* epidemiology (for an overview, see reference 13). While a thorough comparison and correlation of some phenotypic methods and DNA typing remains to be done, a recent comparison of DNA typing and biotyping indicated neither complete nor random association between the two methods (13).

In the present investigation, we applied our DNA typing scheme (8, 13) to the epidemiology of a previously described outbreak of candidiasis in heroin addicts in whom one biotype of *C. albicans* appeared to predominate (10). Unlike the biotyping results, results of the present study showed that no single DNA type is prevalent among the 13 isolates obtained from seven patients. Only two isolates, G2 and G4, from different patients, patients 7 and 9, had the same DNA type. The remainder (11 of 13 isolates) were placed into five different DNA subtypes, each one different for each patient (Table 1). In each instance in which multiple isolates were obtained from a single patient, all isolates had identical DNA subtypes. Of particular interest were the three vitreous isolates (G10 to G12, patient 22); G10 was biotyped as 214, while G11 and G12 were biotyped as 155 (10). By DNA subtyping, these three isolates were identical (IA21), indicating a single strain was responsible for the vitreous infection in this patient. Similarly, another cluster of cases of hospital-acquired candidiasis studied by biotyping and reported to be caused by a single strain were determined to be caused by multiple types by DNA typing (13). However,

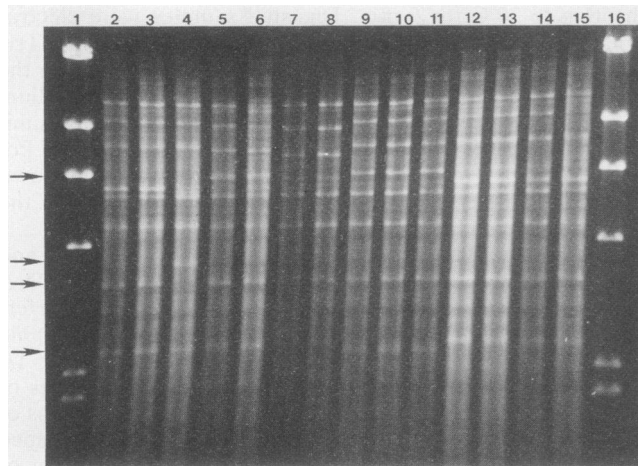


FIG. 1. Gel electrophoresis of *Eco*RI restriction digests of DNA from *C. albicans* isolates from heroin addicts. Lanes: 1 and 16, *Hind*III digest of lambda phage for sizing markers; 2, G2; 3, G4; 4, G5; 5, G6; 6, G7; 7, G8; 8, G9; 9, G10; 10, G11; 11, G12; 12, G13; 13, G14; 14, G15; 15, IA2 reference DNA subtype. Arrows (from top to bottom) are 6 to 7, 4.2, 3.7, and 2.5 to 3 kb. These bands indicate the positions of the rDNA bands used as key bands for strain differentiation by this method. Note the presence of the 4.2-kb band in lane 4 indicating that G5 is a IB subtype, whereas the remaining G isolates have the 3.7-kb band and are IA subtypes. IA2 in lane 15 is shown to illustrate the matching of isolates with a reference subtype.

DNA typing has been used to identify focal outbreaks of candidiasis with a common source of origin (3).

In conclusion, our results indicate that the Glasgow outbreak of endophthalmitis in heroin addicts was most likely due to injection paraphernalia, including preserved lemon juice, contaminated by *C. albicans* that formed part of the addicts' endogenous flora rather than a common exogenous source. Even so, the predominance of one biotype in this outbreak is similar to that reported by Odds et al. (7) from an outbreak in heroin addicts in Spain. It would be of interest to examine the Spanish isolates in our DNA typing system to see whether certain DNA subtypes are associated with disease in heroin addicts.

REFERENCES

1. Lee, W., J. Burnie, and R. Matthews. 1986. Fingerprinting *Candida albicans*. J. Immunol. Methods **93**:177-182.
2. Lehmann, P. F., B. J. Kemker, C. B. Hsiao, and S. Dev. 1989. Isoenzyme biotypes of *Candida* species. J. Clin. Microbiol. **27**:2514-2521.
3. Matthews, R., and J. Burnie. 1989. Assessment of DNA fingerprinting for rapid identification of outbreaks of systemic candidosis. Br. Med. J. **298**:354-357.
4. Odds, F. C., and A. B. Abbott. 1980. A simple system for the presumptive identification of *Candida albicans* and differentiation of strains within the species. Sabouraudia **18**:301-317.
5. Odds, F. C., and A. B. Abbott. 1983. Modification and extension of tests for differentiation of *Candida* species and strains. Sabouraudia **21**:79-81.
6. Odds, F. C., P. Auger, P. Krough, A. N. Neely, and E. Segal. 1989. Biotyping of *Candida albicans*: results of an international collaborative survey. J. Clin. Microbiol. **27**:1506-1509.
7. Odds, F. C., A. Palacio-Hernandez, J. Cuadra, and J. Sanchez. 1987. Disseminated *Candida* infection syndrome in heroin addicts—dominance of a single *Candida albicans* biotype. J. Med. Microbiol. **23**:275-277.
8. 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.
9. Servant, J. B., G. N. Dutton, L. Ong-Tone, T. Barrie, and C. Davey. 1985. Candidal endophthalmitis in Glaswegian heroin addicts: report of an epidemic. Trans. Ophthalmol. Soc. U.K. **104**:297-308.
10. Shankland, G. S., and M. D. Richardson. 1988. Epidemiology of an outbreak of *Candida* endophthalmitis in heroin addicts: identification of possible source of infection by biotyping. J. Med. Vet. Mycol. **26**:199-202.
11. Shankland, G. S., and M. D. Richardson. 1989. Possible role of preserved lemon juice in the epidemiology of *Candida* endophthalmitis in heroin addicts. Eur. J. Clin. Microbiol. Infect. Dis. **8**:87-88.
12. Shankland, G. S., M. D. Richardson, and G. N. Dutton. 1986. Source of infection in *Candida* endophthalmitis in drug addicts. Br. Med. J. **292**:1106-1107.
13. Stevens, D. A., F. C. Odds, and S. Scherer. 1990. Application of DNA typing methods to *Candida albicans* epidemiology and correlation with phenotype. Rev. Infect. Dis. **12**:258-266.