

Restriction Fragment Analysis of a *Candida tropicalis* Outbreak of Sternal Wound Infections

BRADLEY N. DOEBBELING,^{1*} RICHARD J. HOLLIS,² HENRY D. ISENBERG,³ RICHARD P. WENZEL,^{1,4}
AND MICHAEL A. PFALLER^{2,5}

*Departments of Medicine*¹ and *Pathology*,² *The University of Iowa College of Medicine, Program of Epidemiology, The University of Iowa Hospitals and Clinics*,⁴ and *Veterans Administration Medical Center*,⁵ *Iowa City, Iowa 52242*, and *The Long Island Jewish Medical Center, The Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11042-1433*³

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An apparent single-source outbreak of *Candida tropicalis* sternal wound infections in eight patients was investigated by utilizing DNA restriction fragment analysis (RFA) with *Hind*III and *Bst*NI. All eight outbreak isolates appeared to be identical and were easily differentiated from control isolates by DNA RFA. Compared with an arbitrarily selected reference outbreak isolate, $\geq 95\%$ of the bands in the restriction digests identified by a computerized image analysis system from each of the outbreak isolates were identical versus 13 to 53% of the bands in any of the nine control isolates. Outbreak strains were significantly more likely to match the reference outbreak isolate than were controls ($P < 0.0001$). The RFA was greatly facilitated by the use of computerized image analysis and confirmed the epidemiologic link between a scrub nurse and the infected patients.

The *Candida* spp. are among the most important causes of nosocomial bloodstream and urinary tract infections in the United States (1, 4, 19-21). However, although they are a frequent source of dermatitis, the *Candida* spp. are rare causes of wound infection (4). Several recent nosocomial outbreaks of candidiasis have been reported, but transmission of *Candida* spp. from patient to patient has been infrequently documented. Outbreaks of disseminated *Candida albicans* infections have recently been demonstrated in an adult intensive care unit (2), a neonatal care unit (11), a neonatal intensive care unit (18), and in association with parenteral nutrition (9). *Candida parapsilosis* has been implicated in two outbreaks related to parenteral nutrition (16, 19) and one of fungal endophthalmitis (8).

Isenberg et al. recently described the first recognized nosocomial outbreak of *Candida tropicalis* sternal wound infections (5). An epidemiologic investigation suggested a single nurse as the probable source of the yeast infecting eight coronary artery bypass patients. Specifically, the only individual involved in the operative procedure of all eight infected patients was a scrub nurse whose nasopharyngeal and fingertip cultures yielded *C. tropicalis*. None of the environmental cultures or cultures of the fingertips and nasopharynges of the remaining 27 operating room personnel in contact with the patients yielded *C. tropicalis*. No cases occurred after the removal of the nurse from the cardiac team.

Maffei et al. (6) suggested that DNA typing of the *C. tropicalis* isolates reported by Isenberg et al. (5) would have definitively implicated the scrub nurse as the source of the outbreak. However, few data are available regarding the molecular typing of *C. tropicalis*. Previously, Soll et al. utilized restriction endonuclease typing to demonstrate multiple strains of *C. tropicalis* in the course of a single systemic infection in a bone marrow transplant patient (15). The unusual nature of the *C. tropicalis* outbreak described by Isenberg et al. (5) and the solid epidemiologic evidence

linking the patients and scrub nurse in the outbreak make any other source extremely unlikely. However, DNA typing of the isolates would corroborate the epidemiologic findings and would in turn confirm the utility of the typing method for *C. tropicalis*. We report the molecular typing of these isolates by use of DNA restriction fragment analysis (RFA).

***Candida* isolates.** Outbreak isolates included strains of *C. tropicalis* from the sternal wounds of six of eight patients (six isolates, E3 to E8) and the nasopharynx and fingertips (two isolates, E1 and E2) of the epidemiologically linked scrub nurse from the cluster described by Isenberg et al. (5). Isolates from the remaining two patients were not available for further study. Control isolates included nine *C. tropicalis* strains from epidemiologically unrelated hospitalized patients. All yeasts were identified by standard techniques and stored as suspensions in sterile water at ambient temperature until typing. Each isolate was coded and submitted blindly for typing.

DNA RFA. A suspension of each yeast was plated on Sabouraud dextrose agar and then incubated for 16 h at 30°C. Two colonies (>1 mm in diameter) of each isolate were suspended in 2 ml of distilled water. A 30- μ liter aliquot was added to 5 ml of yeast extract peptone dextrose broth (Difco Laboratories, Detroit, Mich.) and incubated for 18 h at 35°C with agitation. Spheroplasts were prepared by digestion with 10,000 U of β -glucuronidase (Sigma Chemical Co., St. Louis, Mo.) by the method of Forte and Fangman (3). DNA was extracted by using the method of Scherer and Stevens (13), but with diethyl pyrocarbonate deleted, and then concentrated by precipitation in 95% ethanol.

DNA samples were digested with RNase A (Sigma) for 1 h at 37°C. Each sample was then digested separately with *Hind*III at 37°C or *Bst*NI at 65°C (New England Biolabs, Beverly, Mass.) according to the instructions of the manufacturer for 4 h. The reaction was terminated by the addition of 20 mM EDTA-0.1% bromophenol blue-50% glycerol-0.1% xylene cyanol. The samples underwent electrophoresis on a horizontal 0.7% agarose gel containing ethidium bromide

* Corresponding author.

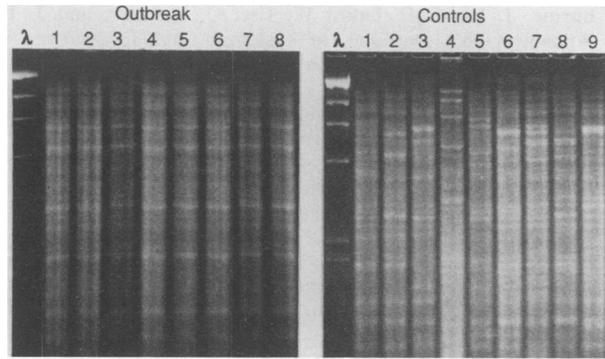


FIG. 1. *Bst*NI RFA of *C. tropicalis* isolates. Photograph demonstrates RFA of outbreak isolates (E1 to E8) on the left and epidemiologically unrelated control strains (U1 to U9) on the right. *Hind*III-digested λ molecular weight markers are present on the left edge of each gel. Note the complicated banding patterns of all isolates and similarity of the outbreak isolates to each other.

and a buffer of 100 mM Tris (pH 8.5), 100 mM boric acid, and 1.0 mM EDTA (TBE buffer) at 45 V for 16 h. Phage lambda DNA digested with *Hind*III (New England Biolabs) was included as a molecular weight standard. All gels were photographed under UV light with Polaroid 107C film.

Photographs of ethidium bromide-stained agarose gels were inspected visually to compare banding patterns. Each major and minor band was identified, and the distance from the origin of the gel to the phage lambda molecular weight standard was measured. Isolates were considered significantly different if any readily detectable major or minor band was different. A computerized video image analysis system (Visage-60; Bioimage, Ann Arbor, Mich.) was also utilized to analyze the DNA restriction fragment banding patterns of the gels. The system scanned all gel patterns and stored the digital images in a data file. The interactive band analysis function of the Visage-60 system identified the major and minor bands for each isolate. The system assigned a molecular weight to each identified band after comparison with the molecular weight standards. The final pattern of each isolate was stored in the computer memory for future reference. The global pattern comparison function of the Visage-60 system compared the banding pattern of each isolate sequentially to all other isolates. The number of bands for each isolate pair which appeared identical (molecular weight, ± 2 to 17%, depending upon location in the gel) was determined and used to calculate a coefficient of similarity (CS) as follows: $CS = 2$ (number of identical bands)/total number of bands.

Thus, for a given comparison isolate, the CS reflected the proportion of bands identical to those in the designated reference isolate. A Fisher's Exact Test was used to compare outbreak and control isolates by utilizing an arbitrary cutoff of $\geq 90\%$ matching (CS, ≥ 0.90).

RFA with either *Bst*NI (Fig. 1) or *Hind*III (not shown) demonstrated essentially identical patterns for all eight of the outbreak isolates. Each of the control isolates had a unique *Bst*NI (and *Hind*III) pattern which was distinctly different from that of outbreak isolates. A schematic representation of the banding patterns, developed by using the image analysis system (Fig. 2), further illustrates the similarities and differences of outbreak and control isolates.

A quantitative analysis of the relationships among the 17 isolates was obtained by examining the CSs calculated by

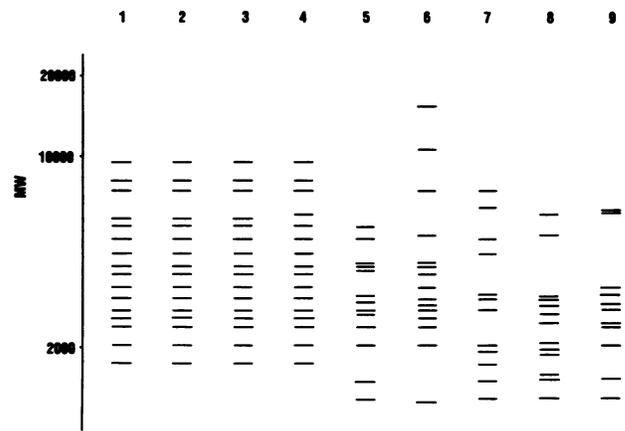


FIG. 2. Schematic representation of RFA banding patterns of study isolates. The computerized image analysis system generated a schematic diagram of the DNA restriction fragments of selected outbreak isolates E6 (lane 1), E1 (lane 2), E4 (lane 3), and E8 (lane 4), and control isolates U1 (lane 5), U4 (lane 6), U5 (lane 7), U6 (lane 8), and U9 (lane 9), allowing easy comparison of the complex banding patterns.

using one of the outbreak isolates (E6) as the reference pattern (Table 1). The CS values of the outbreak isolates were all ≥ 0.95 . The two outbreak isolates (E7 and E8) with CS values less than 1.00 when compared to the reference isolate were identical (CS = 1.00) to each other and differed from the reference isolate by a single faint band in each that was unidentified by the image analysis system. In contrast, the CS values for the control isolates ranged from 0.13 to 0.53. Thus, significantly more of the banding patterns of the outbreak isolates matched the reference isolate than did the controls (seven of seven versus zero of nine, respectively; $P < 0.0001$).

The investigation of an outbreak of *C. tropicalis* sternal wound infections, previously described by Isenberg et al., convincingly implicated the scrub nurse involved (5). The sound epidemiologic evidence of exposure of the nurse to each of the patients and recovery of the same uncommon *Candida* sp. from the fingertips and nasopharynx of the

TABLE 1. CS values for outbreak and control isolates

Group	Isolate no.	CS ^a
Outbreak	E6	1.00
	E1	1.00
	E2	1.00
	E3	1.00
	E4	1.00
	E5	1.00
	E7	0.95
	E8	0.95
Control	U1	0.44
	U2	0.20
	U3	0.36
	U4	0.53
	U5	0.45
	U6	0.13
	U7	0.24
	U8	0.26
	U9	0.27

^a CS values calculated by using outbreak isolate number E6 as the reference pattern.

nurse in the absence of other positive environmental or personnel cultures for the organism strongly implicated the nurse as the likely source. The hypothesis was further supported when the outbreak ended abruptly with the dismissal of the nurse from the operating team. Our data, generated by utilizing RFA, further support the epidemiologic link between the scrub nurse and the infected patients. Moreover, the data are particularly important because they demonstrate that transmission of a single strain of *C. tropicalis* can occur. Similar analyses with epidemiologically well-defined isolates are essential in validating the utility of other molecular typing methods.

Scherer and Stevens have clearly shown the ease of performance, reproducibility, and stability of DNA RFA for differentiating several *Candida* species (13). Subsequently, Stevens et al. found a large number of restriction fragment polymorphisms when the technique that used *EcoRI* was applied to *C. albicans* clinical isolates (17). Matthews and Burnie also studied a large number of *C. albicans* isolates; all were typeable, and DNA RFA was more discriminating than immunoblot typing, morphotyping, serotyping, or biotyping (7). More recently, Pfaller et al. (10) observed that although DNA RFA with *EcoRI* differentiated several strains of *C. albicans*, the combination of the technique with biotyping, utilizing nine agar plate tests, allowed enhanced discrimination of isolates from different patients. As mentioned previously, few data exist regarding the molecular typing of *C. tropicalis* (15).

We found that the restriction endonuclease *BstNI* provided optimal discrimination among epidemiologically related and unrelated isolates of *C. tropicalis*. Other investigators have reported that *EcoRI*, *BstNI*, and *HinfI* are among the most useful enzymes for delineating strains within the more common species, particularly *C. albicans* and *C. tropicalis*, because of the large number of restriction fragment polymorphisms obtained (7, 9, 10, 12, 13, 15, 17, 18). Currently, however, the type and number of restriction enzymes for optimal strain discrimination within the various *Candida* species are unknown (10).

Interestingly, our data demonstrate the usefulness of computerized image analysis in the evaluation of DNA typing results applied to an outbreak of *C. tropicalis* infections. The system greatly facilitated the analysis of the complex banding patterns of *C. tropicalis*. The calculation of the CS by use of data stored in the image analysis system provided a more quantitative analysis of the complex restriction fragment patterns. The ability to display major banding patterns schematically may simplify interpretation and improve communication between laboratories. Since one of the problems with molecular typing of *Candida* spp. is the lack of standardization in terms of methodology, interpretation, and reporting of results (17), the application of computerized image analysis systems may be useful in overcoming this problem (14).

In summary, DNA RFA confirmed the proposed source of *C. tropicalis* sternal wound infections in the outbreak described by Isenberg et al. (5). Furthermore, these data demonstrate the utility of the computerized video image analysis system in providing a quantitative as well as a schematic evaluation of the complex banding patterns of individual isolates of the *Candida* spp.

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