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An outbreak of *Candida parapsilosis* endophthalmitis: analysis of strains by enzyme profile and antifungal susceptibility

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SUMMARY Twenty-two isolates from patients with postsurgical endophthalmitis due to infection with *Candida parapsilosis* as a result of exposure to a contaminated ocular irrigating solution were classified by enzyme profile analysis and antifungal susceptibility. These isolates were identical to a single isolate obtained from a contaminated vial but could be differentiated, on the basis of enzyme profile and antifungal susceptibility, from randomly selected stock isolates. The combination of these tests appears to have value in discriminating epidemic from non-epidemic strains.

The occurrence of a multicentred outbreak of postsurgical endophthalmitis due to *Candida parapsilosis* afforded an unusual opportunity to examine the characteristics of isolates obtained from patients at the various centres involved in the epidemic. The clinical outcome for many of these cases has already been described.[1] All of the patients had undergone cataract surgery in the course of which the anterior chamber of the eye was irrigated with a balanced salt solution (GBR, Lot No. 16738, Maury Biologicals, Los Angeles, CA).[2] An unopened vial of the solution bearing the same lot number was later found to be contaminated with *C. parapsilosis*.[2] In an attempt to confirm the common source of the infection, enzyme profiles and the antifungal susceptibilities of the isolates from patients, a vial of the solution, and the operating room environment, were examined and compared with a random selection of stock *C. parapsilosis* isolates.

Materials and methods

ISOLATE ACQUISITION

Candida parapsilosis isolates were recovered from patients with endophthalmitis by culture of fluid obtained by anterior chamber tap or vitreous aspiration.[1,2] None of the patients had been exposed to antifungal therapy prior to isolate recovery. The isolates on Sabouraud slants were mailed to our laboratory, where they were immediately subcultured on to freshly prepared Sabouraud's agar plates. Plugs from these plates were placed in sterile vials and frozen to −70°C for future use. An isolate of *C. parapsilosis* was recovered from an unopened vial of GBR solution (lot no. 16738). *Candida parapsilosis* was also isolated from the oxygen line used to ventilate patients during surgery at the first institution involved in the epidemic.[2] These isolates were handled in the same way as the human strains.

Eight *C. parapsilosis* isolates were randomly selected from the collection maintained at −70°C in the Microbiology Laboratory at Vanderbilt University Medical Center. Another nine isolates of the same organism were provided from stock maintained at Analytab Products (Plainview, NY). These isolates were also maintained on Sabouraud's agar at −70°C and were not passaged. Inocula for each of the following experiments were taken from these slants.

ENZYME PROFILE ANALYSIS

Enzyme profile analysis was performed by the API 20C clinical yeast system (Analytab Products). This commercially available system for the identification of yeasts from clinical specimens is composed of a series of miniaturised carbohydrate assimilation tests. The result is expressed numerically and is interpreted by reference to a computer generated data base of yeast strains.[3]

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ANTIFUNGAL SUSCEPTIBILITY TESTING
All isolates were evaluated in triplicate for their susceptibility to amphotericin B, ketoconazole, miconazole, and flucytosine.

Amphotericin B. The inoculum was prepared from cultures grown overnight on Sabouraud's agar and diluted in normal saline to give an 85% transmission on the B/L Spect 20 spectrophotometer at 550 nm (Bausch and Lomb). With the Microtiter System (Dynatech Laboratories, Inc.) serial two-fold dilutions of amphotericin B (Fungizone) were prepared in M3 medium to give final concentrations that ranged from 50 µg/ml to 0·05 µg/ml.[4] The plates were wrapped in aluminium foil with a wet sponge and were incubated at 32°C. To determine the minimum inhibitory concentration (MIC) the wells were read at 24 hours.

Flucytosine and the imidazoles. A similar technique was used for these compounds. The medium for determination of flucytosine (Roche Laboratories) susceptibility was yeast nitrogen broth supplemented with asparagine and dextrose. Casein yeast glucose agar in phosphate buffered saline was used for the imidazole studies (Janssen Pharmaceuticals).

Results

Twenty-three isolates of *Candida parapsilosis* were recovered from patients with postsurgical endophthalmitis. Twenty-two were available for testing. Ten of these were from California, four were from Florida, and eight were from Tennessee. All the California isolates came from a group of patients who underwent surgery at the same ambulatory facility. Two separate outpatient surgical centres were the source of the isolates from Florida (2,2), while three hospitals supplied the isolates from Tennessee (3,3,2).

Enzyme profile analysis. All the ocular isolates had the same API 20C enzyme profile (6756171) as the GBR solution isolate and the isolate from the oxygen tubing. Eleven of the 17 stock isolates also shared this profile.

Antifungal susceptibility. The antifungal susceptibility of the epidemic isolates to four antifungal agents is shown in Tables 1 and 2. Individual strains were considered to show a different response to an antifungal agent if the MICs differed by more than four-fold.

All the ocular isolates and the isolates obtained from the GBR solution and the oxygen tubing had the same pattern of susceptibility to each antifungal agent (Table 3). The antifungal susceptibilities of the

Table 1 *Epidemic isolates—San Francisco*

Strain	*Minimal inhibitory concentrations (MIC µg/ml) for 4 antifungal agents*			
	Amphotericin B	*Ketoconazole*	*Miconazole*	*Flucytosine*
1	0·625	0·1	1·56	0·2
2	0·312	0·1	1·56	0·2
3	0·312	0·1	1·56	0·2
4	1·25	0·1	1·56	0·2
5	0·312	0·1	1·56	0·2
6	0·312	0·1	1·56	0·2
7	1·25	0·1	1·56	0·2
8	0·312	0·1	1·56	0·2
9	0·312	0·1	1·56	0·2
10	0·312	0·1	1·56	0·2

Table 2 *Epidemic isolates—Florida, Tennessee*

Strain	*Minimal inhibitory concentrations (MIC µg/ml) for 4 antifungal agents*			
	Amphotericin B	*Ketoconazole*	*Miconazole*	*Flucytosine*
11 Tn(1)	0·312	0·1	1·56	0·2
12 Tn(1)	0·625	0·1	1·56	0·2
13 Tn(1)	0·312	0·1	1·56	0·2
14 Tn(2)	0·625	0·1	1·56	0·2
15 Tn(2)	0·312	0·1	1·56	0·2
16 Tn(2)	0·625	0·1	1·56	0·2
17 Tn(3)	0·625	0·1	1·56	0·39
18 Tn(3)	0·625	0·1	1·56	0·2
19 Fl(1)	0·312	0·1	1·56	0·2
20 Fl(1)	0·625	0·1	1·56	0·39
21 Fl(2)	0·312	0·1	1·56	0·39
22 Fl(2)	0·312	0·1	1·56	0·39

Tn=isolate from Tennessee.
Fl=isolate from Florida.

Table 3 Candida parapsilosis *Isolates (API 20C—6756171)*

Source	*Number of isolates*	In vitro *susceptibility to 4 antifungal agents (µg/ml)*			
		Amphotericin B	*Ketoconazole*	*Miconazole*	*Flucytosine*
Sf	10	0·312–1·25	0·1	1·56	0·2
Fl, Tn	12	0·312–0·625	0·1	0·78–1·56	0·2–0·39
GBR	1	0·625	0·1	1·56	0·2
OL	1	0·312	0·1	1·56	0·2
Stock	11	0·312–0·625	<0·05–>50	3·12–>50	0·1–25

Sf=San Francisco. Fl=Florida. Tn=Tennessee. GBR=Isolate from irrigation solution. OL=Oxygen line isolate.

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Table 4 *Stock isolates*

Strain	*Minimal inhibitory concentration for antifungal agents (MIC μg/m)*				*API 20C Profile*
	Amphotericin B	*Ketoconazole*	*Miconazole*	*Flucytosine*	
A	0·625	0·1	3·12	12·5	6756171
B	0·312	0·2	12·5	25	6756171
C	0·625	0·2	6·25	12·5	6756171
D	0·625	25	>50	0·2	6756171
E	0·625	25	6·25	0·2	6756171
F	0·625	<0·05	6·25	0·2	6756171
G	0·625	12·5	6·25	0·2	6756171
H	0·625	25	12·5	0·2	6756171
J	0·625	<0·05	3·12	0·2	6756171
K	0·625	<0·05	3·12	0·1	6756171
L	0·625	>50	3·12	0·1	6756171
M	0·625	<0·05	1·56	0·2	6756173
N	0·625	0·39	>50	1·56	6756173
O	0·625	25	6·25	0·39	6756131
P	0·625	>50	1·56	0·39	6756131
Q	0·625	>50	12·5	0·1	6756131
R	0·625	0·1	1·56	0·1	6756131

17 stock isolates are listed in Table 4. The 11 isolates with the same API 20C profile as the ocular isolates differed from the ocular isolates in their susceptibility to at least one antifungal agent (Table 5). Five of the six isolates with different enzyme profiles also differed from the ocular isolates in their susceptibility to at least one agent.

Discussion

An epidemic of fungal infection is an unusual event. In most instances epidemiological techniques are used to identify the source of infection, and conclusive mycological evidence linking infected cases with a common source is seldom available.[5,6] During a recent investigation of an outbreak of candidiasis in a special care baby unit Phelps *et al.* used a resistogram typing method to determine the cause of the cross infection.[7] Others have suggested differentiating strains by the killer system.[8] But at present in contrast to bacterial infections, there are no techniques equivalent to phage typing that serve to identify specific fungal isolates.

Traditional methods of yeast identification that utilise carbohydrate fermentations and assimilations are complex and time consuming.[3,9] These methods have now been modified to provide a more practical approach to the problem.[3,10,11] The API 20C system we used is one such modification. It is a rapid and convenient method for identifying clinical yeast isolates. Classification of individual strains is based on the results of a series of carbohydrate assimilation tests. The seven-digit number expressing the result establishes an enzyme profile that serves to identify the strain by reference to a large data bank of previously analysed strains. Six different profiles

Table 5 *Differentiation of 11 randomly selected isolates (API 20C—6756171) from epidemic isolates by MIC (μg/ml)*

Range of differentiation	*Number of isolates differentiated*	*Cumulative number of differential strains*
Amphotericin B		
<0·075–>1·25	0	0
Ketoconazole		
<0·05–>0·39	8	8
Miconazole		
<0·39–>6·25	7	10
Flucytosine		
<0·05–>0·39	3	11

have been identified for *Candida parapsilosis* (Levy I, personal communication, 1986).

In this study it was our purpose to determine whether the combination of enzyme profile analysis and antifungal susceptibility testing could serve as a useful epidemiological tool by separating epidemic from non-epidemic strains. All the ocular isolates had the same profile number (6756171) as the isolates recovered from the GBR solution and the oxygen line. However, this is a common profile for *C. parapsilosis*, being exhibited by up to 50% of isolates (Pincus D, personal communication, 1985). In fact this was the profile for 11 of the 17 randomly selected isolates. Thus the finding of a shared API 20C profile was not enough to link these isolates to each other or to the contaminated solution.

Antifungal susceptibility to the four antifungal agents was remarkably uniform for the strains involved in the epidemic. Although differences were noted with some strains, they were within the range of error of the test. The pattern was in contrast to that observed among the stock isolates with the same

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enzyme profile, each of which differed from the epidemic strains in susceptibility to at least one antifungal agent. Only with amphotericin B was the response uniformly similar. Five of the six isolates with different API 20C profiles also differed in their susceptibility to at least one antifungal agent.

These results suggest that the combination of enzyme profile analysis and antifungal susceptibility has some potential as a means of separating epidemic from non-epidemic strains. Whether any relationship exists between these two tests is unknown at this stage.

The API-20C enzyme analysis is based on the ability of the organism to utilise certain compounds as a sole carbon source, and this ability is genetically determined. Extended testing to additional compounds was not performed in this study but might have revealed differences between these strains. In this outbreak the actual cause of the intrinsic contamination of the irrigating solution was not determined, but it did occur at some stage during the preparation and bottling of the solution. While it is presumed that a single strain was responsible for the contamination, several strains with differences not detected by us may have been involved. Experience with the colonisation patterns in a special care baby unit, a somewhat parallel situation, suggests that this is certainly possible.[7] The origin of the isolate in the oxygen line is also not known with certainty at this stage, though it is likely to be related to the contaminating strain. The oxygen line was probably colonised during the period when the patients were exposed to the contaminating solution. Further studies with extended biochemical analyses of these and additional strains from other contaminated vials are needed to answer some of these questions.

From another point of view the uniformity in the response by all these epidemic strains to this wide battery of tests is remarkable in view of the broad geographic distribution of the cases and the differing periods of human passage prior to isolation.[2]

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