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Epidemiological investigation of patients with vulvovaginal candidosis

Application of a resistogram method for strain differentiation of *Candida albicans*

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SUMMARY The resistogram method was applied to 420 isolates of *Candida albicans* obtained from 30 selected patients undergoing treatment for vulvovaginitis. Of these, 16 patients each harboured a particular strain of *C. albicans* which persisted in the mouth or intestinal tract or both. In three of these patients, this strain persisted in the genital tract, and, in eight patients, it later recolonised the genital tract. Fourteen patients harboured more than one strain of *C. albicans:* one failed to respond to treatment and continued to harbour the same strain in the genital tract; in five the original strain later recolonised the genital tract and a second strain remained confined to the mouth or intestinal tract or both; and in three a second strain, present in the intestinal tract, later colonised the genital tract. Each of the male partners of seven patients harboured a strain of *C. albicans* that was identical to the strain, or to one of the strains, that had been isolated from his female partner.

Introduction

The current prevalence of vaginal infection with *Candida albicans* has led to increased interest in the epidemiological aspects of this condition. The source of this infection is an intriguing problem, and its elucidation might be helpful in treating the patient. Most patients harbouring *C. albicans* in the genital tract are also harbouring this fungus in the mouth and anal tract (Hilton and Warnock, 1975), and Miles *et al.* suggested that the digestive tract could be the principal source of vaginal infection (Miles *et al.*, 1977). Furthermore, transmission of *C. albicans* from the male partner might be contributing to the vaginal infection in certain patients (Rodin and Kolator, 1976; Davidson, 1977).

The differentiation of isolates of *C. albicans* has in the past been restricted to the two serological groups of Hasenclever and Mitchell (1961). In practice, most clinical isolates have been found to belong to one of these groups (Hasenclever and Mitchell, 1963), and this has limited the usefulness of this method for tracing the spread of *C. albicans* infection.

Warnock *et al.* (1979) described a resistogram method for differentiation of strains of *C. albicans* based on differences in the resistance of strains of *C. albicans* to a number of selected chemicals used at critical concentrations. Up to 1092 hypothetical strains of *C. albicans* can be distinguished with this method. The method was tested with isolates from 13 patients undergoing treatment for vaginal infection with *C. albicans,* and it was found that particular strains were consistently present in individual patients, both in different specimens and on different occasions. Furthermore, some patients harboured more than one strain of *C. albicans.*

This paper presents the findings of an epidemiological investigation of 30 patients treated for vulvovaginal infection with *C. albicans.* The strains of *C. albicans* present in the mouth, digestive tract, and genital tract of these patients before and after treatment were characterised by the resistogram method. Isolates of *C. albicans* obtained from the male partners of seven patients were also studied.

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Patients and methods

PATIENTS

Thirty non-pregnant patients were selected from among 100 patients taking part in a clinical trial (Milne and Warnock, 1979) and were included in this investigation provided that a minimum of six isolates of *C. albicans* had been obtained and the patient had attended on at least three occasions. The patients were first seen before starting treatment for vulvo-vaginal infection with *C. albicans;* 24 of them had also had such an infection diagnosed on at least one previous occasion (on the basis of both clinical and mycological findings). The patients were aged from 17 to 35 years, and 25 of them were using oral contraception. Before treatment, all the patients had clinical signs of vaginitis and vulvitis (vaginitis was recorded if the vaginal mucosa was reddened or granular; vulvitis was recorded if the vulva was reddened, swollen, fissured, or ulcerated) together with a positive result to a vaginal culture for *C. albicans.* No other infection was diagnosed. No patient had taken antibiotics or received local antifungal treatment during the preceding fortnight.

MEDICATION

The patients were treated with one clotrimazole vaginal pessary (100 mg) per night for six consecutive nights. Seventeen patients were given additional oral nystatin treatment: four tablets (500 000 IU per tablet) per day for 10 consecutive days. The male partners were not treated. Patients who required further treatment were given one clotrimazole vaginal pessary per night for 12 nights plus four nystatin tablets by mouth per day for 10 days.

FOLLOW-UP ASSESSMENT

The patients returned for assessment on at least two occasions after the initial course of treatment had been completed. Patients who returned with clinical signs of vaginitis or vulvitis and had a positive result to a vaginal culture were given a further course of treatment. Vaginal, oral, and anal swabs were taken at each visit, placed in transport medium (Oxoid Trichomonas Medium No. 2), and sent for mycological investigation. The patients also provided a faecal specimen on each occasion.

The male partners of seven patients attended for investigation. Oral, urethral, and sub-preputial swabs were taken from these men, placed in transport medium, and sent for investigation.

ISOLATION AND IDENTIFICATION

Vaginal, oral, anal, and faecal specimens were inoculated on to plates of glucose peptone agar containing 0·05 mg chloramphenicol per ml and incubated at 37°C for 48 hours. Identification of isolates was based on morphological and biochemical tests according to English (1974).

RESISTOGRAM DETERMINATION

This was performed according to the methods of Warnock *et al.* (1979).

The resistogram of each *C. albicans* strain is a list of the chemicals to which that strain is resistant. Each chemical is alloted a letter of the alphabet so that if a strain is resistant to chemical B, the letter B is included in the description of that strain. If a strain is resistant to all the chemicals used, its resistogram is given as A B C D E F. If chemicals A and D inhibit a strain, the resistogram of that strain is given as B C E F. If a chemical causes partial inhibition of a strain, this is denoted by the letter bracketed.

If two isolates demonstrate a major difference in resistance to one or more chemicals—for example, A B C D E and B C D E—or minor differences in resistance to two or more chemicals—for example, A B C D E and (A)(B) C D E or (A)(B) C D E and C D E—the isolates are considered to belong to distinct strains.

Results

The findings for the 30 female patients in this investigation are summarised in Table 1: 420 colonies of *C. albicans* from the cultures of 420 specimens were subcultured for characterisation. In 26 patients the initial course of treatment eliminated the genital tract infection; in 10 of these patients, genital tract recolonisation did not occur. In the other 20 patients, the genital tract remained colonised or became recolonised once treatment had ceased. Thirteen of these 20 patients developed further clinical signs of genital infection and required a second course of treatment. Five of these 13 patients later received a third course of treatment, and one patient required a fourth course of treatment. In all, 16 patients (Patients 1-5 and 11-21) harboured one particular strain of *C. albicans* throughout the period of investigation, and the other 14 patients (Patients 6-10 and 22-30) harboured two distinct strains.

Five of the 10 patients (Patients 1-5) in whom genital colonisation did not recur harboured a particular strain of *C. albicans* in both the digestive tract and the genital tract. In three of the five other patients in whom genital colonisation did not recur (Patients 6, 7, and 8), two strains of *C. albicans* were isolated before treatment. The strain that was isolated from the anus and genital tract of these three patients before treatment did not reappear after treatment. In Patients 9 and 10, a second strain appeared in the digestive tract after treatment.

Table 1 *Resistograms of 420 isolates of Candida albicans*

Patient no.	Resistogram of strain isolated	Site(s) of isolation at different visits													
		Visit 1	Visit 2		Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		
Each harbouring a single strain of C. albicans (total of 49 isolates)															
1*	C D E (F)	VAFM			AFM	(4)	AFM	(6)	FM	(10)					
2*	C (D) E (F)	VAFM	FM	(3)	FM	(5)	F	(8)							
3*	(A) C D E (F)	VAFM	AFM	(3)					AFM	(16)					
4*	(A) C D E (F)	VAFM	FM	(2)	F	(4)									
5	(A)(B)(C) D E (F)	VAFM	AFM	(2)	FM	(4)	FM	(8)							
Each harbouring two strains of C. albicans (total of 42 isolates)															
6	(A) C (D)(E)(F)	VA													
	C D E (F)	FM	FM	(3)	FM	(5)									
7	C (F)	VA													
	B C (D) E (F)	M	F	(2)	M	(4)	FM	(8)	M	(10)					
8	C D (E)(F)	VA													
	(A) C D E (F)	F			F	(4)	A M	(8)							
9*	(B)(C)(D)(E)(F)	VA M													
	(C) E (F)		AFM	(3)	AF	(5)	F	(8)							
10*	(B) C (D) E (F)	VAFM	FM	(2)	A M	(4)	M	(8)							
	(A) C D E (F)				F	(4)	F	(8)							
Each harbouring a single strain of C. albicans (total of 120 isolates)															
11	(B) C D E (F)	VAF	VA	(2)	V	(6)	VA M	(8)	V	(10)					
12*	(C)	VA	V	(2)	VA	(4)†	V	(8)							
13*	(A) C D E (F)	VA M	VA	(2)†	VA	(4)†			VA	(10)	VA	(14)	VA M	(18)	
14*	(A) C (D) E (F)	VAFM	FM	(3)	V FM	(5)	V FM	(9)							
15	(A)(B) C (D) E (F)	VAFM	FM	(2)	V F	(6)	FM	(8)							
16	(B) C (D) E (F)	VAFM	FM	(2)	VAFM	(4)†									
17*	(C) D E (F)	VAF	F	(3)	VAF	(5)†			FM	(10)	V M	(16)†	M	(18)	
18	(A)(B) C D E (F)	VAFM	AFM	(2)			VAFM	(8)					A M	(18)	
19	(A) C (D) E (F)	VAFM	FM	(2)	AFM	(5)	FM	(9)			VA M	(14)†			
20	(B)(C) D (E)(F)	VAF	F	(3)			AF	(9)					VAFM	(20)†	
21*	(C) D (E)(F)	V FM	M	(2)	F	(4)			M	(10)			V FM	(20)	
Each harbouring two strains of C. albicans (total of 52 isolates)															
22*	(B) C D E (F)	VA	VA	(2)	V	(4)									
	(B)(C)(D) E (F)	M	FM	(2)	FM	(4)	FM	(8)							
23*	(A)(B) C D E (F)	V			V	(4)	VA M	(9)							
	A C D E (F)	AFM	A	(2)	AFM	(4)	F	(9)							
24	(B) C (D) E (F)	V					VA	(8)†	VA	(12)					
	(A) C D (E)(F)	FM	F	(2)	FM	(4)	F	(8)	FM	(12)					
25*	(C)(E)	VA					V	(9)	VA	(15)†			VA	(24)†	
	(A)(B)(C)(D) E (F)	F	F	(2)	FM	(4)			F	(15)	M	(18)	F	(24)	
Each harbouring two strains of C. albicans (total of 57 isolates)															
26	C D E	VA			VA	(6)†			VAF	(12)†	VA	(18)	VA	(21)†	
	(A)(B) C (D) E (F)												M	(21)	
27	(A)(B)(C)(D) E (F)	VA									VA	(19)†			
	(A) C D (E)		F	(3)							F	(19)			
28*	C D E (F)	VAFM	AFM	(2)	M	(4)	AF	(9)†							
	(C)				VA	(4)	V	(9)†							
29*	(A)(C) D E (F)	VAF	A	(2)											
	(A) C (D) E (F)		F	(2)	VA	(6)	M	(8)	VA	(10)†	VAF	(14)	A M	(25)	
30	A C D (E)(F)	VA	F	(2)	M	(4)					F	(18)	FM	(20)	
	(B) C (D) E (F)										VA	(18)	VA	(20)†	

Figures in parentheses indicate time of visit (weeks)
*Patient given oral treatment
†Patient given further treatment
V = vaginal isolate; A = anal isolate; F = faecal isolate; M = oral isolate

Eleven of the 20 patients (Patients 11-21) in whom the genital tract remained colonised, or became recolonised, harboured a particular strain of *C. albicans* throughout the period of investigation. Five of these patients had no further clinical signs of genital infection and did not need further treatment.

Four patients (Patients 22-25) in whom the genital tract remained colonised or became recolonised, harboured two strains of *C. albicans* before treatment. In these patients it was the strain that had been isolated from the genital tract before treatment that later returned. Two of these patients developed signs of genital infection and required a further course of treatment.

Five patients (Patients 26-30) in whom the genital tract became recolonised harboured one strain of *C. albicans* before treatment but later acquired a second strain. In Patient 27, the original strain reappeared in the anus and genital tract and the second strain remained confined to the digestive tract. In Patients 28, 29, and 30, the original strain remained in the digestive tract but a second strain appeared and colonised the anus and genital tract.

Patient 26 received four courses of treatment and on each occasion it was the same strain that was isolated from both the genital tract and the digestive tract. A second strain did appear in the mouth of this patient; this strain had earlier been isolated from the mouth of the male partner of this particular patient (Table 2).

The male partners of seven female patients were studied; 12 isolates of *C. albicans* were obtained for characterisation (Table 2). Each male patient was harbouring a strain of *C. albicans* that was identical to the strain, or one of the strains, that had been isolated from his female partner.

Table 2 *Comparison of resistograms of isolates of Candida albicans from seven pairs of patients*

Female patient no.	*Resistogram of strain isolated*	*Site(s) of isolation*			
		Female patient		*Male patient*	
2	C (D) E (F)	FM	(3)	USM	(3)
4	(A) C D E (F)	VAFM	(0)	S	(0)
11	(B) C D E (F)	VAF	(0)	M	(0)
13	(A) C D E (F)	VA M	(0)	M	(0)
16	(B) C (D) E (F)	VAFM	(0)	USM	(0)
26	(A)(B) C (D) E (F)	M	(21)	M	(12)
30	(B) C (D) E (F)	VA	(20)	US	(20)

Figures in parentheses indicate time of visit (weeks)
U = urethral isolate; S = sub-preputial isolate; M = oral isolate
VAF = as Table 1

Discussion

The findings of our previous investigation (Warnock *et al.*, 1979) suggested that particular strains of *C. albicans* were present in individual patients. One of the patients studied harboured more than one strain of *C. albicans*, and this raised the problem of the number of colonies from each clinical specimen that needed to be tested to detect all the strains that might be present. In its present form, the resistogram method permits 15 isolates to be tested on each occasion. In this investigation, the need to test isolates from all the specimens obtained from a particular patient on the same occasion restricted the number of colonies that could be sampled from each clinical specimen. It cannot, therefore, be assumed that if a strain was not detected in a particular specimen on a particular occasion that strain was not present in that specimen. It should be emphasised that other methods of strain differentiation share this fundamental problem.

There have been no reports that *C. albicans* can become resistant to the antifungal preparations used to treat vaginal candidosis. It has been assumed, therefore, that the failure of some patients to respond to treatment, and the need of others for prolonged or repeated courses of treatment, is due (in part at least) to reinfection from their partner or from their own intestinal tract. Furthermore, the recurrence of genital infection in some patients may possibly be a consequence of incomplete eradication of the initial vaginal infection.

The presence of *C. albicans* in the genital tract is often associated with its presence in the digestive tract (Hilton and Warnock, 1975; Miles *et al.*, 1977). Our findings confirm this association and suggest that most, if not all, patients with genital infection are also harbouring the same strain of *C. albicans* in the digestive tract. The fact that most of our patients harboured the same strain of *C. albicans* in the mouth and intestinal tract suggests that the mouth might be contributing to continuing intestinal colonisation.

The findings suggest that, in patients with profuse vaginal discharge, contamination from the genital tract could be contributing to anal colonisation. Of the seven patients (Patients 6-8 and 22-25) harbouring two distinct strains of *C. albicans* before treatment, five were harbouring one strain in both the anus and the genital tract, and the second strain was present in the mouth or intestinal tract or both. In three (Patients 6, 7, and 8) of these five patients, the strain present in the anus and genital tract before treatment did not reappear after treatment. In the other patients (Patients 22 and 25), the strain that was isolated from the anus and genital tract before treatment later returned; this strain was not isolated from the mouth or intestinal tract. In another patient (Patient 24), the strain that was isolated from the genital tract before treatment was later isolated from the anus and genital tract.

The intestinal tract appears to be a possible source for vaginal reinfection with *C. albicans*. Nine (Patients 14-21 and 26) of the 16 patients in whom genital tract colonisation recurred had continued to harbour the original strain of *C. albicans* in the digestive tract after the initial course of treatment. This strain later recolonised the genital tract. In another patient (Patient 29), it was a second strain, present in the digestive tract, that later appeared in the genital tract.

Incomplete eradication of the initial vaginal infection appears to have occurred in a number of our patients. Patients 11, 12, 13, and 22 were still harbouring the same strain of *C. albicans* in the genital tract at their first visit after completion of treatment. In Patients 23, 24, 25, and 27, the original strain isolated from the genital tract was later isolated from the anus and genital tract but not from the intestinal tract (in the case of Patient 23 the strain concerned was later isolated from the mouth). If the anal colonisation in these patients was derived from the genital tract then the initial vaginal infection might not have been eliminated.

It has sometimes been suggested (Rodin and Kolator, 1976; Davidson, 1977) that *C. albicans* is transmitted to or from the male partner. Our findings support this suggestion; identical strains of *C. albicans* were isolated from the genitalia of three pairs of patients, and in the other four pairs of patients studied both partners harboured the same strain in the mouth or digestive tract. Most of our female patients admitted orogenital contact, which suggests that transmission of *C. albicans* from the mouth might be contributing to the genital colonisation in some patients.

This investigation has shown that most patients with vulvovaginal candidosis harbour the same strain of *C. albicans* in the mouth, intestinal tract, anus, and genital tract and that the male partner often harbours the same strain. Our findings suggest that the failure of patients to respond to treatment is often due to incomplete eradication of the initial infection. In other patients, recurrent vaginal infection is due, in part at least, to reinfection from the intestinal tract or from the male partner.

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