Use of modified resistogram to type *Candida albicans* isolated from cases of vaginitis and from faeces in the same geographical area

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SUMMARY Ninety strains of *Candida albicans* were isolated from vaginal swabs taken by general practitioners from cases of vaginal candidosis. One hundred and nine strains were also isolated from 300 specimens of faeces received from outpatients living in the same geographical area. These strains were typed using a modification of the resistotyping method. We were unable to find any significant differences between the two populations of strains. This suggests that no particular resistotype has an increased ability to cause vaginitis.

Vaginitis due to *Candida albicans* is a common cause of visits to the general practitioner. Little, however, is known of its epidemiology. The importance of an intestinal reservoir for *C. albicans* in the pathogenesis of vaginal candidosis is well established.^{1,2} Further advances have followed the development of typing schemes for *C. albicans*. The isolation of identical resistotypes from the intestinal and genital tracts of patients with candidal vaginitis has supported the view that vaginal infection follows on from intestinal colonisation.³ The question as to whether all strains of *C. albicans* are equally likely to colonise and then infect the vagina is unclear.

This study was undertaken to determine whether strains of *C. albicans* colonising the gut of the general population were equally likely to cause vaginitis. To answer this we compared strains isolated from cases of candidal vaginitis in the community with strains isolated from faecal samples submitted by general practitioners and environmental health officers in the same area.

Material and methods

From June to September 1986 300 specimens of faeces submitted to the Cardiff Public Health Laboratory were cultured for *C. albicans*. Only faeces which had been submitted by general practitioners or environmental health officers within the Cardiff area were

examined. Specimens which were frankly diarrhoeal were excluded from the study. Most of the specimens had been submitted during contact tracing or in the follow up of previously diagnosed cases of enteric infection. During this period all strains of *C. albicans* isolated from vaginal swabs, submitted by Cardiff general practitioners for the investigation of vaginal discharge, were also saved.

Culture of both faeces and vaginal swabs was on Pagano Levin agar with added triphenyltetrazolium chloride (Difco laboratories) for 48 hours at 37°C. Characteristic colonies of *C. albicans* were confirmed by the production of germ tubes in serum after four hours of incubation at 37°C. Strains were then subcultured on to Sabouraud slopes for transfer to the *Candida* reference unit for typing.

The resistotyping scheme used was Medcraft's⁴ modification of McCreight and Warnock's⁵ method. This depends on the ability of strains to grow on agar plates containing various dilutions of toxic chemicals. Table 1 shows the chemicals used, their code, and concentrations. The typing set used varies from that of McCreight and Warnock⁵ in that silver nitrate is not included (E), but malachite green (F) and copper sulphate (G) are. A strain was deemed to be resistant to a chemical when growth was seen on three of the four plates used for each chemical.

Results

One hundred and nine strains of *C. albicans* were isolated from 300 faecal specimens (36%). Ninety strains

Table 1 Chemicals used for resistotyping strains of *C albicans*

Chemical compound	Letter code	Concentration of stock solution (g/l)	Volume added to resistogram plates (ml/20 ml agar)			
Sodium selenite	A	20.0	0.5	0.6	0.7	0.8
Boric acid	B	20.0	1.2	1.3	1.4	1.5
Centrimide	C	20.0	0.05	0.1	0.15	0.2
Sodium periodate	D	20.0	0.3	0.4	0.5	0.6
Malachite green	F	0.02	0.2	0.3	0.4	0.5
Copper sulphate	G	20.0	1.0	1.2	1.4	1.6

were isolated from vaginal swabs during the same period. Table 2 shows the number of strains isolated from faecal and vaginal specimens for the more commonly found resistotypes. No significant difference was found between the incidence of isolation of any resistotype from either site (χ^2 test). Neither were there any significant differences in the resistance of strains to any of the individual chemicals between the two sites (table 3).

Discussion

The addition of the two new chemicals to the typing set has increased the discrimination of the method. These divide what would have been the largest resistotype A-C- (33.7% of all strains) into three resistotypes; A-C-.F (19.6%), A-C-.- (8.0%), and A-C-.FG (6.0%). Silver nitrate has not been included in this set as we have found it to give both poor discrimination and reproducibility.

In a study of patients attending a genitourinary clinic no significant difference was found between the biotypes isolated from the vaginas of women with and without candidosis.⁶ Sexual transmission of *C albicans* is known to occur,⁷ and may be a source of infection in women attending genitourinary clinics. Thus results from a genitourinary clinic may therefore not be applicable to the general population.

It has been suggested that women who suspect a venereal cause for their symptoms tend to attend the more anonymous genitourinary clinic, rather than

their general practitioners.⁸ If this is true then sexual transmission of *C albicans* was less common in our study than in the study of patients attending a genitourinary clinic.⁶ In most of our study group, therefore, infection is likely to have been endogenous and due to organisms already colonising the intestinal tract. That no significant differences were found between resistotypes causing vaginitis and those colonising the intestinal tract in the community as a whole, suggests that no particular resistotype is more virulent than any other. All resistotypes found colonising the human intestine seem equally likely to colonise and then infect the human vagina.

An important virulence factor for *C albicans* in superficial candidal infections is thought to be adherence to epithelial cells.^{9,10} This adherence varies between strains.^{11,12} These studies found some correlation between cellular adherence, pathogenicity for mice, and phospholipase activity¹² or proteinase activity.¹¹ One study¹¹ suggested that strain type --C--. which was the most common isolate, was also the most pathogenic. This resistotype was found only rarely in our study.

In other micro-organisms the association of known types with increased virulence has been shown. This is particularly so for *Escherichia coli*¹³ and also for *Haemophilus influenzae* type b.¹⁴ These associations, however, are usually with antigens on the cell surface which have an important role in the pathogenesis of infection. The association between types designated by other typing schemes and virulence does occur but is less well defined.

Both this work and that of the study of genitourinary patients⁶ have failed to associate any particular type of *C albicans* with vaginal candidosis. This may mean that all strains of *C albicans* are

Table 2 No of strains found of particular resistotypes in faecal and vaginal isolates of *Candida albicans* (%)

Resistotype	Faecal strains	Vaginal strains
A-C-.F-	21 (19.2)	18 (20.0)
ABC-.F-	12 (11.0)	12 (13.3)
A---.F-	9 (8.3)	8 (8.9)
A-C-.-	10 (9.2)	6 (6.7)
A-C-.FG	7 (6.4)	5 (5.6)
AB---.F-	4 (3.7)	6 (6.7)
A---.-	3 (2.8)	6 (6.7)
A---.FG	5 (4.6)	4 (4.4)
-BC-.F-	5 (4.6)	1 (1.1)
ABC-.FG	5 (4.6)	1 (1.1)
----.-	3 (2.8)	3 (3.3)
Others	25 (22.9)	20 (22.2)
Total	109 (100)	90 (100)

Table 3 No of strains resistant to individual agents used (%)

Code	Faecal strains	Vaginal strains
A	96 (88.0)	74 (82.2)
B	41 (37.6)	28 (31.1)
C	76 (69.7)	53 (58.9)
D	0	0
F	77 (70.6)	63 (70.0)
G	30 (27.5)	22 (24.4)

equally virulent for the vagina and that host factors are entirely responsible for the development of candidosis. It is also possible, however, that any particularly virulent strains are only randomly associated with the various known types. No information was available as to whether any of the infections were chronic or recurrent. These cases may yet be found to be due to particularly virulent strains.

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