SENSITIVITY OF YEASTS TO 5-FLUOROCYTOSINE

D. C. E. SPELLER AND M. G. DAVIES

Department of Microbiology, Bristol Royal Infirmary, Bristol BS2 8HW

THE anti-fungal agent, 5-fluorocytosine (5FC), is active *in vitro* against a limited number of species, but these include *Candida* sp., *Torulopsis glabrata*, and *Cryptococcus neoformans*, and the drug has been used successfully in the treatment of clinical infections by these fungi. The oral route of administration and the relative freedom from toxic effects are advantages of 5FC—in contrast to amphotericin-B, the established treatment for systemic mycoses—but primary resistance to 5FC in unexposed fungal isolates and resistance arising during treatment with the drug have been reported (Shadomy, 1970). In some series reported in the United States, a very large number of the isolates tested were primarily resistant to 5FC (Shadomy, 1970; Utz, 1972) though this has not been the experience of workers in Great Britain (Dr J. Garrod, personal communication). This report concerns the sensitivity to 5FC of 778 yeasts isolated consecutively in two general hospital laboratories.

MATERIAL AND METHODS

All yeasts isolated in moderate to heavy growth from specimens received in the Department of Bacteriology of Bristol Royal Infirmary between November 1969 and March 1971 were examined; urinary isolates were included only if the viable particle count was 10^4 per ml or more. In all, 678 isolates were obtained in this way. Another 100 isolates selected in a similar manner at the Radcliffe Infirmary, Oxford, were also examined. No significant differences in sensitivity between the two groups were observed and they are considered together in this report. No patient had previously been treated with 5FC.

In identification of the isolates, they were first subjected to the serum germ-tube test (Taschdjian, Burchall and Kozinn, 1960). Those giving a negative result were further identified by morphology in corn meal agar and by carbohydrate assimilation and fermentation tests after Lodder (1970). Germ-tube-positive isolates were assumed to be *Cand. albicans*, unless they were resistant to 5FC. Resistant isolates were subjected to further tests to distinguish *Cand. albicans* from *Cand. stellatoidea*.

Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were estimated by the methods of Marks and Eickhoff (1971). Known sensitive and resistant strains of *Cand. albicans* were included in each experiment. All isolates were subjected to a screening test with 5FC, $1\mu\text{g}$ per ml, incorporated in Yeast Morphology Agar (Difco), incubated at 37°C for 48 hr. Isolates giving visible growth at this time were tested for MIC and MFC by the broth dilution method, against doubling dilutions of 5FC from $250\mu\text{g}$ per ml to $0.25\mu\text{g}$ per ml in Yeast Nitrogen Base (Difco) with the addition of dextrose and asparagine (Shadomy, 1969), with a final inoculum of approx. 10^4 viable particles per ml and incubation for 48 hr at 37°C . The highest dilution at which there was no visible growth was taken as the MIC; 0.005-ml amounts from each dilution were subcultured on to dextrose peptone agar and incubated for 48 hr, and the highest dilution yielding no growth, or less than four colonies, was taken as the MFC. The MIC was also estimated for 100 isolates randomly selected from those that gave no growth at 48 hr in the screening test.

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TABLE I
Site of origin and species of 778 yeast isolates

Species	Numbers of named species isolated from											Total number examined
	urine			throat and mouth	sputum	faeces	vagina	ear	superficial sites	blood	site not known	
	Catheter	Mid-stream	Total									
67	55	122	129	34	8	162	7	29	1	37	529	
38	44	82	16	6	1	37	1	5	2	3	153	
10	5	15	8	7	0	3	6	12	0	3	54	
6	0	6	1	1	1	2	0	0	0	2	13	
1	6	7	2	2	0	1	0	0	0	0	12	
0	1	1	2	0	0	1	0	0	0	0	4	
2	0	2	0	0	0	0	0	2	0	0	4	
0	0	0	0	0	0	0	0	2	0	0	2	
0	0	0	1	0	0	0	0	1	0	0	2	
0	0	0	0	0	0	0	0	2	0	0	2	
0	0	0	1	0	0	0	0	0	0	0	1	
0	0	0	1	0	0	0	0	0	0	0	1	
0	0	0	1	0	0	0	0	0	0	0	1	
124	111	235	162	50	10	206	14	53	3	45	778	
All species												

RESULTS

The sites of origin of the isolates and their species are given in table I. The observed MIC values and the percentage of the isolates that were resistant

TABLE II
Minimum inhibitory concentrations of 5FC for 778 yeast isolates

Species	Numbers of named species inhibited by concentrations (μg per ml) of 5FC of										Total number examined	Percentage resistant*	
	>250	250	125	62.5	31	16	8	4	2	1			<1
<i>Cand. albicans</i>	20	0	2	2	0	2	1	1	7	3	491	529	4.5
<i>T. glabrata</i>	0	0	0	0	1	0	0	0	0	1	151	153	0.7
<i>Cand. tropicalis</i>	0	2	0	1	0	0	0	0	2	1	48	54	5.6
<i>Cand. parapsilosis</i>	0	0	1	0	0	0	0	0	0	0	12	13	7.7
<i>Cand. krusei</i>	1	0	0	0	0	1	2	0	2	0	6	12	8.3
<i>Trichosporon</i> sp.	0	1	0	1	0	1	1	0	0	0	0	4	50
<i>Cand. rugosa</i>	0	0	0	0	0	1	0	0	0	0	0	1	0
<i>P. membranaefaciens</i>	0	0	0	0	0	0	0	1	0	0	0	1	0
<i>Cand. pseudotropicalis</i>	0	0	0	0	0	0	0	0	0	0	11	11	0
<i>Cand. guilliermondii</i>													
<i>S. cerevisiae</i>													
<i>Rhodotorula</i> sp.													
<i>Cand. pelliculosa</i>													
All species	21	3	3	4	1	5	4	2	11	5	719	778	4.1

* MIC $\geq$ 31 μg 5FC per ml.

TABLE III
Minimum fungicidal concentrations of 5FC for 66 isolates that gave growth on plates containing 1 μg antibiotic per ml

Species	Numbers of named species killed by concentrations (μg per ml) of 5FC of										Total number examined
	>250	250	125	62.5	31	16	8	4	2	1	
<i>Cond. albicans</i>	35	0	2	2	1	1	1	1	1	0	44
<i>T. glabrata</i>	1	0	0	0	0	0	0	1	0	0	2
<i>Cand. tropicalis</i>	5	0	1	0	0	0	0	1	0	0	7
<i>Cand. parapsilosis</i>	1	0	0	0	0	0	0	0	0	0	1
<i>Cand. krusei</i>	1	0	0	1	1	2	0	0	1	0	6
<i>Trichosporon</i> sp.	2	2	0	0	0	0	0	0	0	0	4
<i>Cand. rugosa</i>	0	0	0	1	0	0	0	0	0	0	1
<i>P. membranaefaciens</i>	0	0	0	0	0	0	0	1	0	0	1
All species	45	2	3	4	2	3	1	4	2	0	66

to 5FC (MIC 31 μg per ml or more) are given in table II, and the MFC of the isolates that grew on plates containing 1 μg of 5FC per ml—but excluding five

strains that gave scanty growth and proved to have a MIC and a MFC below 1 μg per ml—in table III. Table IV shows the MIC values of 100 isolates that failed to grow on plates containing 1 μg of 5FC per ml; it will be seen that none had an MIC in broth exceeding 1 μg per ml. All such isolates have therefore been included in table II as having an MIC of less than 1 μg per ml.

No significant differences were found in the percentages of resistant isolates among those from different types of specimen.

TABLE IV

Minimum inhibitory concentration of 5FC for 100 isolates that gave no growth on plates containing 1 μg antibiotic per ml

Number of isolates inhibited by concentrations (μg per ml) of 5FC of								
>1	1	0.5	0.25	0.125	0.06	0.03	0.01	<0.01
0	0	3	8	36	36	14	3	0

DISCUSSION

We examined all yeasts isolated in moderate or heavy growth, but do not suggest that all or even most of them were exercising a pathogenic role in the patients. In many cases, yeasts were not being sought specifically, but had been found in the course of a bacteriological examination. Again, the yeasts isolated will have been selected by the incubation temperature of 37°C used for almost all specimens and by the short incubation times of 20 to 40 hr.

Most of the species found, however, may be pathogenic on occasion, and, because infection is usually endogenous, yeasts colonising body sites are likely to be the invaders when general or local defences are impaired. Ability to grow at 37°C is a requirement for systemic infection. The opportunistic pathogenicity of *Cand. albicans* is undoubted, and *Tor. glabrata* is being reported more frequently in a pathogenic role (Marks *et al.*, 1970). The other species of *Candida* and *Rhodotorula* have been described in systemic infection (Richart and Dammin, 1960; Louria *et al.*, 1967), though infection with *Rhodotorula* may well resolve without treatment when the source of infection—usually an intravenous infusion—is removed. *Trichosporon* spp. have rarely been reported in deep infection (Steer *et al.*, 1972) but were found in the present series repeatedly in heavy growth from the catheter urine in two patients, though admittedly only for a limited period.

Very small variations in the inoculum and the medium used may affect the results of determinations of 5FC sensitivity (Scholer, 1970). Apparent differences in sensitivity between species may result from factors other than intrinsic resistance, for instance the mode of growth and the production of mycelium. We found, as did Marks and Eickhoff (1971), that the incorporation-plate technique is reliable for detecting yeasts with a high MIC in liquid medium, which formed at the least a few colonies on a plate containing 1 μg of 5FC per ml after 48 hr.

The great difference between the MIC and the MFC can be seen by comparison of tables II and III. The antibiotic is only slowly fungicidal in synthetic media *in vitro* (Scholer, 1970), and a longer incubation time than the 40 hr used by us and most others would give a MFC nearer to the MIC. Even some isolates with a very low MIC (0.03–0.06 μg per ml) showed persistence of small numbers of viable organisms after exposure to high concentrations of 5FC. Usually the MIC of these survivors was the same as that of the original strain, but occasionally we observed the selection of highly resistant organisms, remarked upon by Holt and Newman (1972). Because these may be very important in the development of resistance during treatment, careful testing in liquid medium, with a long incubation period and subculture, should be carried out when long-term treatment with 5FC is contemplated.

Serum concentrations of 10–40 μg of 5FC per ml may be achieved for 6–10 hr after a 2-g dose (Utz, 1972). In analysing our results and comparing them with those of other authors who used similar methods we have considered isolates with a MIC of 16 μg per ml or less to be sensitive to 5FC, and those with a MIC of 31 μg per ml or more to be resistant. Our series contained only 4.1 per cent. of resistant isolates, a much lower proportion than has been reported by others. In the series of yeasts described by Marks and Eickhoff, approximately 25 per cent. had MIC values of 31 μg per ml or more, as did at least 67 per cent. of Shadomy's (1970) series of *Candida* spp. *Cand. albicans* was the predominating species in our series and 4.5 per cent. of the 529 isolates were resistant. Steer *et al.* found two of 25 *Cand. albicans* isolates to be resistant, and Shadomy (1970) three of six isolates. We encountered a single resistant *Tor. glabrata* in 153 isolates, and Marks *et al.* (1971) appear to have found one or two very resistant isolates among 35 tested. In our study, the proportion of resistant *Cand. tropicalis* was 5.6 per cent.

It is very difficult to assess the effectiveness of drug treatment in opportunistic infection, when host factors so greatly influence the outcome. There are, however, many reports of successful treatment with 5FC—some after the failure of amphotericin B—in cryptococcal meningitis and pulmonary infection (McGill *et al.*, 1969; Utz *et al.*, 1969), in candida and torulopsis septicaemia and endocarditis (Tassel and Madoff, 1968; Webb *et al.*, 1970; Record *et al.*, 1971; Steer *et al.*), in urinary-tract infection (Davies and Reeves, 1971; Schönebeck, 1971) and in eye infection (Richards *et al.*, 1969). Many of these authors also report treatment failures with 5FC, often but not always with the development of resistance to this agent. In cryptococcosis, the development of resistance seems to be more likely when smaller doses are used (Shadomy, 1970). Some toxic effects on the bone marrow and liver have been observed (Grunberg *et al.*, 1967; Steer *et al.*) but these are usually reversible and of little importance compared with those produced by treatment with amphotericin B (Scholer, 1968).

5-Fluorocytosine certainly has a place in the treatment of systemic yeast infections but the development of resistance puts limitations on its use; primary resistance, however, is uncommon.

SUMMARY

Amongst 778 yeasts isolated from clinical specimens and tested for sensitivity to 5-fluorocytosine, 4.1 per cent. had minimum inhibitory concentrations of 31 μ g of 5FC per ml, or more, a lower percentage of primary resistance than in some reported series. Resistance was found in 4.5 per cent. of *Candida albicans*, 0.7 per cent. of *Torulopsis glabrata* and 5.6 per cent. of *Cand. tropicalis*. The minimum fungicidal concentrations for the resistant candida isolates greatly exceeded the minimum inhibitory concentrations, and some yeasts with low minimum inhibitory concentrations contained small numbers of highly resistant organisms.

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