

## THE SENSITIVITY OF CANDIDA STRAINS TO MYCOSTATIN, ANILINE DYES AND IODINE

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The testing of sensitivity of bacteria to antibiotics is being carried out in every medical laboratory. Thus, whenever a known or even a yet unidentified organism is isolated from pathological material it is tested as a routine procedure to various drugs. This method is not yet in practice with pathogenic fungi, despite their frequent isolation in the laboratory. In cases of fungal diseases the efficiency or inefficiency of a certain drug is measured empirically by the success or failure to treat the disease condition in a patient. Obviously, such a procedure requires more work, time and extra suffering to the patient.

In our opinion, the method of testing the sensitivity of bacteria is equally good for testing the sensitivity of yeast fungi, especially when the latter grow fast and very well on ordinary media. In view of the importance of *Candida* strains in human infections (1, 6, 7, 10 and others) and in view of the widespread use of antibiotics, some possibly provoking such infections (4, 5, 8, 13) it is useful to carry out routine tests on these fungi to the various drugs concerned. It becomes more important because of the possible appearance of resistant pathogenic yeasts to certain antibiotics.

Although this work was done on strains isolated in Israel, it can, however, be of general interest to the medical profession here.

### MATERIALS AND METHODS

The specimens were collected from patients attending the Central Zamenhoff Laboratory in Tel-Aviv during the period 1958-1960. Material was taken for direct microscopy in 20% potassium hydroxide and inoculated on two culture media, agar-dextrose of neutral pH and agar-sucrose of alkaline pH (7.4-7.8). The first medium enhances the growth of *Candida* strains and the second that of dermatophytes, which will not be considered here. The identification of *Candida* strains was based on the fermentation of glucose, maltose, lactose and sucrose in a semisolid medium (12) and on their ability to produce chlamydozoospores in an agar medium containing taurocholate (3). The strains were maintained by subculture on Sabouraud glucose-agar before being identified. The sensitivity test was performed on the same

culture medium in Petri-dishes. For inoculation a twenty-four hour culture on agar-slope was washed in 5 c.c. saline and two drops of this suspension were sown on plates. Eight millimeter holes were bored into which two drops each of the various drugs were introduced in the following concentrations: 5% gentian violet in water, 5% mycostatin in alcohol, 5% basic fuchsin in alcohol-phenol, 1% brilliant green in alcohol and 5% tincture of iodine. At the same time sensitivity was tested in glucose-broth with the same solutions but diluted further from 100 to 1,000,000 times. After an incubation period of 24 hours at 37°C the diameter of the inhibition zone on the plates and the presence of growth in the broth were recorded. Inhibition to a diameter of 15 millimeters was regarded as one plus. Each additional 10 millimeters of inhibition was given one further plus.

Seventy five strains were also tested for sensitivity to griseofulvin and the antibiotic S-68 of Raubitschek (3, 9). The griseofulvin was used in 5% concentration in polyethyleneglycol and S-68 in a solution kindly supplied by Prof. Raubitschek of Jerusalem.

### RESULTS

*Candida* strains were isolated from nails, feet and hand-scrapings, interdigital-webs of the feet, sputum and from vaginal and cervical swabs. The cultures were identified and classified into a number of groups. Only strains frequently isolated and of medical importance (10, 2, 11) are considered here.

In all, 564 cultures were isolated, of which 325 belonged to *C. albicans* and 359 to *C. tropicalis*. Tables 1 and 2 contain data concerning the frequency of isolation of these strains from the various pathological material and their sensitivity to various drugs. The figures are based on minimum sensitivity of one plus (15 millimeters inhibition as mentioned before).

As can be noted the great majority of the strains belonging to *C. albicans* and *C. tropicalis* came from nails and skin-scrapings. These two species showed roughly the same degree of sensitivity, except to mycostatin, which was slightly more effective against *C. tropicalis* (96% as compared

TABLE 1. SENSITIVITY OF *C. ALBICANS* FROM VARIOUS SPECIMENS.

Specimen	Total	Mycostatin	Fuchsin	Gentian Violet	Brilliant Green	Iodine
Nails	190	173 (91%)	119 (63%)	174 (86%)	187 (98%)	181 (95%)
Scrapings	81	72 (89%)	56 (69%)	70 (86%)	76 (94%)	79 (97%)
Interdigital webs	15	13 (87%)	12 (80%)	15 (100%)	15 (100%)	15 (100%)
Sputum	23	19 (83%)	16 (70%)	20 (87%)	22 (96%)	23 (100%)
Vaginal and cervical swabs	16	15 (94%)	11 (69%)	13 (81%)	13 (81%)	16 (100%)
Total	325	292 (90%)	214 (66%)	292 (90%)	319 (96%)	314 (96%)

with 90%). It was also noted, that there were some differences in sensitivity of the same species isolated from different sources. For example, *C. albicans* from sputum was less sensitive to mycostatin than the same candida from vagina and cervix. The strains from nails were less sensitive to fuchsin than the strains from interdigital webs (63% as compared with 80%). Gentian violet and brilliant green were more effective against *albicans* from interdigital webs, but less against

vaginal and cervical cultures (100% as compared with 81%). Iodine was very effective (95-100%) irrespective of the source of isolation of the strain. With *C. tropicalis* mycostatin was better on sputum and interdigital strains than on vaginal and cervical strains (95% against 80%), however, fuchsin was in general of much lower activity (40%).

In order to emphasize more clearly the differences in sensitivity, two hundred and twelve cultures from nails and scrapings were tested on the basis

TABLE 2. SENSITIVITY OF *C. TROPICALIS* FROM VARIOUS SPECIMENS.

Specimen	Total	Mycostatin	Fuchsin	Gentian Violet	Brilliant Green	Iodine
Nails	174	165 (95%)	112 (64%)	155 (89%)	169 (95%)	172 (99%)
Scrapings	46	44 (96%)	46 (78%)	40 (87%)	44 (96%)	45 (98%)
Interdigital webs	8	8 (100%)	5 (62%)	8 (100%)	8 (100%)	8 (100%)
Sputum	21	19 (90%)	15 (71%)	18 (86%)	18 (86%)	18 (86%)
Vaginal and cervical swabs	10	8 (80%)	4 (40%)	9 (90%)	9 (90%)	10 (100%)
Total	259	248 (96%)	172 (66%)	230 (89%)	248 (96%)	253 (98%)

of a minimum of two plus sensitivity. The results are depicted in Table 3.

This table shows that mycostatin is effective against 65% of *C. tropicalis* as compared with 50% against *C. albicans*, whereas brilliant green and iodine retained their potency against 80% of cultures.

Table 4 represents comparative results of sensitivity tests of the two *Candidas* on solid agar and in broth media.

The drug of Raubitschek was similar in action to mycostatin (approx. 90%). Griseofulvin was ineffective on any of the strains tested. Comparative results of examination of seventy-five strains against these drugs are given in Table 5.

TABLE 3. SENSITIVITY OF CANDIDA STRAINS ON THE BASIS OF TWO PLUS INHIBITION.

Specimen	Total	Mycostatin	Fuchsin	Gentian Violet	Brilliant Green	Iodine
<i>Candida albicans</i>						
Nails	86	40 (46%)	10 (12%)	31 (36%)	69 (80%)	65 (78%)
Scrapings	29	18 (62%)	5 (17%)	10 (34%)	23 (79%)	26 (89%)
Total	117	58 (50%)	15 (13%)	41 (35%)	92 (80%)	91 (78%)
<i>Candida tropicalis</i>						
Nails	81	51 (63%)	18 (22%)	26 (32%)	66 (81%)	66 (81%)
Scrapings	14	11 (79%)	6 (43%)	6 (43%)	14 (100%)	13 (93%)
Total	95	62 (65%)	24 (25%)	32 (34%)	80 (84%)	79 (83%)

TABLE 4. ACTION OF DRUGS ON CANDIDA IN LIQUID AND SOLID MEDIA.

	Mycostatin		Fuchsin		G. Violet		Br. Green		Iodine	
	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar
<i>C. albicans</i>	+	+	—	—	+	+++	+++	+++	—	+
<i>C. tropicalis</i>	+	+	+++	+	+	+++	+++	+++	—	+

—... No inhibition in broth or under 15 mm. on plate by drug  $10^{-3}$

+... Inhibition in broth or 15 millimeter on plate by drug  $10^{-3}$

++... Inhibition in broth or 15 millimeter on plate by drug  $10^{-4}$

+++... Inhibition in broth or 15 millimeter on plate by drug  $10^{-5}$

TABLE 5. COMPARISON OF SENSITIVITY WITH S-68 AND GRISEOFULVIN.

Specimen	Total strains	S-68	Mycostatin	Fuchsin	Gentian Violet	Brill. Green	Iodine	Griseofulvin
Nails	47	42	41	33	43	47	47	0
Scrapings	22	20	20	16	20	22	22	0
Interdigital webs	6	6	6	5	6	6	6	0
Total	75	68 (91%)	67 (82%)	54 (72%)	69 (93%)	75 (100%)	75 (100%)	0 (0%)

While carrying out this work we met a strain of *C. albicans* found in sputum of a patient, which was initially sensitive to mycostatin but became resistant to this drug after several months treatment.

#### SUMMARY

1. 564 strains, among which 325 *C. albicans* and 259 *C. tropicalis* were isolated from pathological material of an outpatients dermatological department. About 65% consisted of nail specimens, 23% of skin scrapings and the rest came from interdigital webs, sputum, vaginal and cervical swabs.

2. The cultures were tested for sensitivity on solid media by conventional methods. The following drugs were tested: Mycostatin, Fuchsin, Gentian Violet, Brilliant Green, Iodine, Griseofulvin and S-68 of Raubitschek.

3. The results lead to the conclusion that there exist slight differences in sensitivity among *Candida* strains to different drugs and that the same strain may show further differences in sen-

sitivity if isolated from different pathological sources.

4. The simplicity of testing and the results obtained justify the implementation of this method for yeast fungi as a routine similar to that carried out with bacteria.

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