J. clin. Path. (1957), **10**, 219.

SENSITIVITY OF *CANDIDA* STRAINS TO NYSTATIN

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(RECEIVED FOR PUBLICATION JULY 24, 1956)

The discovery of an effective antifungal antibiotic, nystatin, has been followed by reports of its use in the treatment of various clinical conditions (Drouhet, 1955; Robinson, 1955; Sarewitz, 1955; Sloane, 1955; Stewart, 1956) and also prophylactically to prevent *Candida* infections in patients on long-term treatment with broad-spectrum antibiotics and cortisone (Childs, 1956; Stewart, 1956). So far, there have been no reports of strains of *Candida* which were resistant to nystatin, but it seems to be advisable to look out for the appearance of such strains, especially if the antibiotic is to be used prophylactically on a large scale. Therefore, a rapid routine test for sensitivity to this antibiotic is desirable, and the method used for other antibiotics of a blotting paper disc impregnated with an appropriate amount of antibiotic seems to be the most convenient.

During a recent clinical trial of nystatin vaginal tablets in the treatment of vaginal *Candida* infections (Jennison and Llywelyn-Jones, 1957), the opportunity arose of studying 73 strains of *Candida* isolated from such cases. The results of sensitivity tests by two methods on these organisms, 17 strains isolated from children, and stock strains of *Candida* are reported here.

Methods

(1) Serial Dilution Technique.—Amounts of nystatin, from 25 μg. per ml. down to 0.78 μg. per ml., were incorporated in a broth containing 3% glucose and Andrade's indicator at *p*H 7.2. One hundred units of penicillin and 100 μg. of streptomycin were added to prevent the growth of any extraneous bacteria. In practice, however, it was found that the change in colour of Andrade's indicator was not a suitable method of determining whether growth had occurred or not, because when the organisms were just beginning to multiply or when only slight growth had occurred, insufficient acid was formed appreciably to change the colour of the indicator. Therefore the development of turbidity was used as the first indication of growth in a tube. The relatively high *p*H of 7.2 for *C. albicans* was deemed necessary because nystatin is extremely unstable in an acid *p*H (Drouhet, 1955). The inoculum of *Candida* was 0.02 ml. of a 48-hour broth culture into 5 ml. of test media giving a final concentration of approximately 50,000 organisms/ml. The tubes were incubated at 37° C. After 24 hours' incubation there was no apparent growth in any of the tubes. Readings were therefore taken at two, three, and five days, and the amount of nystatin in the tube in which there was no turbidity or change of indicator was recorded as the minimal inhibitory concentration.

(2) Blotting Paper Disc.—Discs of Ford Mill 428 blotting paper, 8 mm. in diameter, were impregnated with various amounts of nystatin and dried, the technique being essentially the same as that of Fairbrother and Martyn (1951). A blood agar or plain nutrient agar plate was flooded with a six-hour culture of the *Candida* strain being tested, and after removing the excess fluid and allowing the surface of the plate to dry the discs were placed thereon and incubated overnight at 37° C. On examination two zones of inhibition could be seen. The smaller one, showing complete inhibition, was the one taken for measurement. The larger zone surrounding the first zone showed partial inhibition, and the diameter of this zone, as in the case of the smaller one, was proportional to the strength of the disc used. Some difficulty was experienced in the measurement of the zones because the growth of *Candida* after incubation overnight produced practically no pigment. This difficulty was overcome by painting white circles of varying diameters on black cardboard. The plate was then placed on the cardboard and examined by a strong direct light, when it was comparatively easy to match the zone of inhibition to one of the standard white circles on the cardboard.

Nystatin Solutions

Nystatin is supplied as a lyophilized powder which is relatively insoluble in water, approximately 200 μg./ml., but fairly soluble in the alcohols, approximately 5,000 μg./ml. In earlier experiments we used an aqueous suspension, but changed to a solution in 70% isopropanol because although the alcoholic solution is less stable more accurate dilutions could be obtained with the solution than with the aqueous suspension. Results were similar with both preparations. The alcoholic solutions were made strong enough so that when diluted

in broth the final concentration of alcohol was less than 0.3%. For the preparation of blotting paper discs it was found that the " 50 " dropper commonly employed with watery solutions delivered 120 drops per ml. of the 70% propanol solution. The nystatin used in all these tests was batch No. St 695–714 containing 2,800 units per mg.

Results

Serial Dilution Technique.—Stock strains of *Candida* were all sensitive to similar strengths of nystatin (1.56 μg.–6.25 μg. at 48 hr.) and the results are shown in Table I. Strains isolated from patients were inoculated on to cornmeal agar for the detection of chlamydospores; 14 strains did not produce

TABLE I
RESULTS OBTAINED WITH STANDARD STRAINS OF *CANDIDA*

Organism	Serial Dilution: Mean Inhibitory Concentration (μg./ml.)			Disc: Zone of Inhibition (mm.)	
	48 hr.	72 hr.	120 hr.	6·25 μg.	12·5 μg.
Candida albicans (Z 248)	6·25	6·25	12·5	13	15
Candida krusei (Z 70)	3·12	6·25	12·5	12	15
,, *parakrusei*	3·12	3·12	12·5	15	18
,, *tropicalis*	3·12	6·25	12·5	13	15
,, *pseudo-tropicalis*	1·56	3·12	6·25	15	17
Candida guilliermandi	1·56	6·25	6·25	15	17
,, *stellatoidea*	3·12	3·12	3·12	15	18

TABLE II
NUMBER OF STRAINS INHIBITED AT EACH CONCENTRATION

	Hours	Nystatin (μg. ml.) 1·56	3·12	6·25	12·5	25·0
C. albicans (76 strains)	48	10	58	7	1	—
	72	—	14	52	10	—
	120	—	—	10	58	8
C. spp. (14 strains)	48	4	9	1	—	—
	72	1	6	7	—	—
	120	—	—	8	5	1

chlamydospores. Table II shows the mean inhibitory concentration of all the organisms isolated from patients with *Candida* infections. The mean inhibitory concentration at 48 hr. was 3.3 μg. for *C. albicans* and 2.9 μg. for the other species. It will be seen that the action of nystatin is mainly fungistatic as growth is only partially stopped and continues for some days even in the stronger concentrations. But in the majority of cases no further growth occurs in 25 μg./ml. after five days, suggesting either a fungicidal action at this level or death of the organisms from other causes. Subcultures utilizing the whole 5 ml. of media taken from the tubes containing 25 μg./ml. did not grow when the nystatin concentration was diluted to below the fungistatic level.

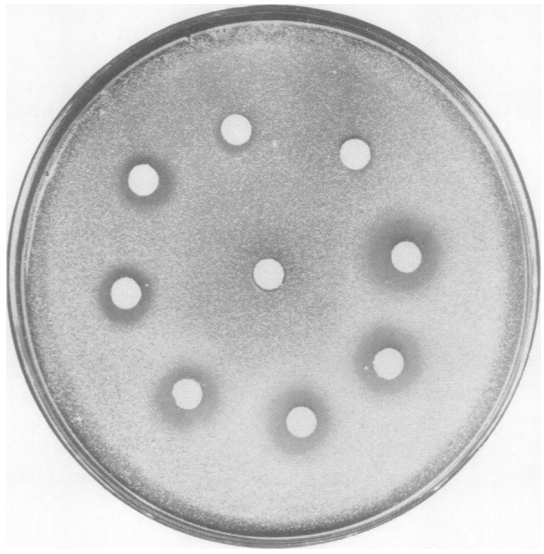

FIG. 1.—A 14 cm. Petri dish showing zones of inhibition obtained with blotting paper discs containing amounts of nystatin ranging from 100 μg. per disc down to 0.78 μg. per disc. The central disc contains solvent only.

Blotting Paper Discs.—At first a range of discs was used as shown in Fig. 1, and a fairly good correlation was obtained between the strength of the disc and the zone of inhibition, which was 20 mm. with the 100 μg. disc down to nothing with the 0.78 μg. disc. Subsequently all the strains were tested against discs containing 12.5, 6.25, 3.125, and 1.56 μg. and the relative zones with all the strains were similar to those in Fig. 1. The distribution of the actual zones of inhibition around 12.5 μg. and 6.25 μg. with 90 strains is shown in Fig. 2. The average for 12.5 μg. was 16 mm. and

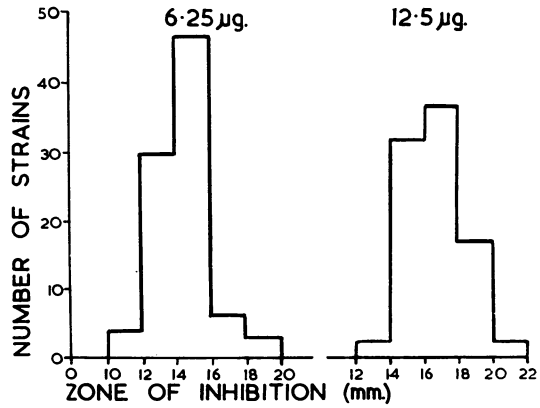

FIG. 2.—Distribution of zones of inhibition around discs containing 6.25 μg. and 12.5 μg.

for 6.25 μg. was 14 mm. Dried discs when stored in the refrigerator keep their potency for at least four months.

Conclusion

The majority of workers have used serial dilution methods for the determination of sensitivity to nystatin. In our series the mean inhibitory concentration of *Candida* strains at 48 hr. was 3.2 μg./ml. with a range of 1.56 μg. to 12.5 μg./ml. These results are similar to those obtained by Drouhet (1955) of 8.4 μg. (3.12 μg.–12.5 μg.) and by Stewart (1956) of 1.6 μg. to 6.4 μg. There was no difference in the sensitivity of strains isolated from infections resistant to treatment with nystatin. On the other hand Drouhet stated that blotting paper discs only work at strengths of 100 μg. per disc, and Stewart found that discs work but does not mention the actual strength used, whereas we find that discs containing as little as 3.125 μg. give a smal but obvious zone of inhibition and discs containing 12.5 μg. give a clear-cut zone of inhibition which is suitable for use as a routine bacteriological test.

Summary

The sensitivity to nystatin of 76 strains of *Candida albicans* and 14 other strains of *Candida* has been estimated using a tube dilution method and blotting paper discs.

The average mean inhibitory concentration at 48 hr. in broth was 3.2 μg./ml., no resistant strains being encountered.

Blotting paper discs containing 12.5 μg./ml. are stable for some months and give an average zone of inhibition of 16 mm. which is not dependent on the type of culture medium employed.

We wish to thank Dr. R. W. Fairbrother for much helpful advice and Messrs. Squibb & Sons for supplies of lyophilized nystatin.

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