SHORT COMMUNICATION

In vitro Development of Resistance to Nystatin by Candida albicans and Torulopsis glabrata

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The levels of resistance to nystatin induced in 16 strains of Torulopsis glabrata by a few subcultures in the presence of the drug were high (minimum inhibitory concentrations > 20000 U ml⁻¹) compared with those obtained in 12 strains of Candida albicans (minimum inhibitory concentrations ≤ 2000 U ml⁻¹). The results suggest a high rate of mutation in the first species.

INTRODUCTION

The results of tests of sensitivity of Candida albicans to nystatin obtained by different authors differ considerably, the mean values of the minimum inhibitory concentration (m.i.c.) varying as much as 10-fold. This probably reflects the disparity of the methods more than differences in the susceptibility of the yeast (Lovgren & Salmela, 1978).

The action of nystatin is greatly influenced by the pH of the medium, the temperature of the incubation (Johnson et al., 1978) and, in particular, by the dilution technique – whether by suspension in sterile distilled water or by solubilization in dimethyl sulphoxide or propylene glycol.

There is no evidence of emergence in vivo of resistance to nystatin in C. albicans even after 20 years of clinical use of the drug (Athar & Winner, 1971; Hamilton-Miller, 1973). However, resistant strains of C. albicans, C. tropicalis, C. pseudotropicalis, C. krusei, C. parapsilosis, C. stellatoidea, C. guilliermondii and Saccharomyces cerevisiae have been reported after subculture in vitro in the presence of gradually increasing concentrations of nystatin (Athar & Winner, 1971; Molzahn & Woods, 1972) and by using mutagenic agents (Hamilton-Miller, 1972a, b).

We have, however, isolated from clinical material two strains of Torulopsis that are relatively resistant to nystatin. For one of these (T. glabrata) the m.i.c. was 250 U ml⁻¹ while the other (T. candida) was not inhibited by 20000 U ml⁻¹, the highest concentration of the drug which has been used in the test.

The purpose of this study was to compare the development of in vitro resistance to nystatin of a number of strains of C. albicans with that of strains of T. glabrata by subculture in the presence of sub-inhibitory concentrations of the drug.

METHODS

The 12 strains of C. albicans and 16 strains of T. glabrata selected for this study were obtained from patients at the Instituto Bacteriológico Câmara Pestana and Centro de Dermatologia Médico-Cirúrgica, Serviços Médico-Sociais, Lisbon. The sources of the isolates were: C. albicans – genital tract 4, skin 8; T. glabrata –
genital tract 14, urine 1, skin 1. Identification of *C. albicans* was based on rapid filamentation on serum. *Torulopsis glabrata* was identified by conventional methods described by Lodder & Kreger-van Rij (1967).

Resistance to nystatin was induced by serial transfer in the presence of increasing concentrations of the drug. Nystatin (Mycostatin, Calbiochem lot 802388, containing 6060 U mg⁻¹) suspended in sterile distilled water, according to the technique of Bodenhoff (1968), was incorporated in Sabouraud maltose agar (pH 7) at the following final concentrations (U ml⁻¹): 5, 10, 15, 25, 50, 75, 100, 250, 500, 1000 and, when necessary, also 2000, 5000, 10000, 15000 and 20000.

The inoculum was a yeast suspension in sterile saline containing approximately 10⁷ cells ml⁻¹. The m.i.c., defined as the lowest concentration of the drug giving complete inhibition of growth, was read after 48 h incubation at 30 °C. Each strain was subcultured 10 times or until resistance to 20000 U nystatin ml⁻¹ was attained.

**RESULTS AND DISCUSSION**

After 10 passages the strains of *C. albicans* were inhibited by nystatin at 2000 U ml⁻¹, or less, whereas all strains of *T. glabrata* were resistant to 20000 U ml⁻¹ (Fig. 1). In addition, the increase in resistance was much more rapid in *T. glabrata*; in all but one strain, resistance to 20000 U nystatin ml⁻¹ was found after the fifth passage.

The results of the work of Athar & Winner (1971) suggest differences in the rate of *in vitro* acquisition and level of resistance to nystatin attained by various species of *Candida*. In the present study a pattern of progressive increase in resistance to nystatin was observed in all the strains of *T. glabrata* tested, a high level of resistance being attained after a few passages. This pattern differed from that found in *C. albicans*.

These results suggest a higher rate of mutation in *T. glabrata*. The possible occurrence of mutants of this species naturally resistant to polyene antibiotics must therefore be considered in clinical practice.
REFERENCES


