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 $> 20000 \text{ U ml}^{-1}$) compared with those obtained in 12 strains of *Candida albicans* (minimum inhibitory concentrations $\leq 2000 \text{ U ml}^{-1}$). 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 (Lövgren & 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, 1972*a, 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* –

Fig. 1. Increase in resistance to nystatin of *C. albicans* (●) and *T. glabrata* (○) on transfer in medium containing the antibiotic.

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.

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