In vitro activity of antimycotic agents determined by E-test method against vaginal Candida species

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Received 5 November 1998; accepted in revised form 22 December 1998

Abstract

Vaginal candidiasis continues to be a common cause of vaginal discharge, pruritus and other local complaints in women worldwide. Although numerous antimycotic agents are available for the treatment of yeast vaginitis there is little comparative data on the in vitro activity of these drugs. The objectives of this study were to isolate and identify the Candida species in the vagina and anus of patients treated in a gynaecology clinic, as well as determine the susceptibility to azolic compounds measured by the E-test method. Vaginal and rectal swabs were collected from 80 adult non-pregnant patients, seen at a gynaecological clinic, aged 18–59 years, with sexual activity, with and without vaginitis. The swabs were processed by methods routinely used for the detection of pathogenic yeasts. The susceptibility of the isolates to fluconazole, ketoconazole and itraconazole, was measured by the agar diffusion method (E-test), using RPMI 1640 medium with 2% glucose and phosphate buffer.

Candida species (33) strains were isolated from 17 patients at similar proportions from both anatomical sites, and 12 patients harboured 24 strains of C. albicans in the vaginal and rectal tracts. Twenty one percent of the strains of C. albicans were resistant to ketoconazole, 54% were resistant to itraconazole and 0% were resistant to fluconazole. The sensitivity of strains isolated from the two sites were similar, indicating that these are strains of the same phenotype.

Key words: C. albicans, C. krusei, C. parapsilosis, C. tropicalis, E-test, yeasts

Introduction

Vaginal candidiasis is an infection caused by Candida albicans or occasionally by other yeasts of the Candida genus [1]. Candida is a saprophytic, opportunistic microorganism and conditions resulting in a decrease in vaginal pH or alteration of the local defense mechanisms favour the appearance of Candida vaginitis [2].

In contrast to the large number of antibacterial drugs commercially available, the number of systemic antifungal therapeutics is rather limited. The most frequently used antimycotics are flucytosine, amphotericin B andazole derivatives [3]. The discovery of the antifungal activity of the azole compounds represented an important development in the treatment of mycoses. In 1969, clotrimazole was launched for topical use followed by miconazole, which is available for topical and systemic use. However, the toxicity of the latter encouraged new research resulting in the development of ketoconazole, which permits oral administration due to the fact that it is easily absorbed by the gastrointestinal tract [3, 4]. More recently, the triazoles fluconazole and itraconazole have been developed. These new compounds are characterized by a wide spectrum of activity. The differences in metabolism, pharmacokinetics, adverse reactions and medicinal interactions guide the physician’s choice of one compound or another [3, 5].

At present, the E-test method, which is a new concept for quantitative sensitivity tests, is used in order to assess the in vitro susceptibility of the yeast forms to antifungal agents. The E-test method is based on a previously defined gradient of the antimycotic, which is incorporated onto a plastic strip. The method can be used to determine the minimum inhibitory con-
concentration (MIC) of antimycotics against yeast forms growing in standardized medium [6,7].

The objectives of this study were to isolate and identify the Candida species in the vagina and anus of patients treated in a gynaecology clinic, as well as determine the susceptibility to azolic compounds measured by the E-test method.

Materials and methods

Patients: Eighty adult patients, aged 18–59 years, who were sexually active participated in this study. They were examined by a gynaecologist from University Campus Medical Assistance Services, SISUSP in Ribeirão Preto. The samples were collected from the vagina and anus of each patient with sterile swabs.

Organisms: The yeast forms were isolated, stored at Sabouraud dextrose agar medium and identified on the basis of sugar fermentation and assimilation reactions, microscopic morphology, production of chlamydospores and germ tubes and, when required, urease activity and nitrate assimilation [8].

Inoculum: Prior to testing, each isolate was grown on Sabouraud dextrose agar for 24 h at 37 °C. Suspensions were prepared from individual colonies in 3 ml of sterile 0.85% saline to a density of a 0.5 MacFarland standard.

Assay medium: The culture medium was used for the testing of each isolate was solidified (1.6% agar) RPMI 1640 medium with L-glutamine and without Sodium Bicarbonate (Sigma). The medium was buffered with phosphate buffer to pH 7.0 which has been shown to provide sharper end points [9].

Antifungal agents: The E-test antifungal gradient strips were provided by the manufacturer AB Biodisk, Solna, Sweden [9]. The strips were stored at −20 °C. The antifungal agents evaluated were ketoconazole, fluconazole and itraconazole.

E-test procedure: The suspensions of the organisms were inoculated onto 150 mm petri plates containing 60 ml of RPMI agar using a sterile swab, streaking it over the entire surface of the agar. The plates were placed at room temperature for 15 min to dry. Following this, the E-test strips of fluconazole, ketoconazole and itraconazole were placed onto the agar surfaces. After 24 h of incubation at 37 °C, the minimum inhibitory concentration (MIC) of the azole compounds was determined [9]. The ATCC reference strains C. krusei (ATCC 6258) and C. parapsilosis (ATCC 22019) were used as quality controls.

Determination of MIC endpoints: The MIC by the E-test was the lowest drug concentration at which the border of the elliptical inhibitory zone intercepted the scale on the antifungal strip. It was possible to identify four different reading patterns for the MICs: a) sharp endpoint reading; b) growth of microcolonies inside the inhibitory zone; c) double halo, illustrated by the growth of microcolonies just close to the border of the inhibitory zone; d) resistant isolate with homogeneous growth around the strip. Patterns a, b and c, can be observed in Figure 1.

Results interpretation: To define interpretive breakpoints of the azole compounds we correlated the data of NCCLS-M27A [10] with the data of the literature available [11, 12]. Table 1 shows the interpretive guidelines we followed. Ketoconazole is known to have a similar mode of action and similar pharmacokinetics to previously studied itraconazole, so we might expect that the breakpoints would be similar [11].

Results

Different species of Candida were isolated in 17 (21.2%) of the 80 patients studied. Candida albicans was the prevailing species; it was isolated from both anatomical sites (vagina and anus) in 12 patients. C. tropicalis was isolated from the vagina in 3 patients and in the anus in 2 patients. C. parapsilosis was isolated from the vagina in 1 patient and from the anus in 1 patient. C. krusei were isolated from both the anus