In vitro susceptibility testing of yeasts to nystatin – low minimum inhibitory concentrations suggest no indication of in vitro resistance of Candida albicans, Candida species or non-Candida yeast species to nystatin

Pietro Nenoff*, Constanze Krüger1, Claudia Neumeister2, Ulrich Schwantes2 and Daniela Koch1

1 Laboratory for Medical Microbiology, Mölbiser Hauptstrasse 8, 04571 Rötha / OT Mölbis, Germany
2 Dr. R. Pfleger GmbH, 96045 Bamberg, Germany

Abstract

In total, 14 yeast strains originating from patients with dermatomycoses, and 7 control strains (isolates from strain collections and collaborative ring trials) were investigated regarding their in vitro susceptibility to the polyene antifungal agent nystatin. Testing was performed using a broth microdilution assay based on the standardized method of susceptibility testing of yeasts per EUCAST (The European Committee on Antimicrobial Susceptibility Testing). Minimum inhibitory concentrations (MIC) for nystatin were measured. The reading of the MIC values was performed by both visual examination, and spectrophotometric measuring after 24 and 48 hours incubation time at 36°C.

The visual read-out of growth inhibition revealed MICs for nystatin in a range from 3.7 to 7.4 IU/mL (0.625 to 1.25 µg/mL) for all Candida species tested. One of the Candida (C.) albicans strains, and both strains of C. glabrata and C. tropicalis, showed low MIC values of 3.7 IU/mL (0.625 µg/mL). Geotrichum candidum and Trichosporon mucoides were also inhibited by nystatin. The control strains (C. albicans, C. glabrata, C. parapsilosis, C. krusei and C. tropicalis) confirmed the values which were found for the wild strains. The spectrophotometric measuring of the turbidimetry revealed slightly lower MIC values for Candida species. Spectrophotometric measurement of Geotrichum candidum and Trichosporon mucoides was unsuccessful or not possible; however, visual reading of the results was carried out effectively.

Nystatin showed very good in vitro activity against these non-Candida yeast species. In conclusion, very good in vitro activity of nystatin against all tested yeast strains could be detected. The in vitro efficacy was independent of the origin of the strains, as both the wild strains isolated from patients in this study, and the control strains originating from strain collections, were inhibited.

Introduction

The common use of azole antifungal agents, in particular oral fluconazole, and also topical azoles, for the treatment of cutaneous and oral candidiasis, and possibly due to the use of azole fungicides in agricultural crop protection, has led to the emergence of azole and oral candidiasis, and possibly due to the use of azole fungicides for the treatment of cutaneous dermatomycoses patients, and seven reference strains (controls), including isolates from the DSMZ strain collection (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) and inter-laboratory ring tests of INSTAND e.V. (the German Society for Promoting Quality Assurance in Medical Laboratories, Düsseldorf, Germany), were tested for their in vitro susceptibility to the polyene antifungal agent nystatin. A broth microdilution assay which corresponded to the method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and allowed for determination of the minimal inhibitory concentration (MIC) of nystatin, was used for this purpose.

Materials and methods

Active substance

Nystatin: Pure nystatin from Dr. R. Pfleger GmbH, Bamberg, Germany was used in this study. Each ampoule contained: nystatin, lyophilized, approximately 195,000 International Units (IU); stored at refrigerating temperature of 4°C. The active substance content corresponded to 5916 IU nystatin per milligram.

Test strains

Sensitivity testing was performed using test strains from routine

Correspondence to: Dr. Pietro Nenoff, Dermatology, Laboratory Medicine, Allergy & Andrology Specialist; Main Focus: Tropical and Travel Dermatology (DDA), Mölbiser Hauptstraße 8, 04571 Rötha/OT Mölbis, Germany; Tel: +49-34347-50 323; Fax +49-34347-50 123; E-mail: pietro.nenoff@gmx.de

Received: October 31, 2016; Accepted: November 17, 2016; Published: November 22, 2016
Nenoff P (2016) In vitro susceptibility testing of yeasts to nystatin – low minimum inhibitory concentrations suggest no indication of in vitro resistance of Candida albicans, Candida species or non-Candida yeast species to nystatin

Volume 1(3): 71-76
Clin Med Invest, 2016         doi: 10.15761/CMI.1000113

In vitro

Clinical isolates and sources of the yeast strains investigated in this study. The activity of nystatin against the specified yeasts was determined by broth microdilution (Table 3). 3-Morpholinopropanesulfonic acid (MOPS, Sigma-Aldrich Chemie GmbH, Schnellendorf, product no. M3183-100G, Germany), adjusted to a final concentration of 0.165 mol/l and a pH of 7.0, was used as the buffer for the RPMI 1640 medium.

Procedure for testing the antifungal sensitivities of Candida and other yeast species to nystatin by broth microdilution assay according to EUCAST

The broth microdilution assay according to EUCAST [Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts, EUCAST DEFINITIVE DOCUMENT EDef 7.2 Revision] was used to assess the antifungal activity of nystatin against the specified yeasts in vitro. This method allows for determination of the minimum inhibitory concentration for each strain in IU/mL (and µg/mL). RPMI 1640 medium was used in sensitivity testing.

Table 3. Method of serial dilution of nystatin and final concentration of the antifungal agent in the serial dilution test.

| No. | Nystatin Working solution | Nystatin Final concentration |
|-----|---------------------------|------------------------------|
|     | IU/ml µg/mL               | IU/ml per well µg/mL-per well |
| I   | 11832 2000                | 59.16 10                     |
| II  | 5916 1000                 | 29.58 5                      |
| III | 2958 500                  | 14.79 2.5                    |
| IV  | 1479 250                  | 7.4 1.25                     |
| V   | 740 125                   | 3.7 0.625                    |
| VI  | 370 62.5                  | 1.85 0.312                   |
| VII | 185 31.25                 | 0.92 0.156                   |
| VIII| 92 15.55                  | 0.46 0.078                   |
| IX  | 46 7.77                   | 0.23 0.039                   |
| X   | 23 3.88                   | 0.115 0.0195                 |
| XI  | Growth control            |                              |
| XII | Sterility control         |                              |

Culture media

In accordance with EUCAST specifications [EUCAST Definitive Document EDef 7.2 Revision], fully synthetic Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine (Sigma-Aldrich Chemie GmbH, Schnellendorf, product R8758-IL, Germany), supplemented with glucose to a final concentration of 20 g/l (2%), was used for susceptibility testing [4]. 3-Morpholinopropanesulfonic acid (MOPS, Sigma-Aldrich Chemie GmbH, Schnellendorf, product no. M3183-100G, Germany), adjusted to a final concentration of 0.165 mol/l and a pH of 7.0, was used as the buffer for the RPMI 1640 medium.

Inoculum

The inoculum was prepared using sterile, flat-bottom 96-good microdilution plates with a total volume of approximately 300 µl. 100 µl of each respective nystatin working solution was diluted with 9.9 ml [2X RPMI / 2% glucose], equivalent to a ratio of 1:100. Next, 100 µl of the respective nystatin solution was pipetted into the respective wells numbered 1 to 10. Thus, each well contained 100 µl of the respective nystatin working solution and 2X concentrated RPMI 1640 / 2% glucose medium with 1% solvent. By subsequent 1:2 dilution with inoculum, the final concentration of nystatin in the test wells was adjusted to 59.16 IU/ml (10 µg/mL) to 0.115 IU/mL (0.0195 µg/mL), respectively (Table 3). Column 11, the growth control, contained antifungal-free RPMI 1640 medium. Column 12, the sterility control, contained antifungal-free RPMI medium (with no nystatin); 100 µl distilled water was added instead of inoculum.

Preparation of microdilution plates

Testing was performed using sterile, flat-bottom 96-good microdilution plates with a total volume of approximately 300 µl.

| Yeast          | Origin          |
|----------------|-----------------|
| Candida albicans 115370/2015 | Oral mucosa    |
| Candida albicans 803972/2015   | Stool / intestine |
| Candida albicans 115323/2015   | Urethral swab  |
| Candida albicans 115369/2015   | Skin at corner of mouth |
| Candida glabrata 703950/2015   | Urine / genital area |
| Candida parapsilosis 115753/2014 | Throat swab |
| Candida parapsilosis 216299/2015 | Fungal nail clippings, first digit (both sides) |
| Candida krusei 703658/2015     | Urine          |
| Candida krusei 113204/2015     | Vaginal swab   |
| Candida kefyr 803965/2015      | Stool          |
| Geotrichum candidum 803545/2015 | Stool |
| Trichosporon mucoides 215610/2015 | Toe nail |
| Trichosporon mucoides 215470/2015 | Toe nail |
In each case, the yeast cell density was checked and confirmed by transferring cells to Sabouraud’s dextrose agar and subsequently counting the colonies (after 24 or 48 hours of incubation).

A MultiPipette was used to dispense the yeast suspensions onto the plates (100 µl inoculum per well, columns 1 to 11). Subsequent 1:2 dilution with nystatin/(RPMI 1640 / 2% glucose) working solution was performed to yield the aforementioned test yeast cell suspension concentrations ranging from 0.5 to 2.5 × 10⁵ CFU/ml in the microplates.

Incubation was performed under aerobic conditions at 36 °C. Visual readings were obtained after 24 and 48 hours of incubation. Turbidity i.e. growth inhibition on the microplates was also determined spectrophotometrically at 450 nm after 24 and 48 hours of incubation. The absorbance measured with the blank sample (background absorbance) was deducted from the absorbance values obtained with the test samples. The MIC was defined as the lowest concentration of the active substance that inhibited growth of the microorganisms.

Growth controls in antifungal-free culture medium (RPMI 1640, column 11) and sterility controls containing no inoculum (column 12) were run with each MIC determination.

The MIC<sub>90</sub> which represents the MIC that inhibits 90% of the isolates, and the MIC<sub>50</sub> which represents the MIC which inhibits 50% of the isolates of the species tested, was calculated.

Results

In each case, the MIC values were read visually and spectrophotometrically (at 450 nm), after 24 and, 48 hours of incubation at 36°C. All yeast strains were tested in duplicate.

Visual reading

After 24 hours of incubation, the growth of the vast majority of studied yeast strains allowed MIC reading which could be considered as conclusive. All Candida species could be fully evaluated after 24 hours of incubation (Tables 4a, 4b and 4c). The Geotrichum candidum strain and the two Trichosporon mucoides strains grew slower per se and thus could not be evaluated until the second day (Table 4b).

The MIC values of nystatin were in the range of 3.7 to 7.4 IU/mL (0.625 to 1.25 µg/mL) for all Candida species (Table 4a). Specifically, the MICs for one C. albicans strain, the two C. glabrata strains, and C. tropicalis were in the low range of 3.7 IU/mL (0.625 µg/mL). The two non-Candida species, Geotrichum candidum and Trichosporon mucoides, were also inhibited well by nystatin; the MIC values for these yeasts remained constant at 7.4 IU/mL (1.25 µg/mL, Table 4b). The five reference strains from the DSMZ collection (Braunschweig, Germany) (C. albicans, C. glabrata, C. parapsilosis, C. krusei and C. tropicalis) confirmed the MIC values obtained for

### Table 4b. Minimum inhibitory concentrations (MIC) of nystatin against various yeast strains (Candida krusei, Candida tropicalis, Candida lusitaniae, Candida kefyr, Geotrichum candidum and Trichosporon mucoides) as determined by broth microdilution assay according to EUCAST. MIC readings (double determination) were taken after 24 and 48 hours of incubation at 36 °C. Visual reading

| Yeast strain       | Nystatin MIC in IU/ml | Nystatin MIC in µg/ml |
|--------------------|-----------------------|-----------------------|
|                    | 24-hour reading | 48-hour reading | 24-hour reading | 48-hour reading |
| Candida krusei     | 7.4 | 7.4 | 1.25 | 1.25 |
| DSM 115368/2015    | 7.4 | 7.4 | 1.25 | 1.25 |
| Candida krusei     | 7.4 | 7.4 | 1.25 | 1.25 |
| DSM 113204/2015    | 7.4 | 7.4 | 1.25 | 1.25 |
| Candida tropicalis | 3.7 | 7.4 | 0.625 | 1.25 |
| DSM 115470/2015    | 3.7 | 7.4 | 0.625 | 1.25 |
| Candida tropicalis | 7.4 | 7.4 | 1.25 | 1.25 |
| DSM 303545/2015    | 7.4 | 7.4 | 1.25 | 1.25 |
| Trichosporon mucoides | Not evaluable | Not evaluable | Not evaluable | Not evaluable |
| DSM 215610/2015    | Not evaluable | Not evaluable | Not evaluable | Not evaluable |
| Trichosporon mucoides | Not evaluable | Not evaluable | Not evaluable | Not evaluable |
| DSM 215470/2015    | Not evaluable | Not evaluable | Not evaluable | Not evaluable |

### Table 4c. Minimum inhibitory concentrations (MIC) of nystatin against various control strains (reference strains/collection strains of Candida albicans, Candida glabrata, Candida parapsilosis, Candida krusei and Candida tropicalis) as determined by broth microdilution assay according to EUCAST. MIC readings (double determination) were taken after 24 and 48 hours of incubation at 36 °C. Visual reading

| Yeast strain       | Nystatin MIC in IU/ml | Nystatin MIC in µg/ml |
|--------------------|-----------------------|-----------------------|
|                    | 24-hour reading | 48-hour reading | 24-hour reading | 48-hour reading |
| Candida albicans    | 7.4 | 7.4 | 1.25 | 1.25 |
| DSM 28719           | 7.4 | 7.4 | 1.25 | 1.25 |
| Candida glabrata    | 7.4 | 7.4 | 1.25 | 1.25 |
| DSM 28718           | 7.4 | 7.4 | 1.25 | 1.25 |
| Candida parapsilosis | 7.4 | 14.79 | 1.25 | 2.5 |
| DSM 28732           | 7.4 | 7.4 | 1.25 | 1.25 |
| Candida krusei      | 7.4 | 7.4 | 1.25 | 1.25 |
| (Pichia kudriavzevii) | 7.4 | 7.4 | 1.25 | 1.25 |
| Candida tropicalis  | 7.4 | 7.4 | 1.25 | 1.25 |
| DSM 28720           | 7.4 | 7.4 | 1.25 | 1.25 |
| Candida tropicalis  | 3.7 | 7.4 | 0.625 | 1.25 |
| DSM 28721           | 3.7 | 7.4 | 0.625 | 1.25 |
| Candida parapsilosis| 7.4 | 7.4 | 1.25 | 1.25 |
| Candida parapsilosis| 7.4 | 7.4 | 1.25 | 1.25 |
| Candida parapsilosis| 7.4 | 7.4 | 1.25 | 1.25 |
| Candida parapsilosis| 7.4 | 7.4 | 1.25 | 1.25 |
the wild strains. The minimum inhibitory concentrations for these DSM Candida strains also ranged from 3.7 IU/mL (0.625 µg/mL, C. tropicalis) to 7.4 IU/mL (1.25 µg/mL, Table 4c).

Spectrophotometric measurement and determination of MIC

The minimum inhibitory concentrations obtained by spectrophotometric absorbance measurement and optical density determination based on turbidity due to growth of the investigated yeast strains are presented in Tables 5a-c. Compared to the visual readings, the spectrophotometrically determined MIC values for the clinical isolates of C. albicans and C. glabrata were slightly lower: 3.7 IU/mL (0.625 µg/mL) at 24 hours. Spectrophotometric readings at 48 hours were also lower: in contrast to the visual readings, only a few strains had a photometric MIC value of 7.4 IU/mL (1.25 µg/mL, Tables 5a, 5b and 5c).

C. parapsilosis: After 24 hours of incubation, spectrophotometric determination of the MIC for this slowly growing yeast was not possible, but visual reading was feasible (Table 4a, Table 5a). After 48 hours, the MIC values determined by spectrophotometry were in the range of 7.4 IU/mL (1.25 µg/mL), which is slightly lower than the visual readings (Table 5a).

C. krusei, C. lusitaniae, C. tropicalis and C. kefyr were well measurable. The spectrophotometrically and visually determined minimum inhibitory concentrations for these yeasts were comparable, but those determined by spectrophotometry tended to be slightly lower (Table 5b).

Geotrichum candidum (known as the “cheese mold”) and Trichosporon mucoides are slow-growing yeasts that do not tend to develop homogeneous growth. Geotrichum candidum, in particular, tends to develop relatively large single colonies at the bottom of microplate wells. Consequently, photometric measurement is problematic and inferior to visual reading in these cases. When determined by spectrophotometry, the measured optical density values are not in the evaluable range and are generally too low (Table 5b). This does not apply to the visual readout method, with which (as described above) a good inhibitory effect of nystatin against the two arthropore-forming yeast species was observed.

In the spectrophotometric evaluation, the in vitro susceptibility of the reference strains from the DSMZ Braunschweig corresponded to that of the wild strains. C. parapsilosis and C. krusei: Spectrophotometric determination of the MICs for these strains was only possible after 48 hours of incubation (Table 5c). The spectrophotometrically determined MIC values for the other yeasts were generally comparable and, in isolated cases, slightly lower than the corresponding visual readings.

**Discussion**

Nystatin is a polypeptide antifungal agent which therefore belongs to the same group of antifungal substances as amphotericin B and natamycin. Structurally, nystatin belongs to the family of polycyclic macrolide antibiotics and was first successfully isolated in 1950 from the bacterium Streptomyces noursei. The drug has subsequently been available as a “classical” topical antifungal agent which is used to treat fungal diseases of the skin and mucous membranes. Nystatin is a yellowish powder which is practically insoluble in water but soluble in propylene glycol and dimethylformamide [5]. Nystatin undergoes slow degradation in aqueous suspensions. The drug is sensitive to light, heat, oxygen and pH shifts below pH 3 and above pH 9.

The mechanism of action of nystatin is based on binding of the polypeptide to sterols in the yeast plasma membrane resulting in a change in their permeability. Consequently, the fungal cells lose potassium, sugar and phosphate ions, which leads to the impairment of glycolysis and cellular respiration. Nystatin has a fungicidal effect but can also show fungicidal activity at high concentrations.

The primary activity of nystatin both in vitro as well as in vivo in patients involves mainly yeasts. C. albicans and other Candida species are significantly inhibited by nystatin. Nystatin is also known to have an inhibitory effect on some molds.

Nystatin is used for the topical treatment of cutaneous and mucosal fungal infections caused by C. albicans and other yeast species. Examples of these infections include cutaneous candidiasis involving intertriginous areas (e.g. groin or between fingers) and the free skin. Candida infections of the orointestinal tract, as well as infections of the mucous membranes of the genital tract caused by C. albicans and other Candida species, can be effectively treated using nystatin preparations [6].

Film-coated tablets for the treatment of orointestinal candidiasis contain, for example, 500,000 IU nystatin. One gram of nystatin oral gel contains, for example, 100,000 IU nystatin. Since nystatin is hardly absorbed from the gut it is not expected to cause systemic effects following oral administration.

Previously, it has been stated that no nystatin resistant yeasts exist, and in particular that there is no C. albicans which is resistant to nystatin.

In India, an in vitro study of antifungal drug susceptibility of Candida species from human immunodeficiency virus (HIV) positive and HIV negative patients with and without oropharyngeal candidiasis has recently been published [3]. Candida isolates from HIV negative
In vitro susceptibility testing of yeasts to nystatin – low minimum inhibitory concentrations suggest no indication of in vitro resistance of Candida albicans, Candida species or non-Candida yeast species to nystatin

### Table Sb. Minimum inhibitory concentrations (MIC) of nystatin against various yeast strains (Candida krusei, Candida tropicalis, Candida lusitaniae, Candida kefyr, Geotrichum candidum and Trichosporon mucoides) as determined by broth microdilution assay according to EUCAST. MIC values (double determination) were read after 24 and 48 hours of incubation at 36 °C. Spectrophotometric readings were taken at 450 nm. The cut-off was defined as an optical density (OD) value of 0.300. All OD values > 0.300 were interpreted as positive for yeast growth.

| Yeast strain | Nystatin MIC in IU/ml | Nystatin MIC in µg/ml |
|--------------|------------------------|-----------------------|
|              | Measurement: 24 hours  | Measurement: 48 hours |
| Candida krusei 703658/2015 | 7.4 | 7.4 |
| Candida krusei 113204/2015 | 7.4 | 7.4 |
| Candida tropicalis Ring Trial 09/2008 Strain 3 | 3.7 | 3.7 |
| Candida lusitaniae Ring Trial 1/2005 Strain 4 | 7.4 | 7.4 |
| Candida kefyr 803965/2015 | 3.7 | 1.85 |
| Geotrichum candidum 803545/2015 | Not evaluable | Not evaluable |
| Trichosporon mucoides 215610/2015 | Not evaluable | Not evaluable |
| Trichosporon mucoides 215470/2015 | Not evaluable | Not evaluable |
| MIC<sub>50</sub> | 3.7 | 7.4 |
| MIC<sub>90</sub> | 7.4 | 7.4 |

### Table Sc. Minimum inhibitory concentrations (MIC) of nystatin against various control strains (reference strains/collection strains of Candida albicans, Candida glabrata, Candida parapsilosis, Candida kruoei and Candida tropicalis) as determined by broth microdilution assay according to EUCAST. MIC values (double determination) were read after 24 and 48 hours of incubation at 36 °C. Spectrophotometric readings were taken at 450 nm. The cut-off was defined as an optical density (OD) value of 0.300. All OD values > 0.300 were interpreted as positive for yeast growth.

| Yeast strain | Nystatin MIC in IU/ml | Nystatin MIC in µg/ml |
|--------------|------------------------|-----------------------|
|              | Measurement: 24 hours  | Measurement: 48 hours |
| Candida albicans DSM 28719 | 3.7 | 3.7 |
| Candida glabrata DSM 28718 | 3.7 | 3.7 |
| Candida parapsilosis DSM 28722 | Not evaluable | Not evaluable |
| Candida krusei (Pichia kudriavzevii) DSM 28721 | Not evaluable | Not evaluable |
| Candida tropicalis DSM 28720 | 1.85 | 1.85 |
| MIC<sub>50</sub> | 3.7 | 3.7 |
| MIC<sub>90</sub> | 7.4 | 7.4 |

Patients were much more susceptible to antifungals when compared to those which were HIV positive. Candida species from HIV patients were susceptible to fluconazole in 86.1% of cases, and dose-dependent susceptible in 13.9%. Resistance to fluconazole was not found. HIV negative patients showed susceptibility, dose dependent susceptibility and resistance to fluconazole in 94.1%, 2.9% and 2.9% of isolates. For amphotericin B in HIV positive patients, 66.7% of Candida species were susceptible, and 22.2% resistant. HIV negative individuals showed 85.3% susceptibility to amphotericin B, 5.9% dose dependent susceptibility, and 8.8% resistance. In HIV positive patients 61.1% of Candida isolates were susceptible to nystatin, 36.1% were dose dependent susceptible, and 2.8% were resistant to nystatin. Otherwise, HIV negative individuals showed nystatin susceptibility in 91.2% of isolates, dose dependent susceptibility in 8.8%, and there were no nystatin resistant isolates.

It should be noted, however, that nystatin-resistant isolates of C. albicans have repeatedly been reported. In a recent study in Uganda, for example, nystatin resistance of C. albicans isolated from women with vulvovaginal candidiasis was observed in 0.61% of patients [7]. The fact that sensitivity testing in the Ugandan study was performed using a simple agar diffusion test can be regarded as a point of criticism. Conformity with recognized methods such as the EUCAST broth microdilution method was not evident.

Recently, Diaz et al. [8] determined the in vitro susceptibility of vaginal Candida isolates to fluconazole, clotrimazole and nystatin by M27-A3 microdilution method. Among the 145 isolates were 126 C. albicans, 16 C. glabrata, 2 C. parapsilosis, and one C. tropicalis. Five isolates of C. albicans and one isolate of C. tropicalis were in vitro resistant to fluconazole. Five C. glabrata and 1 C. tropicalis were in vitro resistant to clotrimazole.

The spectrum and in vitro antifungal susceptibility pattern of yeast isolates were investigated in Ethiopian HIV patients with oropharyngeal candidiasis [9]. The authors found that C. albicans was the most frequent species followed by C. glabrata, C. tropicalis, C. krusei, C. kefyr,
In vitro susceptibility testing of yeasts to nystatin – low minimum inhibitory concentrations suggest no indication of in vitro resistance of Candida albicans, Candida species or non-Candida yeast species to nystatin

Nenoff P (2016) In vitro susceptibility testing of yeasts to nystatin – low minimum inhibitory concentrations suggest no indication of in vitro resistance of Candida albicans, Candida species or non-Candida yeast species to nystatin

Cryptococcus laurentii, and Rhodotorula species. In total, 5.8%, 5.8%, 12.3%, 8.4%, and 0.6% of the isolates were resistant to amphotericin B, clotrimazole, fluconazole, ketoconazole, and miconazole, respectively. 1.3% isolates were resistant to nystatin. Interestingly, the resistance was higher for amphotericin B when compared with nystatin. Not surprisingly, the azole antifungals also exhibited higher resistance levels than nystatin.

In Iran, 120 clinical isolates of C. parapsilosis from blood stream infections were tested for in vitro resistance [10]. Only three (2.5%) C. parapsilosis strains were resistant to fluconazole, three (2.5%) were resistant to itraconazole, and two (1.7%) were amphotericin B resistant. In this study, nystatin was not investigated.

The present study found low MIC values for nystatin against yeasts such as C. albicans, for which the MIC of nystatin was in the range of 3.7 IU/ml (0.625 µg/mL, 24 hour spectrophotometric reading). The various other yeast species were also well inhibited by nystatin in vitro at comparable minimum inhibitory concentrations, which ranged from 1.85 to 7.4 IU/ml (0.3125 to 1.25 µg/mL) and were predominantly at a focal point of 3.7 IU/ml (0.625 µg/mL). Even slow-growing Candida species such as C. parapsilosis were also inhibited well by nystatin in vitro. C. glabrata and C. krusei, two species known to frequently develop resistance to fluconazole, and the latter which has intrinsic resistance to fluconazole, were also inhibited well by nystatin in vitro, as shown by the MIC values of 3.7 and 7.5 IU/ml (0.625 to 1.25 µg/mL), respectively.

The fact that nystatin was also shown to have very good in vitro activity against non-Candida species, such as Geotrichum candidum and Trichosporon mucoides, is worth mentioning. However, it was necessary to resort to visual read-out of growth inhibition test results as optical density measurement is error-prone due to the slow and inhomogeneous growth of these species.

**Conclusions**

In summary, nystatin exhibited very good in vitro activity against all of the tested yeast strains, C. albicans and non-Candida albicans species as well as Geotrichum candidum and Trichosporon mucoides, regardless of whether they were wild strains or control strains from culture collections. There was no evidence of in vitro resistance by C. albicans, other Candida species, or non-Candida yeast species to nystatin, despite the long time period in which it has been used as a topical antifungal agent.

**References**

1. Gonçalves SS, Souza AC, Chowdhary A, Meis JF (2016) Epidemiology and molecular mechanisms of antifungal resistance in Candida and Aspergillus. Mycoses. [Crossref]
2. Yenisehirli G, Bulut N, Yenisehirli A, Bulut Y (2015) In Vitro Susceptibilities of Candida albicans Isolates to Antifungal Agents in Tokat, Turkey. Jundishapur J Microbiol 8: e28057. [Crossref]
3. Dar MS, Sreedar G, Shukla A, Gupta P, Rehan AD, et al. (2015) An in vitro study of antifungal drug susceptibility of Candida species isolated from human immunodeficiency virus seropositive and human immunodeficiency virus seronegative individuals in Lucknow population Uttar Pradesh. J Oral Maxillofac Pathol 19: 205-11.
4. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope W (2012) EUCAST-AFST. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). Clin Microbiol Infect 18: E246-E247.
5. Seebacher C, Blaschke-Heilmessen R, (1990) Epidemiologic Diagnostisk Therapie. Gustav Fischer Verlag Jena 1990
6. Nenoff P, Krüger C, Pausch U, Ginter-Hanselmayer G (2015) Mycology- an update Part 3: Dermatomycoses: topical and systemic therapy. J Disch Dermatol Ges 13: 387-410. [Crossref]
7. Mukasa KJ, Herbert I, Daniel A, Sserunkuma KL, Joel B, et al. (2015) Antifungal Susceptibility Patterns of Vulvovaginal Candida species among Women Attending Antenatal Clinic at Mbarara Regional Referral Hospital, South Western Uganda. Br Microbiol Res J 5: 322-331
8. Díaz MC, Camponovo R, Araya I, Cerda A, Santander MP, et al. (2016) Denitification and in vitro antifungal susceptibility of vaginal Candida spp. isolates to fluconazole, clotrimazole and nystatin. Rev Exp Quimioter 29: 151-154.
9. Moges B, Bitew A, Shewaamare A (2016) Spectrum and the In Vitro Antifungal Susceptibility Pattern of Yeast Isolates in Ethiopian HIV Patients with Oropharyngeal Candidiasis. Int J Microbiol: 3037817. [Crossref]
10. Loffati E, Kordbacheh P, Mirhendi H, Zaini F, Ghajar A, et al. (2016) Antifungal Susceptibility Analysis of Clinical Isolates of Candida parapsilosis in Iran. Iran J Public Health 45: 322-328.