

Determination of Minimum Inhibitory Concentrations of Several Azole Antifungals for *Malassezia furfur*

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Background : There have been several reports which assessed the activity of antifungals including azoles on *Malassezia furfur* by agar dilution method. However, they did not differentiate *M. furfur* into groups. In addition, the media for growth and minimum inhibitory concentration (MIC) determination, incubation temperature and length of incubation differed from each other.

Objective : The aim of this study was to test the antifungal activities of miconazole, clotrimazole, ketoconazole and itraconazole by determining MICs for *M. furfur* serovars A, B and C for these drugs.

Methods : MICs were determined by the agar dilution method. Leeming & Notman's *Malassezia furfur* agar medium was used.

Results : In all strains of serovars A, B and C, the MICs for miconazole were similar to those for clotrimazole ; MICs for ketoconazole were also similar to those for itraconazole ; MICs for miconazole or clotrimazole were higher than those for ketoconazole or itraconazole.

Conclusion : The results suggested that ketoconazole or itraconazole could be used more effectively than miconazole or clotrimazole for the treatment of the diseases caused by *M. furfur*. (Ann Dermatol 8:(3)187~194, 1996).

Key Words : *M. furfur*, Serovars A, B and C, Azoles, MICs

Malassezia furfur (Robin) Baillon¹ is a dimorphic, lipophilic yeast and is a member of the normal cutaneous flora in humans. Historically, *M. furfur* was thought of as two separate organisms, a yeast and a mycelial fungus. The question of dimorphism was resolved by Dorn & Roehnert² and Nazzaro Porro *et al.*³. *M. furfur* has been the name formally accepted for both phases of growth, mycelial and yeast since 1986⁴. Using the medium assessed by Leeming & Notman⁵, Cunningham *et al.*⁶ placed *M. furfur* isolates into one of three cultural groups, which apparently corresponded to

three serological groups.

M. furfur is susceptible to a wide range of antifungal agents including azoles. Numerous methods have been devised to assess the activity and potential effectiveness of different antifungal drugs. Agar dilution MIC tests are similar to broth dilution tests except that the drug dilutions are incorporated into an agar medium. The test strains are spot-inoculated onto the surface of the medium. This is the preferred method for MIC determination when large numbers of strains have to be tested⁷.

The aim of this study is to test the antifungal activities of azoles, miconazole, clotrimazole, ketoconazole and itraconazole by determining MICs of *M. furfur* serovars A, B and C for these drugs. The *Malassezia* agar medium of Leeming & Notman was used.

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MATERIALS AND METHODS

1. Fungal strains.

Strains 21 HK ChL and 24 CC FL of *M. furfur* serovar A, 31 ML ChS and 34 CJ ChS of *M. furfur* serovar B and 19 BD ChS and 35 JR FS of *M. furfur* serovar C were used as fungal strains for the determination of MICs. They were obtained from the skin of healthy volunteers with no history of skin disease. *C. albicans*, strain Y16/1, was used as a sensitive control. *C. glabrata*, strain NCPF 39 31, was used as a resistant control.

2. Antifungal agents.

Miconazole and clotrimazole were obtained from Sigma Ltd. and ketoconazole and itraconazole were kindly provided by the Janssen Research Foundation.

3. Determination of MIC for antifungals for *M. furfur* serovars A, B and C.

The test was done in duplicate. *M. furfur* agar media containing antifungals, miconazole, clotrimazole, ketoconazole and itraconazole, were prepared in Petri dishes as follows. To each of 16 sterile Universal bottles (numbered 1 - 16), 2 ml of sterile distilled water was added. To bottle 1, 2 ml of the 1280 mg/litre antifungal solution dissolved in dimethylsulfoxide (DMSO) was added. The contents were mixed well and 2 ml was transferred to bottle 2. This serial dilution was repeated through to bottle 15. From bottle 15, 2 ml was discarded. Bottle 16 was a medium control. For preparation of the DMSO control, 2 ml of sterile distilled water was added to each of 4 sterile Universal bottles (numbered a, b, c and d). To bottle a, 2 ml of DMSO was added. The contents were mixed well and 2ml was transferred to bottle b. This serial dilution was repeated through to bottle d. Two ml was discarded from bottle d. Bottle d was renumbered as 17 (DMSO control). To each of bottles 5 - 17, 200 μ l of milk was added. Thirteen bottles of *M. furfur* agar media dispensed in 18 ml amounts were melted in a steam bath and placed in a 50 $^{\circ}$ C water bath. Each 18 ml of *M. furfur* agar media was added to the bottles 5 - 17. The contents of each was mixed well and poured into a 9 cm diameter of Petri dish (numbered 5 - 17). (Bottle 1 - 4 were not used, because prelimi-

nary experiments showed that *M. furfur* had not grown in the media containing antifungals at concentrations greater than 4 mg/litre, data not shown). Once the medium had solidified, the plates were stored at 4 $^{\circ}$ C overnight until use. At the time of use, the plates were dried for 30 minutes to remove condensation. Each strain was inoculated in 2 ml amounts of sterile distilled water with a sterile swab. The concentration of the suspension was adjusted to 1×10^8 cells/ml using a counting chamber. Two series of plates were inoculated with 20 μ l of the suspension of inoculum of each strain of *M. furfur* (1 week old) and control strains (2 days old) and incubated at 34 $^{\circ}$ C for 7 days, and the plates were examined daily. The MIC was defined as the lowest drug concentration at which there was definite inhibition of fungal growth after incubation.

RESULTS

The results of MICs of miconazole, clotrimazole, ketoconazole and itraconazole for *M. furfur* serovars A, B, and C are summarised in Tables 1 to 10.

The MIC for miconazole for serovar A, strain 21 HK ChL, obtained on Day 1 was 0.25 μ g/ml, which increased to 0.5 μ g/ml on Day 2 and to 1 μ g/ml on Day 5. For serovar A, strain 24 CC FL, the growth was delayed and the MIC obtained on Day 2 was 0.25 μ g/ml, which increased to 0.5 μ g/ml on Day 3 and to 1 μ g/ml on Day 5. For serovar B, strain 31 ML ChS, the growth was delayed and the MIC obtained on Day 2 was 2 μ g/ml, which increased to 4 μ g/ml on Day 3 and to > 4 μ g/ml on Day 5. For serovar B, strain 34 CJ ChS, the MIC obtained on Day 1 was also 2 μ g/ml, which increased to 4 μ g/ml on Day 3 and to > 4 μ g/ml on Day 5. For serovar C, strain 19 BD ChS, the growth was delayed but the MIC obtained on Day 3 was 2 μ g/ml, which had not increased by Day 5. For serovar C, strain 35 JR FS, the growth was also delayed and MIC obtained on Day 3 was 0.5 μ g/ml, which increased to 1 μ g/ml on Day 5 (Table 1).

The MIC for clotrimazole for serovar A, strain 21 HK ChL, obtained on Day 1 was 0.25 μ g/ml, which increased to 0.5 μ g/ml on Day 2 and to 1 μ g/ml on Day 5. For serovar A, strain 24 CC FL, the growth was delayed but the MIC obtained on

Day 2 was 1 µg/ml, which increased to 2 µg/ml on Day 5. For serovar B, strain 31 ML ChS, the growth was delayed and the MIC obtained on Day 2 was 1 µg/ml, which increased to 2 µg/ml on Day 4 and to 4 µg/ml on Day 5. For serovar B, strain 34 CJ ChS, MIC obtained on Day 1 was 4 µg/ml, which increased to > 4 µg/ml on Day 5. For serovar C, strain 19 BD ChS, the growth was delayed but the MIC obtained on Day 3 was 2 µg/ml, which had not increased by Day 5. For serovar C, strain 35 JR FS, the growth was also delayed and the MIC obtained on Day 3 was 0.5 µg/ml, which increased to 1 µg/ml on Day 5 and to 2 µg/ml on Day 7 (Table 2).

The MIC for ketoconazole for serovar A, strain 21 HK ChL, obtained on Day 1 was 0.0156 µg/ml, which increased to 0.0313 µg/ml on Day 2 but had not increased by Day 5. For serovar A, strain 24 CC FL, the growth was delayed and the MIC obtained on Day 2 was 0.0156 µg/ml, which in-

creased to 0.0313 µg/ml on Day 3 and had not increased by Day 5. For serovar B, strain 31 ML ChS, the growth was delayed, the MIC obtained on Day 2 was 0.0313 µg/ml, which increased to 0.0625 µg/ml on Day 3 and had not increased by Day 5. For serovar B, strain 34 CJ ChS, the MIC obtained on Day 1 was 0.0625 µg/ml, which had not increased by Day 5. For serovar C, strain 19 BD ChS, the growth was delayed but the MIC obtained on Day 3 was also 0.0625 µg/ml, which had not increased by Day 5. For serovar C, strain 35 JR FS, the growth was also delayed and the MIC obtained on Day 3 was 0.0313 µg/ml, which had not increased by Day 5 (Table 3).

MIC for itraconazole for serovar A, strain 21 HK ChL, obtained on Day 1 was 0.0313 µg/ml, which increased to 0.0625 µg/ml on Day 2 and to 0.125 µg/ml on Day 5. For serovar A, strain 24 CC FL, the growth was delayed and the MIC obtained on Day 2 was 0.0313 µg/ml, which in-

Table 1. MICs (µg/ml) for miconazole for *M. furfur*

| <i>M. furfur</i> | | Day1 | Day2 | Day 3 | Day 4 | Day 5** |
|------------------|-----------|--------|-------|-------|-------|---------|
| Serovar A | 21 HK ChL | 0.25 | 0.5 | 0.5 | 0.5 | 1 |
| Serovar A | 24 CC FL | N. O.* | 0.25 | 0.5 | 0.5 | 1 |
| Serovar B | 31 ML ChS | N. O. | 2 | 4 | 4 | > 4 |
| Serovar B | 34 CJ ChS | 2 | 2 | 4 | 4 | > 4 |
| Serovar C | 19 BD ChS | N. O. | N. O. | 2 | 2 | 2 |
| Serovar C | 35 JR FS | N. O. | N. O. | 0.5 | 0.5 | 1 |

* N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7.

Table 2. MICs (µg/ml) for clotrimazole for *M. furfur*

| <i>M. furfur</i> | | Day1 | Day2 | Day 3 | Day 4 | Day 5** |
|------------------|-----------|--------|-------|-------|-------|---------|
| Serovar A | 21 HK ChL | 0.25 | 0.5 | 0.5 | 0.5 | 1 |
| Serovar A | 24 CC FL | N. O.* | 1 | 1 | 1 | 2 |
| Serovar B | 31 ML ChS | N. O. | 1 | 1 | 2 | 4 |
| Serovar B | 34 CJ ChS | 4 | 4 | 4 | 4 | > 4 |
| Serovar C | 19 BD ChS | N. O. | N. O. | 2 | 2 | 2 |
| Serovar C | 35 JR FS | N. O. | N. O. | 0.5 | 0.5 | 1 |

* N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7 except for serovar C strain 35 JR FS which increased to 2 µg/ml on Day 7.

Table 3. MICs ($\mu\text{g/ml}$) for ketoconazole for *M. furfur*

| <i>M. furfur</i> | | Day1 | Day2 | Day 3 | Day 4 | Day 5** |
|------------------|-----------|--------|--------|--------|--------|---------|
| Serovar A | 21 HK ChL | 0.0156 | 0.0313 | 0.0313 | 0.0313 | 0.0313 |
| Serovar A | 24 CC FL | N. O.* | 0.0156 | 0.0313 | 0.0313 | 0.0313 |
| Serovar B | 31 ML ChS | N. O. | 0.0313 | 0.0625 | 0.0625 | 0.0625 |
| Serovar B | 34 CJ ChS | 0.0625 | 0.0625 | 0.0625 | 0.0625 | 0.0625 |
| Serovar C | 19 BD ChS | N. O. | N. O. | 0.0625 | 0.0625 | 0.0625 |
| Serovar C | 35 JR FS | N. O. | N. O. | 0.0313 | 0.0313 | 0.0313 |

* N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7.

Table 4. MICs ($\mu\text{g/ml}$) for itraconazole for *M. furfur*

| <i>M. furfur</i> | | Day1 | Day2 | Day 3 | Day 4 | Day 5** |
|------------------|-----------|--------|--------|--------|--------|---------|
| Serovar A | 21 HK ChL | 0.0313 | 0.0625 | 0.0625 | 0.0625 | 0.125 |
| Serovar A | 24 CC FL | N. O.* | 0.0313 | 0.0313 | 0.0313 | 0.0625 |
| Serovar B | 31 ML ChS | N. O. | 0.0313 | 0.0313 | 0.0313 | 0.0313 |
| Serovar B | 34 CJ ChS | 0.0625 | 0.0625 | 0.0625 | 0.0625 | 0.0625 |
| Serovar C | 19 BD ChS | N. O. | N. O. | 0.0625 | 0.0625 | 0.125 |
| Serovar C | 35 JR FS | N. O. | N. O. | 0.0156 | 0.0156 | 0.0156 |

* N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7.

creased to 0.0625 $\mu\text{g/ml}$ on Day 5. For serovar B, strain 31 ML ChS, the growth was delayed, The MIC obtained on Day 2 were 0.0313 $\mu\text{g/ml}$, which had not increased by Day 5. For serovar B, strain 34 CJ ChS, the MIC obtained on Day 1 were 0.0625 $\mu\text{g/ml}$, which had not increased by Day 5. For serovar C, strain 19 BD ChS, the growth was delayed and MIC obtained on Day 3 was 0.0625 $\mu\text{g/ml}$, which increased to 0.125 $\mu\text{g/ml}$ on Day 5. For serovar C, strain 35 JR FS, the growth was also delayed and the MIC obtained on Day 3 was 0.0156 $\mu\text{g/ml}$, which had not increased by Day 5 (Table 4).

The MIC for sensitive control, *C. albicans* strain Y16/1, is summarised in table 11. This strain was sensitive to miconazole, clotrimazole and ketoconazole until Day 3 (0.25 $\mu\text{g/ml}$ < MIC < 4 $\mu\text{g/ml}$), but became resistant to those antifungals at all concentrations used from Day 4 (MIC > 4 $\mu\text{g/ml}$). This strain, however, was resistant to itraconazole at all concentrations used (MIC > 4 $\mu\text{g/ml}$) from Day 1. The resistant control, *C. glabrata*

was resistant to all antifungals at all concentrations used (MIC > 4 $\mu\text{g/ml}$).

DISCUSSION

There have been several reports which assessed the activity of antifungals including azoles on *Malassezia* by agar dilution method^{8,9,10}. However, they did not differentiate *M. furfur* into groups. In addition, the media for growth and minimum inhibitory concentration (MIC) determination, incubation temperature and length of incubation were different from each other.

Leeming & Notman⁵ assessed several media for their ability to recover *M. furfur* from normal skin. The final medium which incorporated ox bile, glycerol, glycerol monostearate, Tween 60 and cow whole fat milk yielded *M. furfur* recovery comparable with microscopic counts. Using this medium, Cunningham *et al*⁶ placed *M. furfur* isolates into one of three cultural groups. Strains of *Malassezia furfur* from clinically normal skin could

Table 5. MICs ($\mu\text{g/ml}$) for *M. furfur* strain 21 HK ChL (Serovar A) to various antifungals

| Antifungals | Day 1 | Day 2 | Day 3 | Day 4 | Day 5** |
|--------------|--------|--------|--------|--------|---------|
| Miconazole | 0.25 | 0.5 | 0.5 | 0.5 | 1 |
| Clotrimazole | 0.25 | 0.5 | 0.5 | 0.5 | 1 |
| Ketoconazole | 0.0156 | 0.0313 | 0.0313 | 0.0313 | 0.0313 |
| Itraconazole | 0.0313 | 0.0625 | 0.0625 | 0.0625 | 0.125 |

** MICs had not changed by Day 7.

Table 6. MICs ($\mu\text{g/ml}$) for *M. furfur* strain 24 CC FL (Serovar A) to various antifungals

| Antifungals | Day 1 | Day 2 | Day 3 | Day 4 | Day 5** |
|--------------|-------|--------|--------|--------|---------|
| Miconazole | N.O.* | 0.25 | 0.5 | 0.5 | 1 |
| Clotrimazole | N.O. | 1 | 1 | 1 | 2 |
| Ketoconazole | N.O. | 0.0156 | 0.0313 | 0.0313 | 0.0313 |
| Itraconazole | N.O. | 0.0313 | 0.0313 | 0.0313 | 0.0625 |

* N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7.

Table 7. MICs ($\mu\text{g/ml}$) for *M. furfur* strain 31 ML ChS (Serovar B) to various antifungals

| Antifungals | Day 1 | Day 2 | Day 3 | Day 4 | Day 5** |
|--------------|-------|--------|--------|--------|---------|
| Miconazole | N.O. | 2 | 4 | 4 | >4 |
| Clotrimazole | N.O. | 1 | 1 | 2 | 4 |
| Ketoconazole | N.O. | 0.0313 | 0.0625 | 0.0625 | 0.0625 |
| Itraconazole | N.O. | 0.0313 | 0.0313 | 0.0313 | 0.0313 |

* N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7.

be divided into three serological groups (serovars) identified by surface antigens, which corresponded to groupings based on their in vitro cultural characteristics. The serological and morphological groupings showed a remarkable degree of coincidence; three main serological groups apparently corresponding to the morphological groups A, B and C. Group A strains have round cells; large (~5mm), circular, cream coloured, raised, smooth, dentate colonies, are most commonly isolated from the back and chest, and grow well in vitro. Group B cells are also round: typical colonies are circular, medium sized (2-3mm), lighter in colour,

flat with a pointed button centre, friable and crenated. Clearing of the medium is generally observed around their colonies. Group B strains are most commonly isolated from the head region and are more fastidious than group A strains when cultured in vitro. It is important to note that group A and group B strains can not be differentiated microscopically. Group C strains have oval cells, their colonies being medium sized (2-3mm), circular, umbonated and entire. These vary in appearance from dull to glistening and are generally surrounded by an opalescent precipitate in the medium. The isolation and cultivation of group C

Table 8. MICs ($\mu\text{g/ml}$) for *M. furfur* strain 34 CJ ChS (Serovar B) to various antifungals

| Antifungals | Day 1 | Day 2 | Day 3 | Day 4 | Day 5** |
|--------------|--------|--------|--------|--------|---------|
| Miconazole | 2 | 2 | 4 | 4 | >4 |
| Clotrimazole | 4 | 4 | 4 | 4 | >4 |
| Ketoconazole | 0.0625 | 0.0625 | 0.0625 | 0.0625 | 0.0625 |
| Itraconazole | 0.0625 | 0.0625 | 0.0625 | 0.0625 | 0.125 |

** MICs had not changed by Day 7.

Table 9. MICs ($\mu\text{g/ml}$) for *M. furfur* strain 19 BD ChS (Serovar C) to various antifungals

| Antifungals | Day 1 | Day 2 | Day 3 | Day 4 | Day 5** |
|--------------|-------|-------|--------|--------|---------|
| Miconazole | N.O.* | N.O. | 2 | 2 | 2 |
| Clotrimazole | N.O. | N.O. | 2 | 2 | 2 |
| Ketoconazole | N.O. | N.O. | 0.0625 | 0.0625 | 0.0625 |
| Itraconazole | N.O. | N.O. | 0.0625 | 0.0625 | 0.125 |

** N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7.

is similar to that of group B strains. Ashbee *et al.*¹¹ measured population densities of *M. furfur* serovars A, B and C in patients with pityriasis versicolor, seborrheic dermatitis and controls and found serovar A to be the predominant isolate on the chest and back, while there was no difference in the distribution of serovars on the forehead and cheeks.

The MIC was defined as the lowest drug concentration at which there was no visible fungal growth after incubation. Growth must be present in the medium control⁷. This definition was often difficult to apply when the inoculum concentration 1×10^8 cells/ml was used. There were often gradual diminutions of fungal growth, making end-point interpretations difficult. However, careful observation found definite inhibition of fungal growth at a certain concentration which was regarded as the MIC. Therefore, the MIC could be defined as the lowest drug concentration at which there was definite inhibition of fungal growth after incubation. Faergemann⁸ used the inoculum concentration 1×10^8 cells/ml and defined the MIC endpoint as the lowest concentration of drug that inhibited growth.

Leeming & Notman⁵ found 34°C yielded high recovery rates from the samples at both forehead

and back, although optimum temperatures varied according to sites. They also noted that the finding was in contrast with earlier standard descriptions of growth temperature optima in the range of 35°C to 37°C. Faergemann⁸ incubated the inoculated plates at 37°C and read after 1, 2, 3 and 4 days of growth. Marcon *et al.*⁹ used 30°C as the incubation temperature.

Warnock⁷ suggested that MIC tests with azole compounds presented a number of problems. These drugs often cause gradual diminution of fungal growth with increasing concentration, making end-point interpretation difficult. The MIC obtained often depended on the conditions of the test, with the concentration of the fungal inoculum, the composition and pH of the medium and the temperature and length of incubation all having a marked effect on the result. This means that MICs for responsive fungi are often higher than the levels of drug attainable in blood. With the results of preliminary experiments for the effect of inoculum concentration and temperature of incubation on MICs, the optimised inoculum concentration 1×10^8 cells/ml and incubation temperature 34°C was used for the determination of MICs for miconazole, clotrimazole, ketoconazole and itraconazole for *M. furfur*. The inoculum

Table 10. MICs ($\mu\text{g/ml}$) for *M. furfur* strain 35 JR FS (Serovar C) to various antifungals

| Antifungals | Day 1 | Day 2 | Day 3 | Day 4 | Day 5** |
|--------------|-------|-------|--------|--------|---------|
| Miconazole | N.O.* | N.O. | 0.5 | 0.5 | 1 |
| Clotrimazole | N.O. | N.O. | 0.5 | 0.5 | 1 |
| Ketoconazole | N.O. | N.O. | 0.0313 | 0.0313 | 0.0313 |
| Itraconazole | N.O. | N.O. | 0.0156 | 0.0156 | 0.0156 |

** N. O. means MICs were not obtainable because there was no definite growth of *M. furfur* on all plates including medium controls.

** MICs had not changed by Day 7 except in clotrimazole which increased to 2 $\mu\text{g/ml}$ on Day 7.

Table 11. MICs ($\mu\text{g/ml}$) for *C. albicans* strain Y16/1 to various antifungals

| Antifungals | Day 1 | Day 2 | Day 3 | Day 4 | Day 5** |
|--------------|-------|-------|-------|-------|---------|
| Miconazole | 0.5 | 2 | 4 | >4 | >4 |
| Clotrimazole | 0.25 | 1 | 2 | >4 | >4 |
| Ketoconazole | 0.5 | 0.5 | 1 | >4 | >4 |
| Itraconazole | >4 | >4 | >4 | >4 | >4 |

** MICs had not changed by Day 7.

concentration used, $1 \times 10^6/\text{ml}$ which was the concentration suggested by Warnock, seemed to be small for *M. furfur*. The preliminary studies showed that there was very little change of MICs after Day 5.

The MICs for miconazole for *M. furfur* were quite variable. MICs for *M. furfur* serovar A and serovar C, strain 35 JR FS, were 0.5 $\mu\text{g/ml}$ on Day 3, which is similar to the results of Marcon *et al*⁹, 0.4 to 0.8 $\mu\text{g/ml}$. However, MICs for *M. furfur* serovar B and serovar C, strain 19 BD ChS, were 2 or 4 $\mu\text{g/ml}$ on Day 3 (Table 1). The MICs for clotrimazole for *M. furfur* were also variable. MICs for *M. furfur* serovar A, serovar B, strain 31 ML ChS, and serovar C, strain 35 JR FS, were 0.5 or 1 $\mu\text{g/ml}$ on Day 3. However, MICs for *M. furfur* serovar B, strain 34 CJ ChS, and serovar C, strain 19 BD ChS, were 2 or 4 $\mu\text{g/ml}$ on Day 3 (Table 2). The MICs for ketoconazole for *M. furfur* strains were 0.0313 or 0.0625 $\mu\text{g/ml}$ on Day 3, which had not changed by Day 5 (Table 3). These values for ketoconazole were similar to the results of Faergemann⁸, 0.02 to 0.05 $\mu\text{g/ml}$, and Marcon *et al*⁹, 0.025 to 0.05 $\mu\text{g/ml}$. The MICs for itraconazole for *M. furfur* strains were between 0.0156 and 0.0625 $\mu\text{g/ml}$ on Day 3. On Day 5, the MICs of some strains had doubled, while the others re-

mained at the same level. MICs for the other strain of serovar A and one strain of serovar B reached to 0.0625 $\mu\text{g/ml}$. MICs reached 0.125 $\mu\text{g/ml}$ in one of each strain of serovar A and C. The MICs for the other two strains remained at the original level, 0.0156 or 0.0313 $\mu\text{g/ml}$ (Table 4). These values for itraconazole were similar or lower than those of Faergemann⁸, 0.1 to 0.2 $\mu\text{g/ml}$. MICs for *M. furfur* for itraconazole were probably complicated by the inclusion of lipid in the growth medium, required by the organisms' lipophilic nature, because itraconazole itself is lipophilic. However, the results of the study showed that the effect of lipid inclusion did not seem to affect seriously the measurement of MICs.

In all strains of serovars A, B and C, the MICs for miconazole were similar to those for clotrimazole; MICs for ketoconazole were also similar to those for itraconazole; MICs for miconazole or clotrimazole were higher than those for ketoconazole or itraconazole (Table 5 - 10). These findings suggested that ketoconazole or itraconazole could be used more effectively than miconazole or clotrimazole for the treatment of the diseases caused by *M. furfur*.

The growth of some strains of *M. furfur* was delayed and it was not until Day 3 that all strains

grew on medium controls and agar media containing antifungals below the level of MIC. MICs for such strains obtained later due to delayed growth were usually the same or very similar to those for fast growing strains and placed within two fold dilution in ketoconazole and itraconazole. In miconazole and clotrimazole, MICs for some strains of serovars B and C were much higher than those for serovar A regardless of the growth rate. With inoculum concentration 1×10^8 cells/ml, significant MICs for serovars A, B and C could be obtained although the growth was delayed by Day 3.

The antifungals were dissolved in DMSO solution and the growth pattern was observed on the DMSO control. The pattern observed was almost the same as on the medium control. Therefore DMSO did not seem to cause any significant effect on the growth of *M. furfur*.

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