

In vitro susceptibility of the seven *Malassezia* species to ketoconazole, voriconazole, itraconazole and terbinafine

A.K.GUPTA, Y.KOHLI,* A.LI,* J.FAERGEMANN† AND R.C.SUMMERBELL*‡

Division of Dermatology, Department of Medicine, Sunnybrook and Women's College Health Sciences Center (Sunnybrook Site) and the University of Toronto, Toronto, Canada

*Medical Mycology Laboratory, Laboratory Services Branch, Ontario Ministry of Health, Toronto, Ontario, Canada

†Department of Dermatology and Venereology, Göteborg University, Göteborg, Sweden

‡Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

Accepted for publication 30 September 1999

Summary

Fifty-five strains, either authentic or ex-type, of seven *Malassezia* species were investigated for *in vitro* susceptibility to various concentrations (0.03–64.0 µg/mL) of three azole drugs, ketoconazole, voriconazole and itraconazole, as well as the allylamine terbinafine, using the agar dilution method. All strains of the seven *Malassezia* species were susceptible to the three azole drugs at low concentrations. *M. furfur*, *M. sympodialis*, *M. slooffiae*, *M. pachydermatis*, *M. globosa*, *M. obtusa* and *M. restricta* were most sensitive to ketoconazole and itraconazole, with minimum inhibitory concentrations (MICs) ranging from ≤ 0.03 to 0.125 µg/mL. The recently introduced antifungal, voriconazole, was also very effective, with MIC₈₀ values ≤ 0.03 µg/mL for 80% of strains. MICs of terbinafine against the seven *Malassezia* species ranged from ≤ 0.03 to 64.0 µg/mL. There were variations in susceptibility of the seven *Malassezia* species to ketoconazole, voriconazole, itraconazole and terbinafine. Strains of *M. furfur*, *M. globosa* and *M. obtusa* were more tolerant to terbinafine than the remaining *Malassezia* species; *M. sympodialis* was highly susceptible. *M. furfur* strains tested with terbinafine ranged from highly susceptible to relatively resistant. Correct identification of *Malassezia* species could facilitate selection of appropriate antifungal therapy.

Key words: azoles, *in vitro* susceptibility, itraconazole, ketoconazole, *Malassezia*, terbinafine, voriconazole

Yeasts of the genus *Malassezia* are part of the normal mycota of the skin of humans and other warm-blooded animals.¹ *Malassezia* yeasts may also have a role in pityriasis (tinea) versicolor,² seborrhoeic dermatitis,^{3,4} seborrhoeic blepharitis,⁵ dandruff,⁴ 'Malassezia folliculitis',⁶ confluent and reticulate papillomatosis⁷ and, uncommonly, systemic infections. In the last category, *Malassezia* species may be associated with catheter-acquired sepsis, fungaemia and pulmonary infection in neonates; these infections can sometimes be life-threatening.^{8–12} *Malassezia* species, among several other fungi of emerging importance, have been reported as significant causes of fungal infections in patients with cancer^{13,14} and AIDS.¹⁵

Pityriasis (tinea) versicolor was possibly first reported as a cutaneous disease in 1846.¹⁶ In 1873, Rivolta¹⁷

observed the presence of yeasts in skin. Early investigators such as Malassez¹⁸ and Bizzozero¹⁹ observed the diversity and variation in morphology within the genus *Malassezia*. *M. furfur* (Robin) Baillon was the first species identified within the genus.²⁰ The later name *Pityrosporum ovale* (Bizzozero), cited in Castellani and Chalmers,²¹ is considered to be a synonym for the same organism.²² Prior to 1996, only three species were accepted within the genus *Malassezia*. Besides *M. furfur*, the other two species described were *M. pachydermatis* (Weidman) Dodge (= *Pityrosporum canis*)^{22,23} and *M. sympodialis*, cited in Simmons and Guého.²⁴ In 1990, Cunningham *et al.*²⁵ recognized three subdivisions within the lipid-dependent *Malassezia* species, and named them serovars A, B and C on the basis of cultural characteristics and serological differences in cell-surface antigens. Recently, the genus *Malassezia* has been revised based on morphological, ultrastructural, physiological and molecular observations.²² As a

Correspondence: Dr Aditya Gupta, 490 Wonderland Road South, Suite 6, London, Ontario N6K 1L6, Canada. E-mail: agupta@execulink.com

Table 1. Source and origin/location of 55 *Malassezia* strains used for drug sensitivity testing

Identification no.	<i>Malassezia</i> species	Source	Origin/location	Original substrate
ATCC 14522	<i>M. furfur</i>	ATCC ^a	— ^h	—
ATCC 96811		ATCC	—	—
CBS 1878 ⁱ		CBS ^c	—	Dandruff
GM 551		G.Midgley ^b	London, U.K.	—
JF 01		J.Faergemann ^d	Sweden	—
JF 02		J.Faergemann	Sweden	—
JF 03		J.Faergemann	Sweden	—
JF 04		J.Faergemann	Sweden	—
96FR 1991		Mycology, MOH ^e	Toronto, ON, Canada	Catheter tip longline
97F 661		Mycology, MOH	London, ON, Canada	Toenail swab
97F 6726		Mycology, MOH	Cambridge, ON, Canada	Chest
97F 8817		Mycology, MOH	Toronto, ON, Canada	Left arm
97F 9089		Mycology, MOH	Scarborough, ON, Canada	Chest
97F 9734		Mycology, MOH	Toronto, ON, Canada	Fingernail
97FR 1272		Mycology, MOH	Toronto, ON, Canada	Blood culture
97FR 2125		Mycology, MOH	London, ON, Canada	Skin from chest
97FR 3007		Mycology, MOH	Windsor, ON, Canada	Bronchial wash
97FR 3926		Mycology, MOH	—	—
97SF 5952		Mycology, MOH	Brantford, ON, Canada	Knee tissue
98F 5399 ⁱ		Mycology, MOH	Toronto, ON, Canada	Trunk
JF 05 ⁱ		J.Faergemann	Sweden	—
ATCC 96803		ATCC	—	—
CBS 7222 ⁱ		CBS	Atlanta, GA, U.S.A.	Ear
GM 323 ⁱ		G.Midgley	London, U.K.	—
96FR 1602		Mycology, MOH	Willowdale, ON, Canada	Blood
97FR 2426		Mycology, MOH	London, ON, Canada	Skin
97FR 2699		Mycology, MOH	London, ON, Canada	Abdomen
97F 8615		Mycology, MOH	Toronto, ON, Canada	Upper chest
97F 8753		Mycology, MOH	Toronto, ON, Canada	Trunk
98F 8139 ⁱ		Mycology, MOH	Toronto, ON, Canada	Upper back
CBS 7956		CBS	France	Healthy ear of pig
GM 150		G.Midgley	London, U.K.	—
JF 06		J.Faergemann	Sweden	—
TV1 ⁱ		WT ^f	Hong Kong	Upper back
SF3 ⁱ		N.Morar ^g	South Africa	Back
98F 4721 ⁱ		Mycology, MOH	Mount Forest, ON, Canada	Scalp
98F 6419 ⁱ		Mycology, MOH	Etobicoke, ON, Canada	Groin
HK10 ⁱ	<i>M. globosa</i>	WT	Hong Kong	Chest
SF7 ⁱ		N.Morar	South Africa	Arms
SF14 ⁱ		N.Morar	South Africa	Chest
98F 3552 ⁱ		Mycology, MOH	Etobicoke, ON, Canada	Neck
98F 3715 ⁱ		Mycology, MOH	Etobicoke, ON, Canada	Chest
98F 4888 ⁱ		Mycology, MOH	Toronto, ON, Canada	Back
98F 6443 ⁱ		Mycology, MOH	Toronto, ON, Canada	Body
ATCC 14521	<i>M. pachydermatis</i>	ATCC	—	—
CBS 1879		CBS	Sweden	Ear of a dog
GM 420		G.Midgley	London, U.K.	—
96FR 1502	<i>M. restricta</i>	Mycology, MOH	London, ON, Canada	Fingernail
CBS 7877 ⁱ		CBS	Bristol, U.K.	Healthy skin
YKM 8 ⁱ		Mycology, MOH	Etobicoke, ON, Canada	Forehead
YKM 32 ⁱ		Mycology, MOH	Etobicoke, ON, Canada	Scalp
YKM 57 ⁱ	<i>M. obtusa</i>	Mycology, MOH	London, ON, Canada	Forehead
98F 3529 ⁱ		Mycology, MOH	Etobicoke, ON, Canada	Neck
98F 8316 ⁱ		Mycology, MOH	Toronto, ON, Canada	Fingernail
SF5 ⁱ		N.Morar	South Africa	Neck

^a American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A.; ^b strains obtained as a gift from Dr Gillian Midgley, Department of Medical Mycology, St John's Institute of Dermatology, London, U.K.; ^c cultures bought from Centraalbureau voor Schimmelcultures, the Netherlands; ^d strains obtained as a gift from Dr Jan Faergemann, Department of Dermatology and Venereology, Göteborg University, Göteborg, Sweden; ^e Medical Mycology Laboratory, Laboratory Services Branch, Ontario Ministry of Health, Etobicoke, Ontario, Canada; ^f skin scrapings from patients with pityriasis versicolor provided by a physician in Hong Kong; ^g skin scrapings from patients with pityriasis versicolor provided by a physician in South Africa; ^h missing information is represented by '—'; ⁱ strains for which antifungal susceptibility tests were performed on Leeming–Notman agar; ^j strains for which antifungal susceptibility tests were performed on both Diagnostic Sensitivity Testing medium and Leeming–Notman agar medium.

result, the genus has been enlarged taxonomically to include seven species comprising the three former species and four additional species, *M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae*. Four recently described species of *Malassezia*, which are significantly different in their physiological and biochemical features from the three previously described species,^{23,26,27} may also differ in their responses to antifungal therapy.

Recently, *in vitro* antifungal susceptibility testing of yeasts has been standardized using RPMI-1640 based macro- and microdilution methods (NCCLS-M27A²⁸). However, the standardized RPMI-1640 medium does not support growth of the *Malassezia* yeast cells because it lacks the specific lipids they require. A number of previous studies used both agar- and broth-dilution approaches to investigate *in vitro* susceptibility of *M. furfur* and *M. pachydermatis* isolates to several azole antifungal compounds such as bifonazole,^{29–31} climbazole,³⁰ clotrimazole,^{30,31} ketoconazole,^{30,31} miconazole,³¹ flutrimazole³¹ and sertaconazole.^{31,32} Recently, Leeming *et al.*³³ reported on the susceptibility of *Malassezia* species to terbinafine. To our knowledge, the present study is the first to investigate and compare the interspecies variations in *in vitro* antifungal susceptibility of the seven currently recognized *Malassezia* species to ketoconazole, itraconazole, terbinafine and the new antifungal compound voriconazole.

Materials and methods

Samples and growth conditions

Fifty-five strains of *Malassezia* representing seven species were examined for their *in vitro* susceptibility to ketoconazole, voriconazole, itraconazole and terbinafine. Table 1 summarizes the information about the source, origin/location and original substrate of all the strains used in this study. Thirty-six strains were obtained from clinical specimens sent to Toronto Public Health Laboratory for isolation or identification. Identification of different *Malassezia* species was done on the basis of macro- and microscopic features as well as physiological characteristics as described by Guého *et al.*²² and Guillot *et al.*²⁷ Strains of *M. furfur*, *M. sympodialis* and *M. slooffiae* were cultured and maintained on modified (supplemented with 2.5 mL/L glycerol) Diagnostic Sensitivity Testing (DST;²⁹ Oxoid, U.K.) agar medium at 35°C. This modified DST medium was not suitable for three recently described fastidious species, *M. globosa*, *M. obtusa* and *M. restricta*, which were cultured and maintained on Leeming–Notman³⁴

agar (LNA) medium at 32°C. *M. pachydermatis*, which lacks the other species' requirement for exogenous lipids, was maintained on plain Sabouraud agar medium at 35°C. Some strains of *M. furfur*, *M. sympodialis* and *M. slooffiae* were also maintained on LNA for comparative studies (see below). Drug testing for each species was performed on the same medium on which it was stored.

Drug dilution

Voriconazole was generously supplied by the Central Research Division, Pfizer Pharmaceutical Inc., Groton, CT, U.S.A. Voriconazole stock solution was prepared in dimethyl sulphoxide (DMSO; Sigma, St Louis, MO, U.S.A.) at a final concentration of 5120 µg/mL. Ketoconazole and itraconazole were kindly provided by Janssen Pharmaceuticals Corporation (North York, Ontario, Canada). Ketoconazole stock solution was prepared in DMSO at 5120 µg/mL and itraconazole stock was prepared in polyethylene glycol (PEG-200; Sigma). Terbinafine was generously supplied by Novartis Canada Inc. (Dorval, Quebec, Canada). Terbinafine stock solution was prepared in sterile double-distilled water at 5120 µg/mL. All drug stocks were stored in the dark at –70°C. Drug dilutions were initially made in 100 µL of DST, Leeming–Notman or Sabouraud broth with doubling dilution concentrations ranging from 0.3 to 640 µg/mL. To each diluted drug solution, 900 µL of melted agar was added (the agar medium added was the same type as the broth), resulting in the final test concentrations ranging from 0.03 to 64.0 µg/mL.

Yeast inoculation

Malassezia yeast suspensions were prepared in broth. The type of broth used for each strain corresponded to the growth medium, as described above. The broth medium was used only to prepare yeast suspensions for inoculation; all isolates prior to inoculation were grown and maintained on agar media as indicated previously. Yeast concentrations were adjusted to 1×10^4 cells/mL. Fifty microlitres of the yeast suspension were inoculated on to the surface of each agar medium. Incubation was carried out in the dark at 35°C for *M. furfur*, *M. sympodialis*, *M. slooffiae* and *M. pachydermatis* and at 32°C for *M. globosa*, *M. obtusa* and *M. restricta*. Midgley³⁵ recommended the latter temperature for optimal growth of these three species. A drug-free

Table 2. Minimum inhibitory concentrations (MICs^a) of ketoconazole, voriconazole, itraconazole and terbinafine to seven *Malassezia* species

<i>Malassezia</i> spp.	No. strains	Ketoconazole (µg/mL)	Voriconazole (µg/mL)	Itraconazole (µg/mL)	Terbinafine (µg/mL)
<i>M. furfur</i>	20	≤ 0.03 ^b (18) ^c 0.125 (2)	≤ 0.03 (16) 0.06 (3) 0.125 (1)	≤ 0.03 (19) 0.125 (1)	≤ 0.03 (3) 0.5 (1) 1.0 (1) 4.0 (2) 8.0 (4) 16.0 (7) 32.0 (2)
<i>M. sympodialis</i>	10	≤ 0.03 (10)	≤ 0.03 (9) 0.125 (1)	≤ 0.03 (10)	≤ 0.03 (7) 0.125 (1) 0.25 (2)
<i>M. slooffiae</i>	7	≤ 0.03 (7)	≤ 0.03 (6) 0.25 (1)	≤ 0.03 (7)	≤ 0.03 (3) 0.125 (1) 2.0 (2) 8.0 (1)
<i>M. globosa</i>	7	≤ 0.03 (6) 0.125 (1)	≤ 0.03 (7)	≤ 0.03 (5) 0.06 (1) 0.125 (1)	0.06 (2) 0.25 (1) 4.0 (1) 8.0 (2) 16.0 (1)
<i>M. restricta</i>	4	≤ 0.03 (4)	≤ 0.03 (4)	≤ 0.03 (4)	0.06 (2) 0.5 (1) 4.0 (1)
<i>M. pachydermatis</i>	4	≤ 0.03 (4)	≤ 0.03 (1) 0.125 (1) 0.25 (1) Not tested (1)	≤ 0.03 (4)	≤ 0.03 (4)
<i>M. obtusa</i>	3	≤ 0.03 (3)	≤ 0.03 (1) Not tested (2)	≤ 0.03 (3)	0.125 (1) 16.0 (1) 64.0 (1)

^a All the MIC values were recorded after 7 days of incubation. For ketoconazole, voriconazole and itraconazole, the MIC values were interpreted on the basis of 80% growth inhibition standard;²⁸ for terbinafine, the MIC values were read as the lowest drug concentration that prevented any discernible growth. ^b MIC value ≤ 0.03 µg/mL represents no growth observed at the lowest concentration of the drug tested, i.e. 0.03 µg/mL; ^c numbers in parentheses represent number of strains of a particular species with indicated MIC value.

positive growth control was set up for each strain on the appropriate medium.

Criteria for determining minimum inhibitory concentrations

For ketoconazole, voriconazole and itraconazole, all azole antifungals potentially generating trailing end-points, an 80% growth inhibition standard was used as recommended by Galigiani *et al.*²⁸ for reading and interpretation of minimum inhibitory concentration (MIC) results: the MIC₈₀. For terbinafine, an allylamine antifungal with a sharply defined end-point, the MIC was read as the lowest drug concentration that prevented any discernible growth.

Growth of each strain on various concentrations of all four drugs was recorded every 24 h for 7 days and the final MIC reading was recorded at day 7 after inoculation. The degree of yeast growth was always determined in comparison with growth in the drug-free

control according to the following scale: 0 growth, no visible yeast colony on the agar medium; 1 + growth, 25% growth in comparison with control; 2 + growth, 50% of control growth; 3 + growth, 75% of control growth; and 4 + growth, growth similar to that of the control.

Comparative testing of MICs on DST and LNA media was also performed. To test for reproducibility of MICs on different media, *in vitro* antifungal susceptibility was performed for five strains on both media and against all four drugs. These five strains belonged to three species, *M. furfur* (two), *M. sympodialis* (two) and *M. slooffiae* (one). All the strains grew well on both DST and LNA media. Yeast suspensions were prepared and MICs were determined as described earlier.

Results

The final MIC values, at 7 days postinoculation, of the seven *Malassezia* species against the four drugs, are

Table 3. Comparative minimum inhibitory concentrations (MICs) for representatives of three *Malassezia* species on two different media used in this study

Strain no.	Species	MICs ($\mu\text{g/mL}$) on DST/LNA medium ^a			
		Ketoconazole	Voriconazole	Itraconazole	Terbinafine
CBS 1878	<i>M. furfur</i>	$\leq 0.03/0.06$	$\leq 0.03/0.125$	$\leq 0.03/ < 0.03$	16.0/16.0
98F 5399	<i>M. furfur</i>	$0.25/\leq 0.03$	NT ^b /0.06	$0.125/0.125$	32.0/32.0
98F 8139	<i>M. sympodialis</i>	$\leq 0.03/\leq 0.03$	$\leq 0.03/\leq 0.03$	$\leq 0.03/\leq 0.03$	$\leq 0.03/0.125$
JF 05	<i>M. sympodialis</i>	$\leq 0.03/\leq 0.03$	$\leq 0.03/\leq 0.03$	$\leq 0.03/\leq 0.03$	$0.125/0.125$
TV1	<i>M. slooffiae</i>	$\leq 0.03/\leq 0.03$	$\leq 0.03/\leq 0.03$	$\leq 0.03/\leq 0.03$	4.0/2.0

^a DST, Diagnostic Sensitivity Testing medium and LNA, Leeming–Notman agar medium were the two media used for growth of lipophilic *Malassezia* yeasts in this study; ^b NT, not tested; a susceptibility test for the strain 98F 5399 was not done against the antifungal agent voriconazole.

presented in Table 2. Starting from 48 h postinoculation, as the yeast colonies began to mature and MICs were apparent, variations in antifungal susceptibility among *Malassezia* species began to emerge. The data suggest (not shown) that the minimum incubation time for reading an agar-based sensitivity test for different *Malassezia* species varies and is directly proportional to their rate of growth on specific media. For *M. furfur*, *M. sympodialis*, *M. slooffiae* and *M. pachydermatis*, MIC values could be determined as early as day 2 (i.e. 48 h), whereas for *M. globosa*, *M. obtusa* and *M. restricta*, the MIC values could not be determined until day 5 (i.e. 120 h). In general, most *Malassezia* strains were very susceptible to ketoconazole, voriconazole and itraconazole, with MIC₈₀ values of $\leq 0.03 \mu\text{g/mL}$ (Table 2). Among the three azole drugs, strains of all *Malassezia* species were most sensitive to ketoconazole and itraconazole, with MICs ranging from ≤ 0.03 to $0.125 \mu\text{g/mL}$ (Table 2). For both ketoconazole and itraconazole, 95% of strains had an MIC₈₀ value of $\leq 0.03 \mu\text{g/mL}$. The new antifungal, voriconazole, was also very effective, with MIC₈₀ values of $\leq 0.03 \mu\text{g/mL}$ for most strains (80%, Table 2). MIC values of terbinafine against *Malassezia* species ranged from ≤ 0.03 to $64.0 \mu\text{g/mL}$. Strains of *M. sympodialis*, *M. slooffiae*, *M. pachydermatis* and *M. restricta* were more sensitive to terbinafine than those of *M. furfur*, *M. globosa* and *M. obtusa*. Strains of *M. furfur* demonstrated a gradient of responses to terbinafine, with some strains (three of 20) having a MIC value of $\leq 0.03 \mu\text{g/mL}$ and most strains (15 of 20) having a MIC value of $4.0 \mu\text{g/mL}$ or higher (Table 2). MIC tests showed good reproducibility in separate runs for all species and drugs.

Table 3 shows the MIC readings for five *Malassezia* strains, belonging to three species, on DST and LNA media. MIC values for all strains against three azole drugs and terbinafine were very comparable and within the two-dilution range, accepted under NCCLS

standards.²⁸ Only one *M. furfur* strain (98F 5399) showed a three-dilution difference in MIC with ketoconazole (Table 3). While the MIC values were comparable for each strain on the two different media, the actual growth of these strains was substantially slower on DST medium in comparison with their growth on LNA medium.

Discussion

This study shows that the seven currently recognized *Malassezia* species vary in their *in vitro* susceptibility to ketoconazole, voriconazole, itraconazole and terbinafine.

For valid testing of susceptibility *in vitro*, the culture medium must be nutritionally adequate and also free of antagonists to the drugs under study.³⁶ The standard RPMI-1640 synthetic medium for *in vitro* antifungal susceptibility testing of yeasts²⁸ does not support the growth of lipophilic *Malassezia* species. The modified DST,²⁹ LNA³⁴ and Sabouraud agar media used for susceptibility testing in this study supported good growth of the *Malassezia* species. The last two media named, however, are undefined media which include complex organic materials. Hoeprich and Huston³⁷ studied the effect of culture media on antifungal activity and reported that miconazole was antagonized by two complex, undefined media but not by the two synthetic media tested. They suggested that prolongation of the lag phase of growth by the antifungal imidazoles might correlate with accumulation of pyrimidines and purines in the undefined culture media. On the other hand, Swamy *et al.*³⁸ suggested that in synthetic culture medium, divalent metal ions, such as Ca^{2+} and Mg^{2+} , may compete with the imidazole drug miconazole for binding sites, thereby reducing the effective concentration of drug to exert its effects. Ketoconazole is the only imidazole included in

the present study. Neither category of antagonizing effects is known for the triazoles, such as voriconazole and itraconazole, or terbinafine.

In this study, the modified DST medium was first chosen for *in vitro* susceptibility testing of *Malassezia* species because of its defined composition. However, as *M. restricta*, *M. obtusa* and *M. globosa* grew poorly on this medium, the undefined LNA, with homogenized pasteurized cow's milk as a lipid supplement, was substituted. That this substitution did not generate a significant problem with drug antagonism in the medium is suggested by two factors. One is that, in a control experiment designed to detect any drug antagonism, similar MIC values were observed in a test comparing the defined modified DST and the undefined LNA media for five control strains, from three *Malassezia* species, that grew well on both media. The other factor is that MIC values were very low for nearly all isolates and drugs on the DST and LNA media, indicating that drug antagonism was either absent or at least below the threshold of possible detection. The same consideration applies to the Sabouraud agar used to test *M. pachydermatis*.

The MIC data for the three azole drugs tested in this study are comparable with those reported in previous studies of several azole antifungal compounds.^{30,31,39} Our MIC data for susceptibility of strains of *M. furfur* to ketoconazole are most compatible with those reported by Schmidt and Rühl-Hörster.³⁰ These authors used a microdilution test system in modified Leeming–Notman medium to measure MICs of 30 strains of *M. furfur* against four azole compounds. The lowest concentration of ketoconazole tested by Schmidt and Rühl-Hörster was 0.06 µg/mL, with 29 of 30 strains having an MIC₈₀ of ≤ 0.06 µg/mL. In this study, we observed that 95% (52 of 55) of strains tested for susceptibility to ketoconazole had an MIC₈₀ of ≤ 0.03 µg/mL. Van Gerven and Odds³¹ studied *in vitro* activity of six imidazole antifungal agents against 26 isolates of *Malassezia* species and reported that ketoconazole was a more potent inhibitor of the species compared with other topical antifungal agents of its class. Faergemann and Borgers³⁹ found that ketoconazole and itraconazole at concentrations of 1.0 µg/mL were effective in blocking the production of hyphae on human skin. The current study also demonstrates that strains of all seven *Malassezia* species were very susceptible to voriconazole, ketoconazole and itraconazole. However, within the very susceptible range, variations were observed in the susceptibility of *M. furfur*, *M. pachydermatis*, *M. sympodialis* and *M. slooffiae* strains to

voriconazole, with MICs ranging from ≤ 0.03 to 0.25 µg/mL.

In contrast to the three azoles, terbinafine is an allylamine with a primarily fungicidal action *in vitro* against a broad range of dermatophyte, filamentous, dimorphic fungi and some yeast species.⁴⁰ Variations in susceptibility among the *Malassezia* species to terbinafine were greater compared with the azole drugs. Susceptibility results of the *Malassezia* species to terbinafine reported here are similar to those of Leeming *et al.*³³ who used LNA to test susceptibility of five *Malassezia* species to terbinafine. Leeming *et al.*³³ tested 10 strains each of *M. globosa* and *M. restricta* and observed MIC values ranging from 0.25 to 4.0 µg/mL for the former and 0.06–1.0 µg/mL for the latter. Our MIC readings of 0.06–16.0 µg/mL for seven strains of *M. globosa* and 0.06–4.0 µg/mL for four strains of *M. restricta* are in keeping with these results under the acceptable range set by NCCLS standards.²⁸ We also similarly observed that *M. furfur* varies in susceptibility to terbinafine but that many isolates show relatively high MICs, up to 32.0 µg/mL. MICs of terbinafine against *M. slooffiae* strains ranged from ≤ 0.03 to 8.0 µg/mL. Leeming *et al.*³³ presented combined data for *M. furfur* and *M. slooffiae*, thereby preventing distinction between the MICs for the two species to terbinafine. *M. sympodialis* was uniformly highly susceptible to terbinafine, with no isolate having an MIC higher than 0.25 µg/mL.

Although *Malassezia* species show differences in their antifungal susceptibility *in vitro*, the *in vivo* efficacy of these antifungal agents needs to be further evaluated by correlation between *in vitro* MICs and clinical outcomes. Furthermore, the route of administration, i.e. topical vs. oral, may also affect the efficacy of drugs in the treatment of pityriasis versicolor.^{41–43} While topical treatment may be sufficient to kill all superficially located *Malassezia* yeasts, such a treatment might result in an uneven distribution of the drug in the dermis, epidermis, sebum, stratum corneum⁴⁴ and deeper layers of skin such as pilosebaceous follicles.³³ With oral therapy, however, the drug would be more evenly distributed in the pilosebaceous follicles and skin-surface lipids, but if concentrations were marginal, they would only be effective against the most susceptible *Malassezia* strains.³³

The differences observed in the *in vitro* susceptibility of *Malassezia* species to commonly used antifungal agents warrant the need for reiterating the relevance of *Malassezia* species in skin diseases. The most commonly occurring infection due to *Malassezia* species in humans

is pityriasis (tinea) versicolor. It was initially thought that this superficial fungal infection was caused by a single species of *Malassezia*, but later genetic analysis suggested that several species of lipophilic yeasts may exist.^{22–27,45} It has now been demonstrated that pityriasis versicolor may be associated with six of the seven species of *Malassezia*, *M. furfur*, *M. sympodialis*, *M. slooffiae*, *M. globosa*, *M. obtusa* and *M. restricta*.²⁵ In one North American study, *M. sympodialis*, *M. globosa* and *M. furfur* were much more likely to be associated with pityriasis versicolor compared with *M. restricta* (unpublished data). Furthermore, in some instances more than one *Malassezia* species may be isolated from a patient with pityriasis versicolor (unpublished data). Other disease states such as adult-type seborrhoeic dermatitis may also be associated with *Malassezia* yeasts.⁴⁵ In seborrhoeic dermatitis also, more than one *Malassezia* species has been isolated (unpublished data). It is therefore important to be aware that the seven species of *Malassezia* may exhibit differences in *in vitro* sensitivity to antimycotic agents. The data may help explain the differences in mycological cure rates when an antifungal agent is used to treat what appears to be clinically the same disease state, e.g. pityriasis versicolor.

In reporting that *Malassezia* species vary in their *in vitro* antifungal susceptibility, we do not intend to imply that clinical laboratories need to conduct routine *in vitro* susceptibility studies on *Malassezia* species. However, it is important to be aware that the genus *Malassezia* comprises a heterogeneous group of species, which may vary in their susceptibility to different antifungal agents. Over time, as our understanding of the epidemiology of the seven species of *Malassezia* improves, we may have the option, where appropriate, of targeting our choice of antifungal agent depending upon the species that is causing the *Malassezia* infection in the patient.

Acknowledgments

We thank Dr Gillian Midgley for providing cultures of four *Malassezia* species that were used in this study. We also thank Jennifer Mah for technical support and Sal Albreish, Marianna Zylber, Ursula Bunn and Maria Witkowska for their assistance in primary isolations from skin specimens.

References

- Yarrow AE, Ahearn DG. Genus 7: *Malassezia* Baillon. In: *The Yeasts, a Taxonomic Study* (Kreger van Rij NJW, ed.), 3rd edn. Amsterdam: Elsevier, 1984: 882–5.
- McGinley KJ, Lantis LR, Marples RR. Microbiology of tinea versicolor. *Arch Dermatol* 1970; **102**: 168–71.
- Bergbrant IM, Faergemann J. Seborrhoeic dermatitis and *Pityrosporum ovale*: a cultural and immunological study. *Acta Derm Venereol (Stockh)* 1989; **69**: 332–5.
- Bergbrant IM, Johansson S, Robbins D *et al.* An immunological study in patients with seborrhoeic dermatitis. *Clin Exp Dermatol* 1991; **16**: 331–8.
- Parunovic A, Halde C. *Pityrosporum orbiculare*. Its possible role in the seborrhoeic blepharitis. *Am J Ophthalmol* 1967; **63**: 815–20.
- Borton LK, Schwartz RA. *Pityrosporum* folliculitis. A common acneiform condition of middle age. *Arch Med* 1981; **38**: 598–601.
- Kellett JK, MacDonald RH. Confluent and reticulate papillomatosis. *Arch Dermatol* 1985; **121**: 587–8.
- Danker WM, Spector SA, Furer J *et al.* *Malassezia* fungemia in neonates and adults: complication of hyperalimentation. *Rev Infect Dis* 1987; **9**: 743–53.
- Larocco MA, Dorenbaum A, Robinson A, Pickering LK. Recovery of *Malassezia pachydermatis* from eight infants in a neonatal intensive care nursery: clinical and laboratory features. *Pediatr Infect Dis J* 1988; **7**: 398–401.
- Mickelsen RA, Viano-Paulson MC, Stevens DA *et al.* Clinical and microbiological features of infection with *Malassezia pachydermatis* in high risk infants. *J Infect Dis* 1988; **157**: 1163–8.
- Richet HM, McNeil MM, Edwards MC *et al.* Cluster of *Malassezia furfur* pulmonary infections in a neonatal intensive care unit. *J Clin Microbiol* 1989; **27**: 1197–200.
- Welbel SE, McNeil MM, Pramanik A *et al.* Nosocomial *Malassezia pachydermatis* bloodstream infections in a neonatal intensive care unit. *Pediatr Infect Dis J* 1994; **13**: 104–8.
- Francis P, Walsh TJ. Approaches to management of fungal infections in cancer patients. *Oncol* 1992; **6**: 133–48.
- Samonis G, Bafaloukas D. Fungal infections in cancer patients: an escalating problem. *In Vivo* 1992; **6**: 183–93.
- Goodman DS, Teplitz ED, Wishner A *et al.* Prevalence of cutaneous disease in patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC). *J Am Acad Dermatol* 1987; **17**: 210–20.
- Eichstedt E. Pilzbildung in der Pityriasis versicolor. *Fröierp Neue Notis Natur Heilk* 1846; **39**: 270.
- Rivolta S. *Dei Parassiti Vegetali*. Turin: Giulio Speirani, 1873: 469–71.
- Malassez L. Note sur le champignon du pityriasis simple. *Arch Physiol* 1874; **2**: 451–64.
- Bizzozzero J. Über die Mikrophyten der normalen Oberhaut des Menschen. *Arch Pathol Anat Physiol* 1884; **98**: 441–59.
- Baillon H. *Traité de Botanique Médicale Cryptogamique*. Paris: O.Doin, 1889: 234–9.
- Castellani A, Chalmers AJ. *Manual of Tropical Medicine*. London: Baillière Tindall, 1913.
- Guého E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek* 1996; **69**: 337–55.
- Weidman FD. Exfoliative dermatitis in the Indian rhinoceros (*Rhinoceros unicornis*), with description of a new species: *Pityrosporum pachydermatis*. In: *Report of the Laboratory and Museum of Comparative Pathology of the Zoological Society of Philadelphia* (Fox H, ed.). Zoological Society of Philadelphia, Philadelphia, PA, 1925: 36–44.
- Simmons RB, Guého E. A new species of *Malassezia*. *Mycol Res* 1990; **94**: 1146–9.
- Cunningham AC, Leeming JP, Ingham E *et al.* Differentiation of

- three serovars of *Malassezia furfur*. *J Appl Bacteriol* 1990; **68**: 439–46.
- 26 Guillot J, Guého E. The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparisons. *Antonie van Leeuwenhoek* 1995; **67**: 297–314.
 - 27 Guillot J, Guého E, Lesourd M *et al*. Identification of *Malassezia* species: a practical approach. *J Mycol Med* 1996; **6**: 103–10.
 - 28 Galigiani JN, Bartlett MS, Ghannoum MA *et al*. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. *NCCLS Doc M27A* 1997; **17**: 1–29.
 - 29 Faergemann J. Treatment of seborrhoeic dermatitis with bifonazole. *Mycoses* 1989; **32**: 309–11.
 - 30 Schmidt A, Rühl-Hörster B. *In vitro* susceptibility of *Malassezia furfur* against azole compounds. *Mycoses* 1996; **39**: 309–12.
 - 31 Van Gerven F, Odds FC. The anti-*Malassezia furfur* activity *in vitro* and in experimental dermatitis of six imidazole antifungal agents: bifonazole, clotrimazole, flutrimazole, ketoconazole, miconazole and sertaconazole. *Mycoses* 1995; **38**: 389–93.
 - 32 Drouhet E, Dupont B. *In vitro* antifungal activity of sertaconazole. *Arzneim Forsch* 1992; **42**: 705–10.
 - 33 Leeming JP, Sansom JE, Burton JL. Susceptibility of *Malassezia furfur* subgroups to terbinafine. *Br J Dermatol* 1997; **137**: 764–7.
 - 34 Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987; **25**: 2017–19.
 - 35 Midgley G. The diversity of *Pityrosporum* (*Malassezia*) yeasts *in vivo* and *in vitro*. *Mycopathologia* 1989; **106**: 143–53.
 - 36 Hoeprich PD, Merry JM. Influence of culture medium on susceptibility testing with BAY n 7133 and ketoconazole. *J Clin Microbiol* 1986; **24**: 269–71.
 - 37 Hoeprich PD, Huston AC. Effect of culture media on antifungal activity of miconazole and amphotericin B methyl ester. *J Infect Dis* 1976; **134**: 336–41.
 - 38 Swamy KHS, Sirsi M, Rao GR. Studies on the mechanism of action of miconazole: effect of miconazole on respiration and cell permeability of *Candida albicans*. *Antimicrob Agents Chemother* 1974; **5**: 420–5.
 - 39 Faergemann J, Borgers M. The effect of ketoconazole and itraconazole on the filamentous form of *Pityrosporum ovale*. *Acta Derm Venereol (Stockh)* 1990; **70**: 172–6.
 - 40 Balfour JA, Faulds D. Terbinafine. A review of its pharmacodynamics and pharmacokinetic properties, and therapeutic potential in superficial mycoses. *Drugs* 1992; **43**: 259–84.
 - 41 Kagawa S. Clinical efficacy of terbinafine in 629 Japanese patients with dermatomycosis. *Clin Exp Dermatol* 1989; **14**: 114–5.
 - 42 Aste N, Pau M, Pinna AL *et al*. Clinical efficacy and tolerability of terbinafine in patients with pityriasis versicolor. *Mycoses* 1991; **34**: 353–7.
 - 43 Villars V, Jones TC. Present status of the efficacy and tolerability of terbinafine (Lamisil) used systemically in the treatment of dermatomycoses of the skin. *J Dermatol Treat* 1990; **1** (Suppl. 2): 33–8.
 - 44 Faergemann J, Zehender H, Denouel J *et al*. Levels of terbinafine in plasma, stratum corneum, dermis-epidermis (without stratum corneum), sebum, hair and nails during and after 250 mg terbinafine orally once per day for four weeks. *Acta Derm Venereol (Stockh)* 1993; **73**: 305–9.
 - 45 Hay RJ, Moore M. Mycology. In: *Textbook of Dermatology* (Champion RH, Burton JL, Burns DA, Breathnach SM, eds), 6th edn, Vol. 2. Oxford: Blackwell Science Ltd, 1998: 1277–376.