ISSN 2347-6893

In vitro susceptibility testing of dermatophytes with their fragmented mycelia as inoculum

V.A. Czaika[1], A.F. Schmalreck[2]

[1]Universitätsmedizin Berlin, Clinics for Dermatology, Venereology und Allergology, Charité, Campus Benjamin Franklin, 12203 Berlin, Germany

viktor.czaika@charite.de

[2]MBS, Microbiology, 80086 Munich, Germany

muenchen-mbs@t-online.de

ABSTRACT

377 clinical isolates and seven dermatophyte culture collection strains were tested *in vitro* by microdilution against a panel of 18 antifungal agents. A newly introduced method for rapid and reliable inoculum preparation for moulds using the fragmented mycelia of freshly grown isolates as inoculum was firstly applied to dermatophytes. The performance of the method was tested with two different culture media which are recommended by different standardized testing methods for moulds. The standardized fragmented mycelia provided both, countable single colonies (viable units) on solid culture media and distinct readable endpoints (minimum inhibitory concentrations) in the microdilution wells for all tested topical and systemic drugs, including the echinocandins. As with moulds, and as shown by the culture collection strains, reproducible minimum inhibitory concentrations were obtained with an essential agreement (± 1 log_2-dilution) of 97% to 100%, by a significant reduction of the overall testing time. As exemplarily shown for the microdilution technique, this inoculum method should also provide a solid basis for improved agar-based susceptibility testing methods such as disc-, tablet-, or strip-tests.

KEYWORDS

dermatophytes, susceptibility testing, inoculum, fragmented mycelium, azoles, echinocandins, topical antifungal agents

ACADEMIC DISCIPLINE

Microbiology, Mycology

SUBJECT CLASSIFICATION

antifungal susceptibility/resistance

TYPE

susceptibility testing, inoculum preparation

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Biology

Vol. 6, No. 1

editorsjab@gmail.com , editor@cirjab.com

ISSN 2347-6893

INTRODUCTION

In vitro testing of antifungal agents (AFA) paralleled the introduction of antifungal agents. Griseofulvin, detected in 1939, was adopted in 1947 for use against human and animal dermatophytes [1, 2, 3]. However, antimicrobial susceptibility testing technology of fungi is still not as advanced as the in vitro susceptibility testing of bacteria. In particular this applies to the susceptibility testing of filamentous fungi and especially for dermatophytes, where selection of the inoculum form, its preparation, and standardization are major obstacles [4]. Even today a generally accepted and fully standardized susceptibility testing method for dermatophytes is not available.

As for hyphomycetes, several forms of inoculum preparation have been used with dermatophytic fungi: conidial suspensions [1, 5, 6, 7, 8, 9], fragmented mycelium [4], and a mixture of mycelium and conidia [1, 3, 5, 10, 11, 12, 13, 14, 15]. The conidial inoculum method for susceptibility testing of moulds is recommended by CLSI [16] and EUCAST [17]. Probably because spores can be readily counted this is currently the easiest to method to standardize the inoculum. However, the existing methods for moulds (CLSI, EUCAST) are not intended for routine testing. As they require the production of fungal spores these methods are limited to sporogenous strains of fungi. Therefore, this may end up for the slow growing dermatophytes (DMPs) with susceptibility testing (SUST) times of up to 14 days. To shorten the inoculum preparation for SUST fragmentation of hyphae producing fungi was already applied in the early days of fungal SUST by using ball mills with glass beads [10] or ground glass grinders [4]. Due to the technical progress of the appropriate devices it deemed worthwhile to improve the necessitated fragmentation of the mycelium for the following reasons:

- Conidia formation is not a consistent feature of dermatophytes
- Difficulties in harvesting and purifying of conidia are common
- Conidia formation is not particularly fast compared to sufficient mycelium element formation
- Mycelia have less morphological variation than conidia
- Hyphae are the cell form which is found within the infected compartments of individuals
- Appropriate fragmented mycelia can be readily dispersed in equal amounts, and thereof viable units easily to be counted.

Therefore, with the fragmentation technique it can be assured that the microdilution wells will receive approximately the same mass of viable mycelium parts when microdilution testing of hyphae-forming fungi will be performed. Because susceptibility screening and in vitro testing of moulds with fragmented mycelia preparations turned out to be successful [18, 19], in this study susceptibility testing with fragmented mycelia was extended to dermatophytes. By a panel of 18 different antifungal agents, currently used for superficial and systemic infections, more than three hundred clinical isolates and seven culture collection strains were tested by microdilution to evaluate the performance of the fragmented dermatophyte mycelium inoculum (FDMI).

1 MATERIAL AND METHODS

1.1 Clinical isolates

The dermatophyte (DMP) isolates (N=377), 36 (9.6% of total) *Microsporum canis*, 98 (26%) *Trichophyton mentagrophytes*, 200 (53%) *Trichophyton rubrum*, 28 (7.4%) *Trichophyton tonsurans,* and 15 (4%) *Epidermophyton floccosum* strains were derived from the wards of the University Hospitals, mainly from the Charité (Berlin, Germany), partly from LMU and TU Munich, and of the Laboratory for Clinical Research (Prof. Dr. Junge, Raisdorf, Germany). All isolates were identified according to common mycological methods and partly by PCR and FT-IR [20]. The 7 culture collection strains: *Epidermophyton floccosum* DSM 10709, *Microsporum canis* DSM 10708, *Microsporum canis* ATCC 28327, *Trichophyton mentagrophytes* ATCC 18748, *Trichophyton mentagrophytes* ATCC 9533 (≙ DSM 4870), and *Trichophyton rubrum* DSM 4167 were obtained by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig (Germany). For further quality control purposes, the yeast control strains described in a recent collaborative yeast study [21] were used in parallel.

1.2 Antifungal susceptibility testing (AFST)

AFST was performed by microdilution as described for hyphomycetes by Schmalreck et al. [18, 19] using the ready-to-use microdilution panels for yeasts with the antifungal agent (AFA) amphotericin B (AMB), flucytosine (FCY), fluconazole (FLC), posaconazole (POS), voriconazole (VOR), anidulafungin (ANI), caspofungin (CAS), and micafungin (MCA) manufactured by Merlin GmbH (Germany) [21]. Microdilution trays with the antifungal agents (AFA) abafungin (ABA), bifonazole (BIF), ciclopiroxolamine (CIC), clotrimazole (CLO), griseofulvine (GRF), itraconazole (ITR), ketoconazole (KET), miconazole (MCZ), nystatin (NYS), and terbinafine (TER) were prepared in-house. The following AFA were provided free of charge by the following manufacturers: FLC, VOR, and ANF by Pfizer GmbH (Germany), MCS (Astellas GmbH, Germany), CSF (MSD, Germany), POS (Essex Pharma, Germany), ABA (York Pharma, Germany), BIF (Bayer AG, Germany). APH, CIC, CLO, GRF, ITR, FCY, ITR, KET, MCZ, NYS, and TER were obtained by Sigma-Aldrich (Munich, Germany). The latter were solubilised in polysorbate 20 prior the daily log_2-dilution series preparations. The following procedure was crucial for ABA, CLO, MCA, CIC, TER, and also suitable for ITR and POS to yield completely dissolved AFA. To the weighed pure substance 50 µl to max 1.5 ml polysorbate 20 is added (500 µl = 30 drops = 0.52g) to dissolve the substances. It has to be waited for 1-15 min (depending on the amount of AFA, respectively, the desired initial concentration) until the suspension was completely dissolved (optically clear). Further dilutions were prepared with the test

media. For comparison purposes the AFA were tested in parallel with another culture medium using testing conditions as described by Ghannoum et al. [9].

1.3 Inoculum preparation

DMP clinical isolates and control strains were prepared by dropping 0.2 ml of polysorbate 80 onto the pre-culture colonies on Sabouraud-2%-glucose agar (SAB-2-GA). These wetted colonies were transmitted with a sterile transfer pipette with 10ml - 15ml physiological saline containing 1g polysorbate 80/l into (sterile) IKA-BMT-20 (IKA®, Germany) mixing vessels, containing 15 to 20 (sterile) stainless steel balls. These suspensions were fragmented with the IKA®-Ultra-Turrax© Tube-Drive homogenization tool (IKA®, Germany). The Tube drive settings were: time 30s; mode 6; 6000 U/min. The suspensions were controlled microscopically whether a homogenous suspension of about equal particle size has been achieved. The particle density was controlled by appropriated means (e.g. counting in a Neubauer® counting chamber; determination of the optical density at 540 nm with a commercial spectrophotometer; or with a commercially available, miniaturized densitometer for (bacterial) inoculum standardization). According to the issued test protocol, all comparisons should be tested with a final inoculum of 2-5x10^4 viable units (vu) per ml [18], whereby a mean inoculum size of 5.4x10^4 vu/ml has been achieved by the participating laboratories. To ensure a sufficient high starting-inoculum mostly 2-3 pre-culture plates per isolate were necessary. The combined fragmented suspensions had been adjusted with 0.85% NaCl containing 0.1% polysorbate 80 (final concentration) to approx. 1x10^6 vu/ml (initial inoculum). For viability testing, 100 µl aliquots of a 1:1000 dilution thereof were plated onto SAB-2-GA and counted after appropriate growth at 36 °C.

1.4 Endpoint determination (MIC)

The microdilution plates were incubated at 36 °C ±1 °C, stapled to a maximum of five, with the top one covered by a lid, until optimal growth in the control wells was achieved. The MIC was read visually and determined as the first well which showed no growth [8] as compared to the drug-free growth-control well. A second control reading was performed 1d after the first reading.

1.5 Statistical analyses

All calculations were performed with $\log_2$-MIC values and statistical analyses performed with *SAS®* software from *SAS®* Institute (Cary, USA - Heidelberg, Germany). The antilog of the calculations represents the MIC from the calculated result. The epidemiological cut-off value (ECV) was calculated as two $\log_2$-dilutions above the median MIC [22].

2 RESULTS AND DISCUSSION

2.1 General Aspects of Ionoculum Preparation

Antimycotic susceptibility testing of dermatophytes with a standardized fragmented mycelial inoculum has been already described in 1980 by Granade & Artis [4]. Early studies by Guidry & Trelles [23] used a special glass-homogenizer and a grinding time of 4 min per isolate to obtain optimal fragmented hyphae, whereas Savage and Vander Brook determined two minutes for their high-speed blender as a suitable time span to achieve optimal hyphal fragments of *Penicillium notatum* [24]. If more than one strain is tested it has to be noticed that for each strain a sterile grinder respectively, a sterile blending-container has to be available. With the commercially available device used in this study fragmentation time per strain was achieved in only 30 seconds. When aliquots plated onto solid agar with a spiral plater (BIOSYS® GmbH, Germany) the suspension with the hyphal fragments resulted in growth of single, and easily (automatically) countable colonies (viable units). To ensure enough mycelium of the slow growing dermatophytes several pre-cultures per strain under test were prepared in parallel. Thus sufficient viable hyphae fragments of the individual species were obtained on the appropriate medium within 3 to 6 days prior to susceptibility testing.

The reason why fragmented hyphae are not recommended as inoculum by the currently available susceptibility testing methods for filamentous fungi [16, 17] may be multi factorial:

- The fractionated mycelium is thought to more difficult to standardize than conidia suspensions
- Glass (grinders) or Teflon homogenizers using the Potter-Elevhjem method [25] have a low throughput, are labour-extensive, and are logistically and financially expensive because a sterile unit has to be provided for each strain to be tested
- Beating of cells by using glass or stainless steel balls may device-dependent generate much heat and have little throughput for small samples
- High throughput homogenizers are fairly large devices, and require a high financial investment
- The French press or French pressure cell in which the move of cells from extremely high pressure to low pressure through a tiny orifice is used to disrupt them is very effective and efficient; however the delivery rate is very low, and the amount of non-viable cell parts may be high. In addition to the high cost of the device, Additionally, French pressure cells may be expensive relative to the number of samples to be processed [25].

The rapid development of heat is common to all these methods. Additionally the use of shearing and other disruption forces can destroy partly or completely the viable cells. This may affect extremely the viability of the inoculum, and therefore can be diametric to its growth development, respectively may be used to destroy viable cells and cell compounds [26]. However, due to the downsizing of the equipment and the development of miniaturized laboratory beads beaters at

ISSN 2347-6893

very reasonable prices, together with their appropriate sterilized or production-sterile plastic containers, effective laboratory homogenisation at very low heat levels is possible and allows multiple testings per day. As demonstrated, this methodology is suitable to generate reliable and reproducible MICs for hyphomycetes [18, 19]. Therefore this method was evaluated in this study for susceptibility testing of dermatophytes. That incubation temperature has no effect on dermatophyte MICs has been reported [27].

2.2 Mycelia versus Conidia, Incubation and Testing Time

To keep close to infectious conditions, incubation was performed as published for hyphomycetes [18, 19] and yeasts [21], at 35 °C - 37 °C. Within this temperature-range DMP-endpoint reading was possible at the earliest after 6 days incubation. On average the mean MIC reading time was 9 days (mean time for the first MIC reading was after 8.5 d, and the mean time frame for the second reading was after 9.7 d), except for *Epidermophyton floccosum* where the mean endpoint reading time was 12.8 d. In respect to the clinical situation the use of conidia for MIC determinations has been questioned because they are not necessarily the morphological form of the causative fungus *in vivo* [28]. Conidia formation is also not a consistent factor of dermatophytic fungi, and they require a longer period of fungal growth (up to 14 d) before appearing [3]. Thus some of the dermatophytes produce readily conidia (e.g. *T. mentagrophytes*), and the species most frequently causing severe dermatophytic infections, *T. rubrum*, produces only few conidia [4]. Therefore it may be necessary to use special culture media to induce sporulation [7, 8, 29-32]. As mycelia have less morphological variations than conidia, (microaleurospores versus macroaleurospores), and hyphae are the form that are found within the stratum corneum or in compartments of an infected individual [4], susceptibility results thereof may reflect the clinical situations more close.

2.3 Minimum Inhibitory Concentration (MIC)

The results (number of individual MICs and characteristic MIC-values) under these conditions are given for total isolates and *Trichophyton mentagrophytes* and *Epidermophyton floccosum* in Table 1, for *Trichophyton rubrum* and *Trichophyton tonsurans* and *Microsporum canis* in Table 2. All *Trichophyton* spp. isolates were inhibited by low MICs of abafungin, ciclopiroxolamine, terbinafine, and of the azoles miconazole, clotrimazole, posaconazole (order of increasing activity), and with the highest in vitro activity of voriconazole and the echinocandins. Aside of the echinocandins, ciclopiroxolamine, posaconazole, and voriconazole were the most active AFA against *Microsporum canis*. All echinocandins scored with the lowest MICs and the highest in vitro activity against all clinical dermatophyte isolates tested (Tables 1 & 2). This holds also true for the seven culture collection strains tested (Table 5a – 5c). For moulds and echinocandins disturbed and aberrant mycelial growth is reported for outgrown conidia [33]. These morphological changes have to be detected microscopically for the determination of MIC endpoints [34], and had also has been applied for dermatophytes [35]. Although echinocandins exhibit only a fungistatic effect on moulds [36], however, due to the possibility of distinct endpoint reading of the visible growth, the determination of the minimum effective concentration (MEC) of ANF, CSF and MCF for moulds and DMPs was not necessary because distinct echinocandin MICs were obtained due to the fractionated mycelium method.

No inoculum effect, respectively, influence on susceptibility test results were observed with the Tween concentrations used (data not shown). This is confirmed by studies of Gomez-Lopez et al. [37] who found no inhibitory effect of Tween 20 below 0.5%.

2.4 Comparison of MICs from different Culture Media

To further evaluate the performance of the FDMI method, apart from YST medium [19], RPMI 1640 was tested in parallel. This medium has been shown to produce also suitable visible growth of well characterized clinical dermatophyte isolates [8] and in addition, is recommended by CLSI [16] and EUCAST [17] for testing filamentous fungi. However, as the glucose concentration is critical for optimal growth [25] the RPMI medium was adjusted to the same level of glucose as YST medium (2% final concentration). With the AFA-panel containing the newer azoles (FLC, POS, VOR), echinocandins (ANF, CSF, MCF), flucytosine, and amphotericin B, both media were tested with the fragmented hyphae inoculum. In Table 3 the characteristic MIC-values (MIC range, geometric mean of the MICs, the percentage where 50% (MIC_{50}), or 90% (MIC_{90}) of the isolates are inhibited, respectively, both, the percentage at the MIC ≤ the ECV and the percentage at ≤ the MIC_{75} are exemplarily shown for the common dermatogenic pathogens *T. rubrum* and *T. mentagrophytes,* and *T. tonsurans*. As far as available these results were compared with the sparsely available data of the literature. As none of the tested AFA exhibit a in vivo efficacy of >70%-80%, instead of the MIC_{90} the 75th percentile (MIC_{75}) is presented, indicating the MIC at which 75% of the DMP-isolates are inhibited. Interestingly, the percentage of isolates Tests with YST and RPMI medium resulted in very similar MICs for all AFA. Although mostly not directly comparable due to different ways of data presentation, the results with the new inoculum are very similar to the data obtained by the “conidia” techniques (Table 3). This holds also true for the topical agents and other systemically used AFA [38-41].

The agreement of the performance on both susceptibility testing media, YST, and RPMI, either comparing the 1st and 2nd endpoint determination with each test medium and comparison of the endpoints of both media at the 1st and the 2nd MIC reading, is shown exemplarily for four dermatophyte species in Table 4. There is no statistically significant difference between the 1st and the 2nd endpoint reading, as there is no significant difference observed between both susceptibility testing media. The essential agreement (± 1log2-dilution) was for the individual media at the first MIC reading (Ø 8.5d) 97%, for the second reading (Ø 9.7d) 97% - 99%, and for both media compared 98-100% for the first, and 99% - 100% for the second MIC reading and each species. Thus, an incubation time for the tested species of10 days can be recommended, except for *E. Floccosum*, where at least 13d at 36°C may be appropriate. However, a lager collaborative study with more isolates and species would be necessary to verify the optimal medium and endpoint reading time.

The method presented obtained similar results as the conidia method described by EUCAST for moulds (data not shown), which will be presented elsewhere.

2.5 Quality Control Strains

As with testing of moulds, for internal quality control commonly “yeast” control strains are recommended [19]. The reported QC-strains, which need 10 days pre-growth to obtain their spores [9] were at the time of the study not readily available. Therefore seven different dermatophyte culture collection strains were included as quality control (QC) strains (Tables 5a-5c). Possible QC-ranges for *Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Trichophyton tonsurans* strains were based on the mode MIC of the repeat AFA-tests with a QC-range of ±1 log_2-dilutions if the standard deviation of the mean was 0 or 1, and a ±2 log_2-dilution QC-range if the standard deviation of the mean MIC was ≥ ±2. As these preliminary results are promising for establishing species specific QC-strains and QC-ranges, they should be defined and validated on an international basis with an international standard for dermatophytes.

3 CONCLUSIONS

This investigation confirmed finally the findings for hyphomycetes [18, 19], and that the semi-automatic inoculum preparation by fragmenting freshly grown mycelia can be also favourable applied to dermatophytes. The in vitro results are comparable to those in the literatures with only conidia as inoculum [15, 38, 41-42]. That this method of inoculum preparation is reliable, reproducible, and easy to perform and can shorten the in vitro testing up to 14 days could be demonstrated. In addition, due to the successful production of single colony agglomerates from the fragmented inoculum, this technique may be successfully applied for agar based test methods to accelerate and ease disc-, tablet-, and strip-test diffusion susceptibility testings.

ACKNOWLEDGMENTS

To perform and report this study no financial support from pharmaceutical or other companies or from national or international funding sources were received.

No writing assistance was utilized in the production of this manuscript.

The authors declare that no conflict of any interests exists.

REFERENCES

[1] Emmons, C.W. 1945. Fungicidal and fungistatic agents: proposed method for testing fungicides against *Trichophyton*. Am. Public Health, 35, 844-846.

[2] Bergmann, S. 1955. In vitro studies on antimycotics. A comparison of different methods. Acta Pathol. Scand. Suppl. 104.

[3] Roth, F.J., Sallman, B., and Blank, H. 1959. In vitro studies of the antifungal antibiotic griseofulvin. J. Invest. Dermatol. 33, 403-418.

[4] Granade, T.C., and Artis, W.M. 1980. Antimycotic Susceptibility Testing of Dermatophytes in Microcultures with a Standardized Fragmented Mycelial Inoculum. Antimicrob. Ag. Chemother. 17, 725-729.

[5] Aytoun, R.S.C., Campbell, A.H., Napier EJ, Seiler, D.A.L. 1960. Mycological aspects of action of griseofulvin against dermatophytes. AMA Arch. Dermatol. 81, 52-58.

[6] Qualman, S.J., Jones, H.E., and Artis, W.M. 1976. An automated radiometric microassay of fungal growth: quantitation of growth of *T. mentagrophytes*. Sabouraudia, 14, 287-297.

[7] Jessup, C.J., Warner, J., Isham, N., Hasan, I., and Ghannoum, M.A. 2000. Antifungal Susceptibility Testing of Dermatophytes: Establishing a Medium for Inducing Conidial Growth and Evaluation of susceptibility of Clinical Isolates. J. Clin. Microbiol. 38, 341-344.

[8] Fernández-Torres, B., Cabañes, F.J., Carrillo-Muñoz, A.J., Esteban, A., Inza, I., Abarca, L., and Guarro, J. 2002. Collaborative evaluation of optimal antifungal susceptibility testing conditions for dermatophytes. J. Clin. Microbiol. 40, 3999-4003.

[9] Ghannoum, M.A., Chatuverdi, V., Espinel-Ingroff, A., Pfaller, M.A., Rinaldi, M.G., Lee-Yang, W., and Warnock, D.W. 2004. Intra- and Interlaboratory Study of a Method for Testing the Antifungal Susceptibilities of Dermatophytes. J. Clin. Microbiol. 42, 2977-2979.

[10] Young, C.N. 1972. Sensitivity patterns to griseofulvin of *Trichophyton rubrum* and other ringworm fungi. Trans. St. Johns Hosp. 58, 226-234.

[11] Sekhon, A.S., and Funk, A. 1977. In vitro inhibitory studies of phacidin against some dermatophytes, opportunistic and systemic fungi, and yeasts pathogenic on man. J. Antimicrob. Chemother. 3, 95-100.

[12] Favre, B., Hofbauer, B., Hildering, K.-S., and Ryder, N.S. 2003. Comparison of In Vitro Activities of 17 Antifungal Drugs Against a Panel of 20 Dermatophytes by Using a Microdilution Assay. J. Clin. Microbiol. 41, 4817-4819.

[13] Ciebiada-Adamiec, A., Ciebiada, I., Malafiej, E., and Denys, A. 2008. Susceptibility testing of dermatophytes - proposition of inoculum standardization. Mikologia Lekarska, 15, 17-19.

[14] Shathele, M.S., and Fadlelmula, A.. 2010. In vitro Effectivness of Some Antifungal Drugs in Treatment of *Trichophyton verrucosum*; Dermatophytic Fungi. Asian J. Anim. Vet. Adv. 2010, 1-13.

[15] Korting, H.C., Ollert, M., Abeck D. 1995. Results of a German Multicenter Study of Antimicrobial Susceptibilities of *Trichophyton rubrum* and *Trichophyton mentagrophytes* Strains Causing Tinea Unguium. Antimicrob. Ag. Chemother. 39, 1206-1208.

[16] CLSI M38-A2. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard – Second Edition. Clinical Laboratory Standards Institute, USA.

[17] EUCAST Definitive Document E.DEF 9.1. 2012. Method for determination of broth dilution minimum inhibitory concentration of antifungal agents for conidia forming moulds.

[18] Schmalreck, A., Willinger, B., Czaika, V., Fegeler, W., Becker, K., Blum, G., and Lass-Flörl, C. 2012. Susceptibility Screening of Hyphae-Forming Fungi with a New, Easy, and Fast Inoculum preparing Method. Mycopatholgia 174, No. 1, July 2012: DOI 10.1007/s11046-012-9570-7.

[19] Schmalreck, A.F., Fegeler W., Becker K., Lass-Flörl C., and Czaika, V. 2014. Cross-Resistance of Azoles, Echinocandins, Flucytosine, and Amphotericin B in Clinically Important Hyphomycetes. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN:0975-9174.

[20] Bastert, J., Korting, H.C., Tränkle, P., and Schmalreck, A.F. 1999. Identification of dermatophytes by Fourier-Transform Infrared-Spectroscopy (FT-IR). Mycoses, 4, 525-528.

[21] Schmalreck, A.F., Willinger, B., Haase, G., Blum, G., Lass-Flörl, C., Fegeler, W., Becker, K., and the Antifungal Susceptibility Testing (AFST) Study Group. 2012. Species and susceptibility distribution of 1062 clinical yeast isolates to azoles, echinocandins, flucytosine and amphotericin B from a multi-centre study. Mycoses, 55, e124–e137. DOI: 10.1111/j.1439-0507.2011.02165.x.

[22] Arendrup, M.C., Garcia-Effron, G., Lass-Flörl, C., Gomez Lopez ,A., Rodriguez-Tudela, J.-L., Cuenca-Estrella, M., and Perlin D.S. 2009. Susceptibility testing of *Candida* species to echinocandins: comparison of EUCAST EDef 7.1, CLSI M27-A3, Etest, disk diffusion and agar-dilution using RPMI and IsoSensitest medium. Antimicrob. Agents Chemother. 54, 426-439.

[23] Guidry, D.J., and Trelles, G.H. 1962. Evaluation of a new Method for the Preparation of Homogenous Mycelial Suspensions. J. Bacteriol. 83, 53-60.

[24] Savage, G.M., and Vander Brook, M.J. 1946. The frangmentation of the Mycelium of *Penicillium notatum* and Penicillium chrysogenum by a HighSpeed Blender and the Evaluation of Blended Seed. J. Bacteriol. 52, 385-391.

[25] Burden, D.W. 2008. Guide to the Homogenization of Biological Samples. Random Primers 7, 1-14.

[26] Schmalreck, A.F., and J. Hoiß. 1984. Offenlegungsschrift DE 3308325 A1: Verfahren und Vorrichtung zur vollständigen oder partiellen Zerstörung von Mikroorganismen. Deutsches Patentamt München.

[27] Zhang, J., Chen, J., Huan, H.-G., Xi, L.-Y., Lai, W., Xue, R.-Z., Zhang, X.-H., and Chen ,R.-Z. 2010. Comparison of a glucose consumption based method with the CLSI M38-A method for testing antifungal susceptibility of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Chin. Med. J. 123, 1909-1914.

[28] Barchiesi, F., Arzeni, D., Camiletti, V., Simonetti, O., Cellini, A., Offidani, A.M., and Scalise, G. 2001. In Vitro Activity of Posaconazole against Clinical Isolates of Dermatophytes. J. Clin. Microbiol. 39, 4208-4209.

[29] Bejak, V. 1985. Standarization of Hyphal Inoculum of Aspergilli for Amphotericin B Testing. J. Clin. Microbiol. 21, 509-512.

[30] Nweze, E.I., Mukherjee, P.K., and Ghannoum, M.A. 2010. Agar-based Disk Diffusion Assay for Susceptibility Testing of Dermatophytes. J. Clin. Microbiol. 48, 3750-3752.

[31] Santos, D.A., Barros, M.E.S, and Hamdan, J.S. 2006. Establishing a method of inoculum preparation for susceptibility testing of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. J. Clin. Microbiol. 44, 98-101.

[32] Santos, D.A., and Hamdan, J.S. 2005. Evaluation of Broth Microdilution Antifungal Susceptibility Testing Conditions for *Trichophyton rubrum*. J. Clin. Microbiol. 43, 1917-1920.

[33] Fortwendel, J.R., Juvvadi, P.R., Pinchai, N., Perfect, B.Z., Alspaugh, J.A., Perfect, J.R., and Steinbach, W.J. 2009. Differential effects of inhibiting chitin and 1,3-{beta}-D-glucan synthesis in ras and calcineurin mutants of *Aspergillus fumigatus*. Antimicrob. Agents Chemother. 53: 476-482. doi: 10.1128/AAC.01154-08.

http://aac.asm.org/content/53/2/476.full.pdf+html.

[34] Espinel-Ingroff, A., Canton, E., and Penman, J. 2009. Updates in antifungal susceptibility testing of filamentous fungi. Cur Fungal Infect Rep. 3, 133-41.

[35] Bao, Y.Q., Wan, Z., and Li, R.Y. 2013. In vitro antifungal activity of micafungin and caspofungin against dermatophytes isolated from China. Mycopathologia, 175, 141-145. doi: 10.1007/s11046-012-9571-6.

[36] Pound, M.W., Townsend, M.L., Richard, H., and Drew, R.H. 2010. Echinocandin pharmacodynamics: review and clinical implications. J. Antimicrob. Chemother. 65, 1108-1118.

doi: 10.1093/jac/dkq081. http://jac.oxfordjournals.org/content/65/6/1108.full.pdf+html.

[37] Gomez-Lopez ,A., deOliveira, A.N., de Oliveira, L.A., and Andrade, J.S. 2010. Partial characterization of amylases of two indigenous Central Amazonian rhizobia strains. Braz. Arch. Biol. Technol. 53, no.1 Curitiba Jan./Feb. 2010. http://dx.doi.org/10.1590/S1516-89132010000100005.

[38] Fernández-Torres, B., Carrillo, A.J., Martín, E., Del Palacio, A., Moore, M.K., Valverde, A., Serrano, M., and Guarro, J. 2001. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrob. Ag. Chemother. 45, 2524-2528.

[39] Siqueira, E.R., Ferreira, J.C., dos Santos, P.R., Lavrador, M.A.S., and Candido, R.C. 2008. Dermatophyte Susceptibilities to Antifungal Azole Agents Tested by In Vitro Broth Macro and Microdilution Methods. Rev. Inst. Med. Trop. S. Paulo, 50, 1-5. http://www.scielo.br/pdf/rimtsp/v50n1/a01v50n1.

[40] Jha, B.K., Murthy, S.M., Mathur, M., and Gupta,. B.P. 2013. Antifungal Susceptibility Testing of Dermatophytes from all Tinea cases by Broth Micro Dilution Method. J. Pharm. Res., 2, 1-4.

http://www.jprinfo.com/index.php?option=com_mtree&task=att_download&link_id=136&cf_id=24.

[41] Araújo, C.R., Miranda, K.C., Fernandes, O. deFatima, L., Soares, A.J., Silva, M., and do Rosario, R. 2009. In vitro susceptibility testing of dermatophyte isolates in Goiania, Brazil, against five antifungal agents by broth microdilution method. Rev. Inst. Med. Trop. S. Paulo, 51, 1-12.

http://www.readcube.com/articles/10.1590/S0036-46652009000100002.

[42] Pujol, I., Capilla, J., Fernandez-Torres, B., Ortoneda, M. And Guarro, J. 2002. Use of the sensititre colorimetric microdilution panel for antifungal susceptibility testing of dermatophytes. J. Clin. Microbiol.,40, 2618-2621.

ISSN 2347-6893

Table 1: MIC distribution (number/percentage in rounded figures), geometric mean of MIC (**MIC$_{gmean}$**), mode MIC (**MIC$_{mod}$**), the 50th, 75th, and 90th percentile (**MIC$_{50}$, MIC$_{75}$, MIC$_{90}$**), and epidemiological cut-off value (**ECV**) of the antifungal agents (AFA) voriconazole (VOR), posaconazole (POS), ketoconazole (KET), fluconazole (FLC), itraconazole (ITR), miconazole (MCZ), clotrimazole (CLO), anidulafungin (ANI), caspofungin (CAS), micafungin (MCA), ciclopiroxolamine (CIC), terbinafine (TER), abafungin (ABA), flucytosine (FCY), amphotericin B (APH), and partly for bifonazole (BIF), and griseofulvin (GRF) for total clinical isolates, *Epidermophyton floccosum*, and of *Trichophyton mentagrophytes*.

			Number and % of isolates at individual log$_2$-dilution (mg/l):																Characteristic MIC-values					
Organism	**AFA**	**N**	**0.004** n/%	**0.008** n/%	**0.016** n/%	**0.031** n/%	**0.063** n/%	**0.125** n/%	**0.25** n/%	**0.5** n/%	**1** n/%	**2** n/%	**4** n/%	**8** n/%	**16** n/%	**32** n/%	**64** n/%	**128** n/%	**MIC$_{gmean}$** mg/l	**MIC$_{mode}$** mg/l	**MIC$_{50}$** mg/l	**MIC$_{75}$** mg/l	**MIC$_{90}$** mg/l	**ECV** mg/l
Total isolates N=377	FLC	377							1/0.5	2/0.5	16/4	40/10	53/14	75/20	74/20	74/20	22/6	20/5	5.3	8.0	16.0	32.0	64.0	64.0
	CLO	298					119/40	96/32	52/17	19/6	0/0	0/0	1/0.5	1/0.5	5/2	2/0.5	3/1	1/0.5	0.3	0.063	0.125	0.25	0.5	0.5
	MCO	326					97/29	58/18	59/18	42/13	49/14	9/3	1/0.5	2/0.5	0/0	4/1.5	2/0.5	5/2	0.4	0.063	0.25	0.5	1.0	1.0
	ITR	28								4/14	13/46	11/40							1.1	1.0	1.0	2.0	2.0	4.0
	KET	298					1/0.5	1/0.5	3/1	8/2	40/14	42/14	45/15	61/21	49/16	48/16			3.2	8.0	8.0	16.0	32.0	32.0
	POS	51			12/24	13/25	15/29	0/0	3/6	4/8	0/0	4/8							0.1	0.063	0.063	0.5	2.0	0.25
	VOR	377		3/1	46/12	86/23	89/24	83/22	36/10	10/2	2/1	0/0	0/0	0/0	13/3	9/2			0.2	0.063	0.063	0.125	0.25	0.25
	CIC	326				2/1	30/9	38/12	50/15	77/24	73/22	33/10	18/5	1/0.5	3/1	1/0.5			0.6	0.5	0.5	1.0	2.0	2.0
	TER	326		81/24	42/13	60/18	101/31	19/6	7/2	3/1	2/1	0/0	5/2	0/0	1/0.5	4/1	1/0.5		0.1	0.063	0.031	0.063	0.125	0.125
	ABA	298		13/4	24/8	57/19	104/35	68/23	14/5	4/1	3/1	0/0	5/2	0/0	1/0.5	4/1	1/0.5		0.2	0.063	0.063	0.125	0.25	0.25
	BIF	28							1/4	4/14	17/61	6/21							1.0	1.0	1.0	1.0	2.0	4.0
	GRS	28						8/29	8/29	9/32	3/10								0.4	0.5	0.25	0.5	1.0	1.0
	NYS	28								17/61	11/39								0.8	0.5	0.5	1.0	1.0	2.0
	AMB	51					1/2	14/27	10/20	3/6	1/2	0/0	0/0	22/43					1.0	8.0	0.5	8.0	8.0	2.0
	FCY	51														3/6	48/94		17.4	64.0	64.0	64.0	64.0	256.0
	ANF	51	24/47	21/41	0/0	4/8	2/4												0.03	0.004	0.008	0.008	0.031	0.031
	CSF	51		1/2	16/31	15/30	18/35	1/2											0.09	0.063	0.031	0.063	0.063	0.125
	MCF	51	26/51	19/37	1/2	5/10													0.03	0.004	0.004	0.008	0.016	0.016
Epidermophyton floccosum N=15	FLC	15											1/7	4/26	9/60	1/7			5.8	16.0	16.0	16.0	16.0	64.0
	CLO	15					1/7	8/53	6/40										0.3	0.125	0.125	0.25	0.25	0.5
	MCO	15					1/7	1/7	0/0	7/46	6/40								0.6	0.5	0.5	1.0	1.0	2.0
	KET	15											4/27	10/66	1/7				3.8	8.0	8.0	8.0	8.0	32.0
	VOR	15					3/20	8/53	4/27										0.2	0.125	0.125	0.25	0.25	0.5
	CIC	15					1/7	1/7	0/0	3/20	10/66								0.7	1.0	0.5	1.0	1.0	2.0
	TER	15		2/13	0/0	1/7	9/60	3/20											0.1	0.063	0.063	0.063	0.125	0.25
	ABA	15			1/7	0/0	2/13	10/67	2/13										0.2	0.125	0.125	0.125	0.25	0.5
Trichophyton mentagrophytes N=98	FLC	98								2/2	0/0	6/6	18/18	26/27	13/13	25/26	6/6	2/2	5.4	8.0	8.0	32.0	32.0	32.0
	CLO	51					24/47	19/37	6/12	2/4									0.2	0.063	0.125	0.125	0.25	0.5
	MCO	79					8/10	12/15	17/22	13/16	22/28	7/9							0.5	1.0	0.5	1.0	1.0	2.0
	ITR	28								4/14	13/47	11/39							1.1	1.0	1.0	1.0	2.0	4.0
	KET	51						1/2	0/0	0/0	2/4	8/16	13/25	11/21	8/16	8/16			3.6	4.0	8.0	16.0	32.0	32.0
	POS	19			10/53	3/16	6/31												0.08	0.016	0.016	0.063	0.063	0.063
	VOR	98		1/1	7/7	30/31	19/19	28/29	11/11	2/2									0.2	0.031	0.063	0.125	0.25	0.5
	CIC	79					1/1	3/4	12/15	7/9	21/27	20/25	15/19						1.1	1.0	1.0	2.0	4.0	4.0
	TER	79	11/14	22/28	13/16	33/42													0.09	0.063	0.031	0.063	0.063	0.125
	ABA	51			7/14	6/11	26/51	8/16	3/6	1/2									0.1	0.063	0.063	0.063	0.125	0.25
	BIF	28							1/4	4/14	17/61	6/21							1.0	1.0	1.0	1.0	2.0	4.0
	GRS	28						8/29	8/29	9/31	1/11								0.04	0.5	0.25	0.5	1.0	1.0
	NYS	28								17/61	11/39								0.8	0.5	0.5	1.0	1.0	2.0
	AMB	19						5/26	6/32	3/16	1/5	0/0	0/0	4/21					0.6	0.25	0.25	1.0	8.0	1.0
	FCY	19															19/100		17.9	64.0	64.0	64.0	64.0	256.0
	ANF	19	16/84	3/16															0.02	0.004	0.004	0.004	0.008	0.016
	CSF	19			4/21	13/68	2/11												0.09	0.031	0.031	0.031	0.063	0.125
	MCF	19	18/95	1/5															0.02	0.004	0.004	0.004	0.004	0.016

ISSN 2347-6893

Table 2: MIC distribution (number/percentage in rounded figures), geometric mean of MIC (**MIC$_{gmean}$**), mode MIC (**MIC$_{mod}$**), the 50th, 75th, and 90th percentile (**MIC$_{50}$, MIC$_{75}$, MIC$_{90}$**), and epidemiological cut-off value (**ECV**) of the antifungal agents (AFA) voriconazole (VOR), posaconazole (POS), ketoconazole (KET), fluconazole (FLC), itraconazole (ITR), miconazole (MCZ), clotrimazole (CLO), anidulafungin (ANI), caspofungin (CAS), micafungin (MCA), ciclopiroxolamine (CIC), terbinafine (TER), abafungin (ABA), flucytosine (FCY), amphotericin B (AMB), and partly for bifonazole (BIF), and griseofulvin (GRF) for the clinical isolates of *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Microsporum canis*.

			Number and % of isolates at individual log$_2$-dilution (mg/l):																Characteristic MIC-values					
Organism	**AFA**	**N**	**0.004**	**0.008**	**0.016**	**0.031**	**0.063**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**MIC$_{gmean}$**	**MIC$_{mode}$**	**MIC$_{50}$**	**MIC$_{75}$**	**MIC$_{90}$**	**ECV**
			n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Trichophyton rubrum N=200	FLC	200							1/1	0/0	12/6	33/16	32/15	38/19	43/21	31/16	3/2	7/4	4.3	16.0	8.0	16.0	32.0	32.0
	CLO	180					88/48	60/33	27/15	3/2	0/0	0/0	0/0	0/0	1/1	0/0	0/0	1/1	0.2	0.063	0.125	0.125	0.25	0.5
	MCO	180					86/47	42/23	34/19	11/6	3/2	0/0	0/0	1/1	0/0	1/1	0/0	2/1	0.3	0.063	0.125	0.25	0.5	0.5
	KET	180					1/1	0/0	2/1	8/4	38/21	33/18	28/16	25/14	22/12	23/13			2.6	1.0	4.0	16.0	32.0	16.0
	POS	20			1/5	9/45	8/40	0/0	2/10										0.1	0.063	0.063	0.063	0.125	0.25
	VOR	200		2/1	36/18	53/26	62/31	37/19	4/2	2/1	0/0	0/0	0/0	0/0	2/1	2/1			0.1	0.063	0.063	0.063	0.125	0.25
	CIC	180				2/1	26/14	33/18	36/20	56/31	18/10	5/2	1/1	1/1	1/1	1/1			0.4	0.5	0.25	0.5	1.0	1.0
	TER	180		63/35	17/9	45/25	44/24	6/3	2/1	1/1	0/0	0/0	1/1	0/0	0/0	1/1			0.08	0.008	0.031	0.063	0.063	0.125
	ABA	180		13/7	13/7	47/26	63/35	36/20	4/2	2/1	0/0	0/0	1/1	0/0	0/0	1/1			0.1	0.063	0.063	0.063	0.125	0.25
	AMB	20						9/45	1/5	0/0	0/0	0/0	0/0	10/50					1.0	8.0	2.0	8.0	8.0	8.0
	FCY	20														2/10	18/90		17.0	64.0	64.0	64.0	64.0	256.0
	ANF	20	4/20	14/70	0/0	0/0	2/10												0.04	0.008	0.008	0.008	0.031	0.031
	CSF	20			1/5	2/10	16/80	1/5											0.1	0.063	0.063	0.063	0.063	0.25
	MCF	20	4/20	14/70	0/0	2/10													0.04	0.008	0.008	0.008	0.016	0.031
Trichophyton tonsurans N=28	FLC	28										1/4	2/7	3/10	2/7	8/29	11/39	1/4	10.1	64.0	32.0	64.0	64.0	128.0
	CLO	24						4/17	9/38	11/45									0.4	0.5	0.25	0.5	0.5	1.0
	MCO	24					2/8	1/4	4/17	6/25	11/46								0.6	1.0	0.5	1.0	1.0	2.0
	KET	24							1/4	0/0	0/0	1/4	0/0	11/46	20/42	¼			4.7	8.0	8.0	8.0	16.0	32.0
	POS	4			1/25	1/25	1/25	0/0	1/25										0.1	0.125	0.063	0.125	0.25	0.25
	VOR	28			1/4	2/7	2/7	5/18	10/36	6/21	2/7								0.3	0.25	0.25	0.25	0.5	1.0
	CIC	24							2/8	1/4	4/17	6/25	11/46						0.8	1.0	1.0	1.0	1.0	4.0
	TER	24		2/8	0/0	1/4	9/38	6/25	3/13	2/8	1/4								0.2	0.063	0.125	0.25	0.5	0.5
	ABA	24				1/4	8/33	8/33	4/17	1/4	2/9								0.3	0.063	0.125	0.25	0.5	0.5
	AMB	4							1/25	0/0	0/0	0/0	0/0	3/75					2.3	8.0	8.0	8.0	8.0	32.0
	FCY	4														1/25	4/75		15.8	64.0	64.0	64.0	64.0	256.0
	ANF	4	2/50	2/50															0.04	0.004	0.016	0.031	0.031	0.063
	CSF	4		1/25	3/75														0.05	0.016	0.016	0.016	0.016	0.063
	MCF	4	2/50	1/25	1/25														0.04	0.004	0.008	0.031	0.031	0.031
Microsporum canis N=36	FLC	36									4/11	0/0	0/0	4/11	7/19	9/25	2/6	10/28	9.3	128.0	32.0	128.0	128.0	128.0
	CLO	28					6/21	5/18	4/14	3/11	0/0	0/0	1/4	1/4	4/14	2/7	2/7		0.9	0.063	0.25	16.0	32.0	1.0
	MCO	28						2/7	4/14	5/18	5/18	2/7	1/4	1/4	0/0	3/10	2/7	3/11	1.9	0.5	1.0	32.0	128.0	4.0
	KET	28												4/14	8/29	16/57			8.4	32.0	32.0	32.0	32.0	128.0
	POS	8								4/50	0/0	4/50							1.0	0.5	1.0	1.0	2.0	4.0
	VOR	36			2/6	1/3	3/8	5/14	7/19	0/0	0/0	0/0	0/0	0/0	11/31	7/19			1.3	16.0	2.0	16.0	32.0	8.0
	CIC	28					2/7	1/4	0/0	4/14	10/36	7/25	2/7	0/0	2/7				1.1	1.0	1.0	2.0	4.0	4.0
	TER	28		3/11	3/11	0/0	6/20	4/14	2/7	0/0	1/4	0/0	4/14	0/0	1/4	3/11	1/4		0.5	0.063	0.125	4.0	32.0	0.5
	ABA	28			3/11	3/11	5/17	6/20	1/4	0/0	1/4	0/0	4/14	00	1/4	3/11	1/4		0.5	0.125	0.125	4.0	32.0	0.5
	AMB	8					1/13	0/0	2/25	0/0	0/0	0/0	0/0	5/63					1.5	8.0	8.0	8.0	8.0	32.0
	FCY	8															8/100		17.9	64.0	64.0	64.0	64.0	256.0
	ANF	8	2/25	4/50	2/25														0.031	0.008	0.008	0.016	0.031	0.031
	CSF	8			8/100														0.063	0.016	0.016	0.016	0.016	0.063
	MCF	8	2/25	4/50	0/0	2/25													0.031	0.008	0.008	0.016	0.031	0.031

ISSN 2347-6893

Table 3: Comparison of characteristic MIC-values of the antifungal agents (AFA) amphotericin B (APH), flucytosine (FCY), fluconazole (FLC), posaconazole (POS), voriconazole (VOR), anidulafungin (ANF), caspofungin (CSF), and micafungin (MCF) obtained by microdilution testing of *Trichophyton rubrum* and *T. tonsurans,* and *T. mentagrophytes* with YST medium (DIN) and RPMI medium (CLSI), together with available MIC-data from the literature (Lit. Ref.).

Organism	AFA	N	Comparison of characteristic MIC-values: YST Medium % ≤ MIC_{75}	% ≤ ECV	MIC_{range} mg/l	MIC_{50} mg/l	MIC_{90} mg/l	MIC_{mean} mg/l	RPMI Medium % ≤ MIC_{75}	% ≤ ECV	MIC_{range} mg/l	MIC_{50} mg/l	MIC_{90} mg/l	MIC_{mean} mg/l	AFA	MIC-data from literature N	MIC_{range} mg/l	MIC_{50} mg/l	MIC_{90} mg/l	MIC_{mean} mg/l	Lit. Ref.
Trichophyton rubrum	APH	20	100	100	).125-8.0	0.125	8.0	1.0	100	100	125-0.25	0.125	0.25	0.3	FLC	132		1.0			[7]
	FCY	20	100	100	ʻ4.0-64.0	64.0	64.0	17.0	100	100	64.0	64.0	64.0	64.0		5	1.0-4.0	4.0		6.3	[12]
	FLC	20	90	100	25-128.0	8.0	32.0	4.3	90	100	1.0-32.0	2.0	16.0	2.3		20		0.5	4.0		[38][a]
	POS	20	65	85	).008-0.5	0.063	0.125	0.1	65	85	0.08-0.5	0.063	0.5	0.08		30		16.0	32.0		[39]
	VOR	20	90	90	008-32.0	0.063	0.125	0.1	90	90	016-0.25	0.031	0.125	0.03		27	2.0-32.0	8.0	32.0	7.6	[40]
	ANF	20	95	100	04-0.125	0.008	0.031	0.04	90	90	04-0.125	0.008	0.031	0.04	ITR	30		0.125	0.5		[39]
	CSF	20	10	90	16-0.125	0.063	0.063	0.1	15	90	).031-0.5	0.063	0.25	0.06		27	0.031-4.0	0.125	0.5	0.1	[40]
	MCF	20	100	100	04-0.031	0.008	0.016	0.04	100	100	004-0.31	0.008	0.016	0.04	KET	27	0.031-4.0	0 .063	4.0	0.13	[40]
															VOR	5	016-0.063	0.031		0.03	[12]
															CSF[b]	82			1.0		[35]
															MCF[b]	82			0.03		[35]
Trichophyton tonsurans	APH	4	100	100	).125-0.5	8.0	8.0	0.3	100	100	125-0.25	0.25	0.25	0.3	FLC	42		1.0			[7]
	FCY	4	100	100	ʼ2.0-64.0	64.0	64.0	17.9	100	100	ʻ4.0-64.0	64.0	64.0	64.0		5	2.0-8.0	4.0		6.3	[12]
	FLC	28	100	100	:.0-128.0	32.0	64.0	3.3	100	100	2.0-8.0	8.0	8.0	3.3	VOR	5	008-0.031	0.031		0.03	[12]
	POS	4	75	100	016-0.25	0.063	0.25	0.8	75	100	031-0.25	0.063	0.25	0.2	CSF[b]	82			1.0		
	VOR	28	100	100	).016-1.0	0.25	0.5	0.1	75	100	16-0.125	0.031	0.063	0.1	MCF[b]	82			0.03		
	ANF	4	100	100	04-0.031	0.016	0.031	0.04	100	100	04-0.031	0.016	0.031	0.04							
	CSF	4	100	100	16-0.031	0.016	0.016	0.06	100	100	16-0.031	0.016	0.031	0.06							
	MCF	4	100	100	04-0.031	0.008	0.031	0.04	100	100	04-0.031	0.008	0.031	0.04							
Trichophyton mentagrophytes	APH	19	100	100	).125-8.0	0.25	8.0	0.6	100	100	).125-0.5	0.25	0.5	0.4	FLC	20		0.5	4.0		[38][a]
	FCY	19	100	100	ʻ4.0-64.0	64.0	64.0	64.0	100	100	ʻ4.0-64.0	64.0	64.0	64.0		52		32.0	64.0		[39]
	FLC	19	100	100	).5-128.0	8.0	32.0	5.4	94	100	0.5-16.0	4.0	8.0	4.0		14	4.0-16.0	16.0	16.0	11.3	[40]
	POS	19	94	94	016-0.25	0.031	0.063	0.08	94	100	016-0.25	0.031	0.063	0.09	ITR	14	).031-0.25	0.125	0.25	0.09	[40]
	VOR	19	100	100	08-0.063	0.0631	0.25	0.09	100	100	16-0.063	0.016	0.063	0.08	KET	14	0.031-1	0.125	0.25	0.12	[40]
	ANF	19	100	100	04-0.008	0.004	0.008	0.02	89	100	04-0.008	0.004	0.008	0.03	CSF[b]	82			0.5		[35]
	CSF	19	100	56	16-0.063	0.031	0.063	0.09	78	100	16-0.063	0.031	0.063	0.09	MCF[b]	82			0.03		[35]
	MCF	19	100	100	04-0.008	0.004	0.004	0.02	89	100	04-0.008	0.004	0.008	0.02							

ISSN 2347-6893

Table 4: Percentage (%) of concordance (**±0**) and/or differences in MIC-log$_2$-dilutions (**±1, ±2)** of the antifungal agents (AFA) amphotericin B (APH), flucytosine (FCY), fluconazole (FLC), posaconazole (POS), voriconazole (VOR), anidulafungin (ANF), caspofungin (CSF), and micafungin (MCF), when they are tested in parallel with YST and RPMI medium by microdilution against clinical isolates of *Microsporum canis, Trichophyton mentagrophytes, T. rubrum*, and *T. tonsurans,* and the two mean endpoint readings (8.5=9d and 9.7=10d) are compared.

		AFA-specific MIC log$_2$-differences when both endpoint readings are compared from the same medium (YST / RPMI) or between both media (YST – RPMI)																			
		YST medium 9d - 10d					RPMI medium 9d - 10d					YST - RPMI 9d					YST - RPMI 10d				
Organism	**AFA**	Δ log$_2$MIC			Correlation		Δ log$_2$MIC			Correlation		Δ log$_2$MIC			Correlation		Δ log$_2$MIC			Correlation	
		±0	±1	±2	*r*	p	±0	±1	±2	*r*	p	±0	±1	±2	*r*	p	±0	±1	±2	*r*	p
Microsporum canis N=36	APH	66	98	100	0.5851	<.0001	72	98	100	0.5851	<.0001	84	96	100	0.9561	0.0002	92	98	100	0.9562	0.0002
	FCY	98	100		-	-	98	100		-	-	98	100		-	-	98	100		-	-
	FLC	76	98	100	-0.1631	0.2577	70	98	100	0.4088	0.0032	82	100		-0.3614	0.3791	100			1.0	<.0001
	POS	72	88	97	0.9145	<.0001	84	98	100	0.9259	<.0001	86	96	100	0.9562	0.0004	86	98	100	0.9561	0.0002
	VOR	80	98	100	0.9021	<.0001	0.0032	98	100	0.9150	<.0001	100			1.0	<.0001	92	100		1.0	<.0001
	ANF	92	98	100	0.9200	<.0001	0.0032	100		0.9201	<.0001	90	100		0.8982	0.0024	90	100		0.8982	0.0024
	CSF	82	96	100	0.9229	<.0001	84	96	100	0.9413	<.0001	92	100		1.0	<.0001	94	100		1.0	<.0001
	MCF	92	100		0.8974	<.0001	90	100		0.8960	<.0001	96	100		1.0	<.0001	96	100		1.0	<.0001
	Mean	82	97	100			84	99	100			91	99	100			90	99	100		
Trichophyton mentagrophytes N=50	APH	67	98	100	0.5746	<.0001	72	98	100	0.5851	<.0001	78	89	100	0.7261	0.0004	83	94	100	0.7262	0.0006
	FCY	98	100		-	-	98	100		-	-	100			1	-	100			1	-
	FLC	76	98	100	0.9220	<.0001	70	98	100	0.9132	<.0001	89	100		1.0	<.0001	100			1.0	<.0001
	POS	72	88	94	0.9145	<.0001	76	86	94	0.9288	<.0001	95	100		0.9138	<.0001	89	100		0.9138	<.0001
	VOR	80	98	100	0.9021	<.0001	84	98	100	0.9151	<.0001	100			1.0	<.0001	100			1.0	<.0001
	ANF	92	98	100	0.9200	<.0001	89	100		0.9201	<.0001	83	100		0.5701	0.0135	83	100		0.5701	0.0135
	CSF	82	96	100	0.9220	<.0001	89	100		0.9413	<.0001	100			1.0	<.0001	100			1.0	<.0001
	MCF	92	100		0.8974	<.0001	89	100		0.8926	<.0001	89	100		0.6614	0.0028	89	100		0.6614	0.0028
	Mean	82	97	99			83	98	99			92	98	100			93	99		100	
Trichophyton rubrum N=50	APH	66	98	100	0.6545	<.0001	72	98	100	0.6180	<.0001	100			1.0	<.0001	100			1.0	<.0001
	FCY	100			-	-	100			-	-	100			1	-	100			-	-
	FLC	76	98	100	0.9413	<.0001	70	98	100	0.9349	<.0001	75	100		1.0	<.0001	100			1.0	<.0001
	POS	72	88	94	0.9117	<.0001	76	86	94	0.9120	<.0001	90	100		0.9419	<.0001	85	100		0.9419	<.0001
	VOR	80	98	100	0.9234	<.0001	84	98	100	0.9334	<.0001	100			0.8785	<.0001	85	100		0.8785	<.0001
	ANF	92	98	100	0.9356	<.0001	92	100		0.9201	<.0001	100			0.9994	<.0001	95	95	100	0.9994	<.0001
	CSF	82	98	100	0.8866	<.0001	84	96	100	0.9413	<.0001	100			0.6474	0.0014	85	10		0.6647	0.0014
	MCF	92	100		0.9555	<.0001	90	100		0.8960	<.0001	100			1.0	<.0001	100			1.0	<.0001
	Mean	83	97	99			84	97	99			96	100				94	99	100	19/19	2/4
Trichophyton tonsurans N=4	APH	66	98	100	0.5747	<.0001	72	98	100	0.5851	<.0001	50	100		1.0	<.0001	100			1.0	<.0001
	FCY	100			-	-	100			-	-	100			1	-	100			1	-
	FLC	76	98	100	0.9221	<.0001	70	98	100	0.9132	<.0001	75	100		1.0	<.0001	100			1.0	<.0001
	POS	72	88	94	0.9145	<.0001	76	86	94	0.9253	<.0001	50	100		0.8333	0.1667	75	100		0.8333	0.1667
	VOR	80	98	100	0.9021	<.0001	84	98	100	0.9150	<.0001	100			0.7918	0.1717	75	100		0.8165	0.1835
	ANF	92	98	100	0.9326	<.0001	92	100		0.9211	<.0001	100			1.0	<.0001	100			1.0	<.0001
	CSF	82	96	100	0.9228	<.0001	84	96	100	0.9421	<.0001	50	100		1.0	<.0001	100			1.0	<.0001
	MCF	92	100		0.8971	<.0001	90	100		0.9060	<.0001	100			1.0	<.0001	100			1.0	<.0001
	Mean	83	97	99			84	97	99			78	100				94	100		3/3	6/1

ISSN 2347-6893

Table 5a: Characteristic MIC-values of repeated (N) testings (MIC-range with minimum MIC (**MIC_{min}**), maximum MIC (**MIC_{max}**), log2-difference of maximum MIC and minimum MIC (nxlog$_2$), geometric mean of MIC (**MIC_{mean}**), standard deviation of the mean (**STD of MIC_{mean}**), mode MIC (**MIC_{mode}**), the percentage MICs within ±1, ±2, and ±3 log2-dilutions **(% within log$_2$-dilution**), and possible QC ranges (**QC MIC-range)** for *Epidermophyton floccosum*, and *Microsporum canis*.

Control strain	AFA	N	MIC-range		Δ	MIC_{mean}	STD of MIC_{mean}	MIC_{mode}	% within n log$_2$-dilution			Possible QC MIC-range
			MIC_{min}	MIC_{max}					±1	±2	±3	
			mg/l	mg/l	nxlog$_2$	mg/l	±nxlog$_2$	mg/l	%	%	%	mg/l
Epidermophyton floccosum DSM 10709	VOR	8	0.016	0.125	3	0.06	2	0.125	75	100	100	0.031-0.5
	KET	8	2.0	4.0	1	3.4	1	4.0	100	100	100	2.0-8.0
	FLC	8	4.0	8.0	1	5.7	1	4.0	100	100	100	2.0-8.0
	MCZ	8	0.063	0.125	1	0.09	1	0.063	100	100	100	0.031-0.125
	CLO	8	0.063	0.125	1	0.09	1	0.063	100	100	100	0,031-0.125
	CIC	8	0.063	0.5	3	0.2	3	0.063	75	100	100	0.016-0.25
	TER	8	0.008	0.063	3	0.02	2	0.016	100	100	100	0.004-0.063
	ABA	8	0.016	0.125	3	0.04	2	0.016	75	100	100	0.004-0.063
Microsporum canis ATCC 28327	VOR	9	0.125	0.5	2	0.2	2	0.125	100	100	100	0.031-0.5
	POS	2	0.5	0.5	0	0.5	1	0.5	100	100	100	0.25-1.0
	KET	9	8.0	32.0	2	23.5	2	32.0	89	100	100	8.0-128.0
	FLC	9	0.5	32.0	6	5.9	8	32.0	78	100	100	8.0-128.0
	ITR	1	2.0	2.0	0	2.0	-	2.0	100	100	100	1.0-4.0
	MCZ	9	0.125	1.0	3	0.5	2	0.5	100	100	100	0.125-2.0
	CLO	9	0.063	0.5	3	0.2	2	0.125	100	100	100	0.031-0.5
	ANF	2	0.008	0.008	0	0.008	1	0.125	100	100	100	0.063-0.25
	CSF	2	0.016	0.016	0	0.016	1	0.016	100	100	100	0.008-0.031
	MCF	2	0.008	0.008	0	0.008	1	0.008	100	100	100	0.004-0.016
	CIC	9	0.5	2.0	2	0.9	2	0.5	78	100	100	0.125-2.0
	TER	9	0.063	0.25	2	0.1	2	0.063	100	100	100	0.016-0.25
	ABA	9	0.125	0.25	1	0.2	1	0.125	100	100	100	0.063-0.25
	BIF	1	2.0	2.0	0	2.0	1	2.0	100	100	100	1.0-4.0
	GRF	1	0.25	0.25	0	0.25	1	0.25	100	100	100	0.125-0.5
	FCY	2	64	64	0	64	1	64.0	100	100	100	32.0-128.0
	AMB	2	0.25	0.25	0	0.25	1	0.25	100	100	100	0.125-0.5
Microsporum canis DSM 10708	VOR	2	0.25	0.25	0	0.25	1	0.25	100	100	100	0.125-0.5
	KET	2	16.0	16.0	0	16.0	1	16.0	100	100	100	8.0-32.0
	FLC	2	16.0	16.0	0	16.0	1	16.0	100	100	100	8.0-32.0
	MCZ	2	0.25	0.25	0	0.25	1	0.25	100	100	100	0.125-0.5
	CLO	2	0.063	0.063	0	0.063	1	0.063	100	100	100	0.031-0.125
	CIC	2	2.0	2.0	0	2.0	1	2.0	100	100	100	1.0-4.0
	TER	2	0.125	0.125	0	0.125	1	0.125	100	100	100	0.063-0.25
	ABA	2	0.25	0.25	0	0.25	1	0.25	100	100	100	0.125-0.5

ISSN 2347-6893

Table 5b: Characteristic MIC-values of repeated (N) testings (MIC-range with minimum MIC (**MIC_{min}**), maximum MIC (**MIC_{max}**), log2-difference of maximum MIC and minimum MIC (nxlog2), geometric mean of MIC (**MIC_{mean}**), standard deviation of the mean (**STD of MIC_{mean}**), mode MIC (**MIC_{mode}**), the percentage MICs within ±1, ±2, and ±3 log2-dilutions (**% within log_2-dilution**), and possible QC ranges (**QC MIC-range**) for *Trichophyton mentagrophytes*.

Control strain	AFA	N	MIC-RANGE MIC_{min} mg/l	MIC_{max} mg/l	Δ $nxlog_2$	MIC_{mean} mg/l	STD of MIC_{mean} $\pm nxlog_2$	MIC_{mode} mg/l	% within n log_2-dilution ±1 %	±2 %	±3 %	Possible QC MIC-range mg/l
	VOR	31	0.031	0.5	4	0.1	2	0.125	84	100	100	0.031-0.5
	POS	19	0.031	2.0	6	0.2	4	0.5	84	100	100	0.125-2.0
	KET	31	1.0	32.0	5	2.3	2	1	61	94	100	0.25-4.0
	FLC	31	2.0	32.0	4	13.7	2	32	59	87	100	8.0-128.0
	ITR	7	2.0	4.0	1	2.4	1	2	100	100	100	1.0-4.0
	MCZ	31	0.063	0.5	3	0.1	2	0.063	55	90	100	0.016-0.25
	CLO	31	0.063	0.125	1	0.08	1	0.063	100	100	100	0.031-0.125
Trichophyton mentagrophytes ATCC 9533	ANF	15	0.004	16.0	12	0.01	9	0.004	80	87	93	0.001-0.016
	CSF	15	0.031	16.0	9	0.07	5	0.031	80	93	100	0.008-0.125
	MCFA	15	0.004	16.0	12	0.02	10	0.004	73	93	100	0001-0.016
	CIC	31	0.063	2.0	5	0.3	4	0.063	42	55	68	0.016-0.25
	TER	31	0.008	0.063	3	0.04	2	0.063	84	87	100	0.016-0.25
	ABA	31	0.031	0.125	2	0.07	1	0.063	100	100	100	0.031-0.125
	BIF	2	1.0	2.0	1	1.4	2	-	100	100	100	1.0-4.0
	GRF	2	0.125	0.5	1	0.2	2	-	100	100	100	0.125-0.5
	FCY	15	64	64	0	64.0	1	64	100	100	100	32.0-128.0
	APH	15	0.125	0.5	2	0.2	2	0.25	100	100	100	0.063-1.0
	VOR	18	0.031	0.25	3	0.1	2	0.125	89	100	100	0.031-0.5
	POS	18	0.125	0.5	2	0.3	2	0.25	100	100	100	0.063-1.0
	KET	18	1.0	32.0	5	4.7	3	4.0	78	100	100	1.0-16.0
	FLC	18	8.8	64.0	3	25.4	3	64.0	50	72	100	16.0-256.0
	ITR	4	2.0	2.0	0	2.0	1	2.0	100	100	100	1.0-4.0
	MCZ	18	0.063	1.0	4	0.2	2	0.25	78	100	100	0.063-1.0
	CLO	18	0.063	125	1	0.09	1	0.063	100	100	100	0.031-0.125
Trichophyton mentagrophytes ATCC 18748	ANF	6	0.004	0.063	4	0.02	4	0.004	100	10	100	0.001-0.016
	CSF	6	0.125	4.0	5	0.4	6	0.125	100	100	100	0.031-0.5
	MCF	6	0.004	0.008	1	0.006	1	0.008	100	100	100	0.004-0.016
	CIC	18	0.25	2.0	3	0.7	2	0.25	78	100	100	0.063-1.0
	TER	18	0.016	0.125	3	0.07	2	0.063	100	100	100	0.016-0.25
	ABA	18	0.063	0.25	2	0.09	2	0.063	89	100	100	0.016-0.25
	BIF	1	1.0	1.0	0	1.0	1	1.0	100	100	100	0.5-2.0
	GRF	1	0.5	0.5	0	0.5	1	0.5	100	100	100	0.25-1.0
	FCY	6	64.0	64.0	0	64.0	1	64.0	100	100	100	32.0-128.0
	APH	6	0.125	0.125	0	0.1	1	0.125	100	100	100	0.063-0.25

Table 5c: Characteristic MIC-values of repeated (N) testings (MIC-range with minimum MIC (**MIC_{min}**), maximum MIC (**MIC_{max}**), log2-difference of maximum MIC and minimum MIC (nxlog_2), geometric mean of MIC (**MIC_{mean}**), standard deviation of the mean (**STD of MIC_{mean}**), mode MIC (**MIC_{mode}**), the percentage MICs within ±1, ±2, and ±3 log2-dilutions **(% within log_2-dilution**), and possible QC ranges (**QC MIC-range)** for *Trichophyton rubrum and Trichophyton tonsurans.*

Control strain	AFA	N	MIC-RANGE MIC_{min} mg/l	MIC-RANGE MIC_{max} mg/l	nxlog_2	MIC_{mean} mg/l	STD of MIC_{mean} ±nxlog_2	MIC_{mode} mg/l	% within log_2-dilution ±1 %	±2 %	±3 %	Possible QC MIC-range mg/l
Trichophyton rubrum DSM 4147	VOR	18	0.063	0.25	2	0.1	1	0.125	100	100	100	0.063-0.25
	POS	18	0.25	0.5	1	0.3	1	0.125	100	100	100	0.063-0.25
	KET	18	1.0	32.0	5	4.2	3	1.0	56	83	89	0.25-4.0
	FLC	18	2.0	64.0	5	21.0	2	16.0	50	94	100	4.0-64.0
	ITR	4	2.0	2.0	0	2.0	1	2.0	100	100	100	1.0-4.0
	MCZ	18	0.063	0.5	3	0.2	2	0.063	44	72	100	0.016-0.25
	CLO	18	0.063	1.0	4	0.2	3	0.063	56	83	89	0.016-0.25
	ANF	8	0.032	0.063	1	0.04	1	0.031	100	100	100	0.016-0.063
	CSF	8	0.125	0.125	0	0.125	1	0.125	100	100	100	0.063-0.25
	MCF	8	0.031	0.063	1	0.04	1	0.031	100	100	100	0.016-0.063
	CIC	18	0.063	1.0	4	0.4	2	0.25	89	100	100	0.063-1.0
	TER	18	0.008	0.25	5	0.07	3	0.063	78	100	100	0.016-0.25
	ABA	18	0.031	0.25	2	0.06	1	0.063	100	100	100	0.031-0.125
	FCY	8	64.0	64.0	0	64.0	1	64.0	100	100	100	32.0-128.0
	APH	8	0.125	0.125	0	0.125	1	0.125	100	100	100	0.063-0.25
Trichophyton tonsurans DSM 12285	VOR	12	0.063	0.25	2	0.1	2	0.063	75	100	100	0.016-0.25
	POS	12	0.25	1.0	2	0.5	2	0.25	58	100	100	0.063-1.0
	KET	12	8.0	32.0	2	16.0	2	16.0	100	100	100	4.0-64.0
	FLC	12	4.0	64.0	4	22.6	3	64.0	58	67	83	16.0-256.0
	ITR	2	4.0	4.0	0	4.0	1	4.0	100	100	100	2.0-8.0
	MCZ	12	0.25	2.0	3	0.5	2	0.5	92	100	100	0.125-2.0
	CLO	12	0.125	0.25	1	0.2	1	0.25	100	100	100	0.125-0.5
	ANF	4	0.004	0.004	0	0.004	1	0.004	100	100	100	0.002-0.008
	CSF	4	0.008	0.008	0	0.008	1	0.008	100	100	100	0.004-0.016
	MCF	4	0.004	0.004	0	0.004	1	0.004	100	100	100	0.002-0.008
	CIC	12	0.5	2.0	2	0.09	1	1.0	100	100	100	0.5-2.0
	TER	12	0.016	0.063	1	0.05	2	0.063	67	100	100	0.016-0.25
	ABA	12	0.063	64.0	8	0.2	8	0.125	100	100	100	0.031-0.5
	FCY	4	64.0	64.0	0	64.0	1	64.0	100	100	100	32.0-128.0
	APH	4	0.25	0.25	0	0.25	1	0.25	100	100	100	0.125-0.5