Antifungal Susceptibility of Japanese Isolates of *Nannizia fulva* (Formerly *Microsporum fulvum*)

Rui Kano¹, Karin Oshimo¹, Teru Fukutomi² and Hiroshi Kamata¹

¹Department of Veterinary Pathobiology, Nihon University College of Bioresource Sciences School of Veterinary Medicine
²Bright Pet Clinic

ABSTRACT

Human and animal dermatophytoses are most commonly treated with systemic antifungal drugs such as itraconazole (ITZ) and terbinafine (TRF). The antifungal susceptibility of *Nannizia fulva*, however, remains poorly documented. In the present study, we investigated the *in vitro* susceptibility of *N. fulva* to ITZ and TRF using the CLSI M38-A2 test. The mean MICs for the 12 tested strains were 0.6542 mg/L (range: 0.0625-1 mg/L) for ITZ and 0.15625 mg/L (range: < 0.003125-0.5 mg/L) for TRF. These results indicate that ITZ and TRF at standard veterinary doses should be efficacious against *N. fulva*.

Key words: Antifungal susceptibility, geophilic dermatophyte, itraconazole, *Nannizia fulva*, terbinafine

Members of the *Microsporum gypseum* complex are geophilic dermatophytes with worldwide distribution and occasionally have been isolated as infectious agents in humans and animals¹⁻³. The teleomorphs of the complex consist of *Nannizia fulva* (formerly *Microsporum fulvum* and *Arthroderma fulvum*), *N. gypsea*, and *Nannizia incurvata*¹⁻⁴. In 1982, Hironaga et al. identified *N. gypsea* and *N. incurvata*, but not *N. fulva*, among *M. gypseum* strains isolated from human skin lesions in Japan⁵. However, in our previous study, we reported the first isolation of *N. fulva* from soils in rabbit hutches in public primary schools in Japan⁶. Given that *N. fulva* occasionally may cause infection in humans and animals, the prevalence of this organism should be assessed.

Human and animal dermatophytoses are most commonly treated with systemic antifungal drugs such as itraconazole (ITZ) and terbinafine (TRF)³⁻⁷. However, the antifungal susceptibility of *N. fulva* remains poorly documented. Antifungal susceptibility of dermatophytes must be investigated to determine the correct dosing for treatment of animal dermatophytoses.

In the present study, the *in vitro* susceptibility of *N. fulva* to ITZ and TRF was investigated using the CLSI M38-A2 test. *N. fulva* CBS 167.64 (+) mating type and CBS 168.64 (-) mating type were used as reference strains in this study (Table 1). The isolates of *N. fulva* examined in this study are listed in Table 1. These isolates were obtained from normal rabbit hair and soils in rabbit hutches in public primary schools in Yokohama, Japan⁶.

The isolates were maintained on diluted Sabouraud’s glucose agar¹ prior to being subjected to antifungal susceptibility tests. The broth microdilution (BM) assays for testing the susceptibility of dermatophytes to antifungal drugs ITZ and TRF were performed according to the CLSI M38-A2 guidelines⁸. MICs were determined after a 72 h incubation at 28°C. For ITZ, the MIC was defined as the lowest concentration that induced prominent inhibition of growth (approximately 80% inhibition); for TRF, the MIC was defined as the lowest concentration showing 100% growth inhibition⁹. Each isolate was tested in duplicate on two separate occasions.

The MIC ranges of ITZ for the reference strains of *Candida parapsilosis* (ATCC22019) and *Candida krusei* (ATCC 6258) were within the value range standardized based on the CLSI document M38-A2⁹. The MIC ranges for the isolates by the CLSI M38-A2 test are summarized in Table 1. The mean MICs for the 12 strains were 0.6542 mg/L (range: 0.0625-1 mg/L) for ITZ and
Table 1. Strains and MICs (mg/L) of anti-fungal drugs

| Species | Strain number (mating type) | MICs (mg/L) |
|---------|-----------------------------|-------------|
| N. fulva | CBS\(^1\) 392.58 (+) | 0.0625 0.03125 |
| N. fulva | CBS 168.64 (-) | 0.125 0.03125 |
| N. fulva | NUBS\(^4\) 16002 | 0.125 0.125 |
| N. fulva | NUBS16004 | 0.5 0.5 |
| N. fulva | NUBS16005 | 1 0.03125 |
| N. fulva | NUBS16006 | 1 0.125 |
| N. fulva | NUBS16007 | 1 0.125 |
| N. fulva | NUBS16009 | 0.25 <0.03125 |
| N. fulva | NUBS16010 | 0.25 0.03125 |
| N. fulva | NUBS16012 | 0.25 <0.03125 |
| N. fulva | NUBS16013 | 1 0.0625 |
| N. fulva | NUBS16016 | 1 <0.03125 |

\(^1\)ITZ: itraconazole  
\(^2\)TRF: terbinafine  
\(^3\)CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands  
\(^4\)NUBS: Nihon University College of Bioresource Sciences

0.15625 mg/L (range: <0.003125 - 0.5 mg/L) for TRF (Table 1).

In our previous study, we investigated the MICs of animal isolates of the *M. gypseum* (*N. gypsea* and *N. incurvata*) complex in Japan\(^9\). In that study, the MICs of ITZ and TRF were (respectively) 0.125 mg/L (range: 0.0625-2 mg/L) and 0.03125 mg/L (range: 0.003125-0.5 mg/L) in the tested strains. Thus, the MICs of ITZ and TRF in *N. fulva* (as determined in the present work) were similar to those previously determined in animal isolates of *N. gypsea* and *N. incurvata*. These results indicate that ITZ and TRF should be effective against *N. fulva*, and that dosing with ITZ and TRF should also be applicable for animal dermatophytes caused by *N. fulva*.

*N. fulva* has been isolated rarely from human and animal dermatophytoses\(^5,\)^1\(^0\), however, this species is the most abundant geophilic dermatophyte species in the soils of Eurasia\(^1\)\(^1\), \(^1\)\(^2\). Therefore, epidemiological study is needed about infections by this dermatophyte in humans and animals.

Acknowledgments

This study was supported by a grant (“International joint research and training of young researchers for zoonosis control in the globalized world”) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan.

Conflict of interest

The authors declare no conflict of interest.

References

1) Kwon-Chung KJ, Bennett EJ: Dermatophytosis. *In Medical Mycology*, pp. 136-161 and 816-826, Lea & Febiger, Philadelphia, 1992.
2) Ellis D, Davis S, Alexiou H, Handke R, Bartley R: Description of Medical Mycology, 2nd ed, p. 92, Nexus Print Solutions, Adelaide, Australia, 2007.
3) Reiss E, Shadowy HJ, Lyon IIIGM: Dermatophytosis. *In Fundamental Medical Mycology*, pp. 527-566, Wiley-Blackwell, New Jersey, 2012.
4) de Hoog GS, Dukik K, Monod M, Packeu A, Stubbe D, Hendrickx M, Kupsch C, Stielow JB, Freeke J, Göker M, Rezaei-Matehkolaei A, Mirhendi H, Gräser Y: Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. *Mycopathologia* **182**: 5-31, 2017.
5) Hironaga M, Tanaka S, Watanabe S: Distribution of mating types among clinical isolates of the *Microsporum gypseum* complex. *Mycopathologia* **77**: 31-35, 1982.
6) Fukutomi T, Kano R, Kamata H: First isolation of *Arthroderma fulvum* in Japan. *Med Mycol* **58**: E115-E118, 2017.
7) Moriello KA, DeBoer DJ: Dermatophytosis. *In Infectious Diseases of the Dog and Cat* (Greene, CE ed), 4th ed, pp. 588-602, Saunders Elsevier, St. Louis, 2012.
8) CLSI M38-A2. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard-second edition. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, 2008.
9) Itoi S, Kano R, Hasegawa A, Kamata H: *In vitro* activities of antifungal agents against clinical isolates of dermatophytes from animals. *J Vet Med Sci* **74**: 1067-1069, 2012.
10) Demange C, Contet-Audonneau N, Kombila M, Miegeville M, Berthonneau M, De Vroey C, Percebois G: *Microsporum gypseum* complex in man and animals. J Med Vet Mycol 30: 301-308, 1992.

11) Papini R, Mancianti F, Grassotti G, Cardini G: Survey of keratinophilic fungi isolated from city park soils of Pisa, Italy. Mycopathologia 143: 17-23, 1998.

12) Rezaei-Matehkolaei A, Jahangiri A, Mahmoudabadi AZ, Najafzadeh MJ, Nouri-pour-Sisakht S, Makimura K: Morpho-molecular characterization of soil inhabitant dermatophytes from Ahvaz, southwest of Iran, a high occurrence of *Microsporum fulvum*. Mycopathologia 182: 691-699, 2017.