

Drug susceptibility of 64 strains of *Rhodotorula* sp.

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ABSTRACT. *Rhodotorula* sp. have emerged as opportunistic pathogens, particularly in immunocompromised patients. Knowledge about the susceptibility of *Rhodotorula* strains to the common antifungal drugs is essential for the treatment of such new infections. The 68 isolates identified as: *Rhodotorula mucilaginosa* (47 strains; 69%), *R. minuta* (14; 21%) and *R. glutinis* (7; 10%) obtained from various sources (feces, skin and nails, vagina and hospital environment) were tested for susceptibility to 5-fluorocytosine (5FC), amphotericin B (AMB), fluconazole (FLC) and itraconazole (ITR). All of the isolates had low MICs for AMB and 5FC. For ITR, the *R. minuta* isolates had the lowest MICs within a range 0.125–0.25 mg/l and for FLC all isolates affected within the range 2–64 mg/l. The majority of *R. mucilaginosa* isolates (82.2%) had MICs in the range 64–128 mg/l for FLC and 95.6% of isolates had MICs above or equal to 2 mg/l for ITR.

Key words: *Rhodotorula*, drug susceptibility testing, ATB Fungus INT2

Introduction

The species belonging to the genus *Rhodotorula* have been increasingly recognized as important human pathogens. Case reports include fungemia, endocarditis, peritonitis, meningitis associated with infection of catheters and other intravenous devices which shows that these species have emerged as opportunistic pathogens, particularly in immunocompromised patients [1–3].

Knowledge concerning the susceptibility of *Rhodotorula* strains to the commonly administered antifungal drugs is essential for the treatment of those new infections.

The objective of this study was to evaluate the susceptibility patterns among *Rhodotorula* strains isolated from humans and human-related environments.

Materials and methods

A total of 68 strains were collected at the Dept. of Mycology Collegium Medicum Jagiellonian University during a three year period, from 2006 till 2008. After isolation, the strains were identified using a carbon assimilation test (API 20C AUX bioMerieux) and an additional nitrate assimilation test.

The susceptibility to 5-fluorocytosine (5FC), Amphotericin B (AMB), Fluconazole (FLC) and Itraconazole (ITC) was evaluated using the ATB Fungus INT 2 microdilution test. Prior to the test, the strains were cultured on Sabouraud Glucose medium for 3–5 days. Then, the yeasts were suspended in sterile 0.85% NaCl solution to reach a turbidity equivalent to that of 2 McFarland standard units using a densitometer DEN-1 (BioSan), and 20 µl of the suspension were added to the specific growth medium (ATB F2 medium). After homogenization, 135 µl were inoculated into each well. After 48 hour of incubation at 27°C, the strips were read visually. If in sufficient growth was observed at this time, especially in the slow growing isolates of *R. minuta,* the time of incubation was prolonged until the growth was seen in control wells. The minimal inhibitory concentration was recorded after incubation.

Results

The 68 isolates tested were identified as: *Rhodotorula mucilaginosa* (47 strains; 69%),

Table 1. Origin of isolated *Rhodotorula* strains

Origin of material	*R. mucilaginosa*	%	*R. glutinis*	%	*R. minuta*	%
feces	7	14.9	–		–	
vagina	5	10.6	–		–	
fingernails	3	6.4	–		2	14.3
toenails	11	23.4	1	14.3	5	35.7
hand skin	5	10.6	1	14.3	1	7.1
foot skin	6	12.8	2	28.6	–	
other skin localization	6*	12.8	1**	14.3	–	
hospital environment	4 (2)***	8.5	2 (0)***	28.6	6	42.9
Total	47		7		14	

Explanations: *isolated from head skin (3 isolates) and chest skin (3 isolates); **isolated from foreskin; ***the numbers of isolates tested for antifungal susceptibility are shown in the brackets

R. minuta (14; 21%) and *R. glutinis* (7; 10%) on the basis of morphological and biochemical features. They were obtained from various sources: feces, skin and nails, vagina and hospital environment. *R. mucilaginosa* was most frequently isolated from toenails while *R. minuta* from both hospital environments and toenails. All strains isolated from feces and vagina belonged to the *R. mucilaginosa* species. Other sites of isolation are shown in Table 1.

Drug susceptibility was evaluated for 64 strains. The remaining four strains (2 of *R. mucilaginosa* and 2 of *R. glutinis*) failed to grow even in control wells; all those strains originated from hospital environments. All of the isolates had low MICs for AMB and 5FC at a level lower or equal to 0.5 mg/l, except one isolate of *R. glutinis* from hand skin that had a MICs 2 mg/l for 5FC and single isolates of *R. minuta* and *R. mucilaginosa* obtained from toenails that had MICs equal to 1 mg/l. For ITC, the *R. minuta* isolates had the lowest MICs within a range of 0.125–0.25 mg/l and for FLC all isolates were within a range of 2–64 mg/l. The majority of *R. mucilaginosa* isolates (82.2%) had MICs within the range 64–128 mg/l for FLC and 95.6% isolates had MICs above or equal to 2 mg/l for ITC. Detailed data are shown in Table 2 and Table 3.

Discussion

To evaluate drug susceptibility in this study, a commercial ATB Fungus INT 2 test was used because it is simple to use, relatively non time consuming, and its results agree wellt with the CLSI methods. Torres-Rodriguez and Alvardo Ramirez [4] compared the results of drug susceptibility test performed with the CSLI M27-A2 procedure with results obtained by use of the ATB test and confirmed good agreement between the two methods: 100% for AMB and 5FC, 97% for FLU and 98% for ITC.

Catheter-related *Rhodotorula* fungemia (CRF) is the most common form of infection and the cause of death among all diseases associated with this yeast [5]. Hazen [2] suggests that a likely source of the organism is the skin, as opposed to the gastrointestinal tract responsible for many candidemias. *Rhodotorula mucilaginosa* was reported as the causative agent in 71.7% of cases of CRF and *Rhodotorula glutinis* in 7.5% (18.6% remained unidentified) [5]. Those data correspond well with the prevalence of *Rhodotorula* species on

Table 2. *Rhodotorula* drug susceptibility [mg/l]

Species		*R. glutinis*	*R. minuta*	*R. mucilaginosa*
n		5	14	45
5FC	range	0.5–2	0.5	0.5
	g. mean	0.66	0.5	0.5
	MIC90	–	0.5	0.5
AMB	range	0.5	0.5–1	0.5–1
	g. mean	0.5	0.525	0.508
	MIC90	–	0.5	0.5
FLU	range	32–64	8–64	2–>128
	g. mean	53.8	23.776	82.8
	MIC90	–	64	>128
ITC	range	0.125–2	0.125	0.125–>4
	g. mean	0.8	0.125	3.1
	MIC90	–	0.138	>4

Table 3. *Rhodotorula* drug susceptibility in terms of number of isolates and cumulative percentage values

MICs [mg/l]		n	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
AMB	*R. glutinis*	5	–	–	5 100%	0	0	0	0	0	–	–	–	–
	R. mucilaginosa	45	–	–	44 97.8%	1 100%	0	0	0	0	–	–	–	–
	R. minuta	14	–	–	13 92.9%	1 100%	0	0	0	0	–	–	–	–
5FC	*R. glutinis*	5	–	–	4 80%	0 80%	1 100%	0	0	0	0	0	–	–
	R. mucilaginosa	45	–	–	45 100%	0	0	0	0	0	0	0	–	–
	R. minuta	14	–	–	14 100%	0	0	0	0	0	0	0	–	–
FLU	*R. glutinis*	5	–	0	0	0	0	0	0	1 20%	1 40%	3 100%	0	–
	R. mucilaginosa	45	–	0	0	0	1 2.2%	0 2.2%	1 4.4%	0 4.4%	1 6.7%	16 42.2%	21 88.9%	5* 100%
	R. minuta	14	–	0	0	0	0	0	2 14.3%	5 50%	4 78.6%	3 100%	0	–
ITC	*R. glutinis*	5	1 20%	0 20%	0 20%	1 40%	3 100%	0	–	–	–	–	–	–
	R. mucilaginosa	45	1 2.2%	0 2.2%	0 2.2%	1 4.4%	10 26.7%	17 64.4%	16* 100%	–	–	–	–	–
	R. minuta	14	12 85.7%	2 100%	0	0	0	0	–	–	–	–	–	–

* these values mean that the MIC is higher than MIC values included in test

human skin obtained in the present study. *Rhodotorula minuta* fails to grow at human body temperature and for this reason cannot be a causative agent of fungemia but occurs on human skin more frequently than the second causative agent of *Rhodotorula* fungemia – *R. glutinis*. *R. minuta* is also related to endophthalmitis [3] and hip-joint infections [6].

All of the strains examined were susceptible to AMB and 5FC. Zaas et al. [7] found that AMB preparations, in addition to catheter removal, are acceptable therapies for *Rhodotorula* infection, with excellent *in vitro* activity, and their use was reported to be successful. 5FC possesses excellent activity *in vitro* [7]. In those findings, *Rhodotorula* strains were resistant to azoles except isolates of *R. minuta* that were susceptible to ITR. Zaas et al. [7] also concluded, on the basis of their *in vitro* data, that narrow spectrum azoles are not appropriate therapy. Kofteridis et. al. [8] reported *Rhodotorula glutinis* species septicemia successfully treated with FLC in two patients. This strain was also resistant to 5FC: MICs obtained by the E-test exceeded 32 mg/l, while for FLC amounted to 1.5 mg/l.

Gomez-Lopez et. al. [9] noted that the susceptibility of *R. glutinis* strains was higher than that of *R. mucilaginosa* but they made it clear that there were too few isolates to generalize their conclusion. Different results obtained by Galan-Sanchez et. al [10] show that the majority of *R. glutinis* strains had MICs≥256 mg/l and all isolates of *R. mucilaginosa* had MIC within the range 64–128 mg/l for FLC, however there were no differences between those species' susceptibility to other antifungals. Diakena et al. [11] investigated a large number of *Rhodotorula* isolates (*R. glutinis* 29, *R. mucilaginosa* 24, *R. minuta* 5, unidentified 6). In their study, there were no significant differences in susceptibility between *R. mucilaginosa* and *R. glutinis.*

There is no published for interpreting MICs as clinical categories (susceptible, intermediate or dose dependent, resistant) for *Rhodotorula* sp. On the basis of the data for *Candida* sp. and *Cryptococcus neoformans* [12], we assume that the lowest values obtained for these fungi could be breakpoints also for *Rhodotorula* species susceptibility. For 5FC, MICs≤4mg/l suggest susceptibility. For AMB there are no defined categories but MICs≥2 mg/l are considered as resistant. All of the *Rhodotorula* isolates investigated in this study were susceptible to 5FC and AMB. In the case of FLC, the breakpoint for resistance is ≥64 mg/l for *Candida* and ≥6 mg/l for *C. neoformans.* Only about 5% of *R. mucilaginosa* isolates and about 15% of *R. minuta* ones had MICs below 16 mg/l.

Resistance to azoles can give *Rhodotorula* an advantage facilitating colonization of niches when other fungi susceptible to e.g. FLC or ITC are killed or their growth is restricted. Now that the first proven case of onychomycosis due to *Rhodotorula* have been described, the role of *Rhodotorula* as an accompanying mycobiota e.g. in onychomycosis [13] can be changed to primary causative agents [14]. These latest findings also provide important knowledge about drug susceptibility of mycobiota from skin and toenails.

Currently, infections of *Rhodotorula* are still rare, however, we can expect evolution of the virulence factors and probably in future *Rhodotorula* infections will be more frequent as is already becoming evident through more and more case reports in the microbiological literature [15–16].

References

[1] Samonis G., Anatoliotaki M., Apostolakou H., Maraki S., Mavroudis D., Georgoulias V. 2001. Transient fungemia due to *Rhodotorula rubra* in cancer patient: case report and review of the literature. *Infection* 29: 173-176.

[2] Hazen K.C. 1995. New and emerging yeast pathogens. *Clinical Microbiology Reviews* 8: 462-478.

[3] Tuon F.F., Costa S.F. 2008. *Rhodotorula* infection. A systematic review of 128 cases from literature. *Revista Iberoamericana de Micologia* 25: 135-140.

[4] Torres-Rodriguez J.M., Alvarado-Ramirez E. 2007. *In vitro* susceptibilities to yeasts using the ATB FUNGUS 2 method, compared with Sensititre Yeast One and standard CLSI NCCLS) M27-A2 methods. *Journal of Antimicrobial Chemotherapy* 60: 658-661.

[5] Tuon F.F., de Almeida G.M.D., Costa S.F. 2007. Central venous catheter-associated fungemia due to *Rhodothorula* spp. – a systematic review. *Medical Mycology* 45: 441-447.

[6] Cerikcioglu N., Tetik C., Mulazimoglu L. 2005. *Rhodotorula minuta*: uncommon yeast isolated as the causative agent of a right hip joint infection. *Journal of Medical Mycology* 15: 52-55.

[7] Zaas A.K., Boyce M., Schell W.A., Miller J.L., Alexander-Lodge B., Perfect J.R. 2003. Risk of fungemia due to *Rhodotorula* and antifungal susceptibility testing of *Rhodotorula* isolates. *Journal of Clinical Microbiology* 41: 5233-5235.

[8] Kofteridis D., Mantadakis E., Christidou A., Samonis G. 2007. *Rhodotorula glutinis* fungemia successfully treated with fluconazole: report of two cases. *International Journal of Infectious Diseases* 11: 179-180.

[9] Gomez-Lopez A., Mellado E., Rodriguez-Tudela J.L., Cuenca-Estrella M. 2005. Susceptibility profile of 29 clinical isolates of *Rhodotorula* spp. and literature review. *Journal of Antimicrobial Chemotherapy* 55: 312-316.

[10] Galan-Sanchez F., Garcia-Martos P., Rodriguez-Ramos C., Marin-Casanova P., Mira-Gutierrez J. 1999. Microbiological characteristics and susceptibility patterns of strains of *Rhodotorula* isolated from clinical samples. *Mycopathologia* 145: 109-112.

[11] Diekema D.J., Petroelje B., Messer S.A., Hollis R.J., Pfaller M.A. 2005. Activities of available and investigational antifungal agents against *Rhodotorula* species. *Journal of Clinical Microbiology* 43: 476-478.

[12] Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard Third Edition CLSI document M27-A3. Wayne, P:. CLSI, 2008.

[13] Mügge C., Haustein U.F., Nenoff P. 2006. Causative agents of onychomycosis – a retrospective study. *Journal der Deutschen Dermatologischen Gesellschaft* 4: 218-228.

[14] da Cunha M.M.L., dos Santos L.P.B., Dornelas-Ribeiro M., Vermelho A.B., Rozental S. 2009. Identification, antifungal susceptibility and scanning electron microscopy of a keratinolytic strain of *Rhodotorula mucilaginosa*: a primary causative agent of onychomycosis. *FEMS Immunology and Medical Microbiology* 55: 396-403.

[15] Fung H.B., Martyn C.A., Shahidi A., Brown S.T. 2009. *Rhodotorula mucilaginosa* lymphadenitis in a HIV-infected patient. *International Journal of Infectious Diseases* 13: 27-29.

[16] Savini V., Sozio F., Catavitello C., Talia M., Manna A., Febbo F., Balbinot A., Bonaventura G., Piccolomini R., Parruti G., D'Antonio D. 2008. Femoral prosthesis infection by *Rhodotorula mucilaginosa*. *Journal of Clinical Microbiology* 46: 3544-3545.

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