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Short Communication

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In vitro antifungal activity of antipsychotic drugs and their combinations with conventional antifungals against *Scedosporium* and *Pseudallescheria* isolates

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Abstract

In the present study, *in vitro* antifungal activities of five antipsychotic drugs (i.e., chlorpromazine hydrochloride, CPZ; trifluoperazine hydrochloride, TPZ; amantadine hydrochloride; R-(-)-deprenyl hydrochloride, and valproic acid sodium salt) and five conventional antifungal drugs (i.e., amphotericin B, AMB; caspofungin, CSP; itraconazole; terbinafine, TRB and voriconazole, VRC) were investigated in broth microdilution tests against four clinical and five environmental *Scedosporium* and *Pseudallescheria* isolates. When used alone, phenothiazines CPZ and TPZ exerted remarkable antifungal effects. Thus, their *in vitro* combinations with AMB, CSP, VRC, and TRB were also examined against the clinical isolates. In combination with antifungal agents, CPZ was able to act synergistically with AMB and TRB in cases of one and two isolates, respectively. In all other cases, indifferent interactions were revealed. Antagonism was not observed between the tested agents. These combinations may establish a more effective and less toxic therapy after further *in vitro* and *in vivo* studies for *Scedosporium* and *Pseudallescheria* infections.

Key words: *Scedosporium* spp., *Pseudallescheria* spp., antipsychotic drugs, drug interactions.

Introduction

Members of the genus *Scedosporium* (teleomorph: *Pseudallescheria*) are associated with a wide spectrum of human infections, including trauma-associated, localized diseases (e.g., mycetoma, corneal-, soft tissue- and bone infections) in otherwise healthy people; pulmonary infections in patients with predisposing pulmonary disorders (such as cystic fibrosis or pneumonia) and systemic invasive diseases in immunocompromised patients or in near-drowning victims [1–3]. Systemic infections are more severe and have a tendency for dissemination and central nervous system (CNS) involvement, which is commonly fatal

Table 1. Overview of the tested non-antifungal agents: mode of action, traditional application, achievable plasma/brain levels (μg/ml), secondary antifungal activity.

Drug	Mode of action	Traditional application	Achievable level (μg/ml) in plasma	Achievable level (μg/ml) in brain	Antifungal activity	Antifungal mechanism	References
Amantadine	NMDA receptor antagonist	Influenza, Multiple sclerosis, Parkinson's disease	–	~80	Mucorales spp. *Aspergillus* spp.	–	[15–19]
Phenothiazines: Clorpromazine, Trifluoperazine	Postsynaptic dopaminerg receptor inhibitor	Schizophrenia	0.5–1	50–100	*Candida* spp. Mucorales spp. *Aspergillus* spp. *Scedosporium* spp.	Inhibitor of calmodulin/ Membrane modifier/ DNA intercalator	[20–27]
Selegiline (/R-deprenyl)	Dose-dependent inhibitor of monoamine oxidase type A and B	Depression Parkinson's disease	$\sim 2–6 \cdot 10^{-3}$	–	Mucorales spp. *Aspergillus* spp.	–	[17, 28, 29]
Valproic acid	Inhibitor of the arachidonic acid - arachidonoyl-CoA conversion	Bipolar disorder	–	~140–210	Mucorales spp. *Aspergillus* spp.	–	[17, 30, 31]

without treatment [4,5]. The clinical presentations of *Scedosporium*/*Pseudallescheria* infections are similar to those of aspergillosis, thus scedosporiosis can be mistakenly treated with the generally accepted antifungals for *Aspergillus* spp. [6]. This may have severe consequences because *Scedosporium* spp. are generally less susceptible to the commonly used antifungal agents than *Aspergillus* spp. [7] When facing with CNS infections the spectrum of eligible antifungal agents is still narrow; the most common approach is an aggressive surgical treatment and/or a high dosage of AMB (often combined with azole compounds), which penetrates across the blood-brain barrier (BBB) poorly and may cause serious adverse effects [8–11]. Moreover, *Scedosporium*/*Pseudallescheria* species typically respond poorly to AMB with a minimum inhibitory concentration (MIC) range of 2–32 μg/ml [7,12,13]. Echinocandins proved to be active against *Scedosporium* spp. [2]; however, they have poor ability to cross the BBB [2,14]. For these reasons, it is especially challenging to find the appropriate therapeutic agents to treat these brain infections and there is an urgent need for new, safely applicable antifungal strategies against *Scedosporium* and *Pseudallescheria* spp. An alternative way to cure these types of infections is the use of non-antifungal drugs with secondary antifungal activity, as monotherapeutic agents or in combination with conventional antifungal drugs. Thus, instead of developing novel antifungal agents, we could save the time and the costs of drug design and clinical trials. According to previous studies, there are a number of medications, which are originally used to treat mental illnesses (e.g., selegiline for depression) or degenerative CNS disorders (e.g., amantadine for Parkinson's disease) but may also have antifungal activity (Table 1). These antipsychotic drugs can easily penetrate the BBB and accumulate in the CNS [20,21]. Thus, they represent promising, novel agents in the treatment of cerebral fungal diseases.

The aim of the present work was to investigate the *in vitro* antifungal activities of five non-antifungal drugs and five traditional antifungal agents against *Scedosporium* spp. either alone or in combination with each other.

Materials and methods

Fungal strains and culture conditions

Nine *Scedosporium* and *Pseudallescheria* isolates obtained from CBS (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) were involved in this study. The isolates derived from a variety of environmental and clinical sources (i.e., dung, human lung, sputum, soil, sewage, and wound exudate) from different parts of the world (Table 2). The strains were maintained on malt extract slants (MEA, Biolab, Hungary) at 4°C. Susceptibility tests were performed in RPMI 1640 medium (Sigma-Aldrich, USA) supplemented with 0.3 g/l L-glutamine and buffered to pH 7.0 with 0.165 M 4-morpholinopropanesulfonic acid (Sigma-Aldrich, USA).

Antifungal susceptibility tests

The following five antipsychotic drugs were involved in this study: chlorpromazine hydrochloride (CPZ), trifluoperazine hydrochloride (TPZ), amantadine hydrochloride

Table 2. Antifungal activity of five antipsychotic drugs and five conventional antifungal drugs against clinical and environmental *Scedosporium* and *Pseudallescheria* isolates.

Strain number[a]	Species	Source	Mean MICs of antipsychotic drugs (μg/ml)[b]					Mean MICs of conventional antifungal agents (μg/ml)[c]				
			AMD	CPZ	NaVAP	RDEP	TPZ	AMB	CSP*	ITC	TRB	VRC
CBS 136046	*S. aurantiacum*	Human lung/Australia	>1024	32	>1024	512	16	128	64	128	128	32
CBS 136047	*S. aurantiacum*	Soil/Australia	>1024	32	>1024	1024	32	32	32	128	128	16
CBS 136049	*S. aurantiacum*	Soil/Austria	>1024	32	>1024	1024	16	128	32	128	128	32
CBS 116910	*S. aurantiacum*	Wound exudate/Spain	512	16	<64	256	8	128	32	32	128	8
CBS 120157	*P. boydii*	Human lung/France	1024	32	1024	256	8	64	32	32	128	16
CBS 117410	*P. boydii*	Soil/Spain	1024	16	>1024	>1024	16	8	32	16	128	64
CBS 117432	*P. boydii*	Sputum/France	1024	16	>1024	1024	32	128	64	32	128	16
CBS 254.72	*P. angusta*	Sewage/USA	1024	32	256	1024	8	128	32	128	128	32
CBS 301.79	*P. ellipsoidea*	Dung/Netherlands	1024	32	>1024	1024	32	64	32	32	128	16
	MIC[d] range		512–>1024	16–32	<64–>1024	256–>1024	8–32	8–128	32–64	16–128	128	8–64

[a]CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

[b]AMD, amantadine hydrochloride; CPZ, chlorpromazine hydrochloride; NaVAP, valproic acid sodium salt; RDEP, (R-)-deprenyl-hydrochloride; TPZ, trifluoperazine hydrochloride.

[c]AMB, amphotericin B; CSP, caspofungin; ITC, itraconazole; TRB, terbinafine; VRC, voriconazole.

[d]MIC, minimum inhibitory concentration required to inhibit the total growth of a certain isolate.

*Mean MEC values of CSP were also determined: CBS 136046 - 13.3 μg/ml; CBS 136047 - 4.7 μg/ml; CBS 136049 - 10.7 μg/ml; CBS 116910 - 16.0 μg/ml; CBS 120157 - 1.7 μg/ml; CBS 117410 - 1.5 μg/ml; CBS 117432 - <1.0 μg/ml; CBS 254.72 - 5.3 μg/ml; CBS 301.79 - 12.0 μg/ml.

(AMD), R-(-)-deprenyl hydrochloride (RDEP) and valproic acid sodium salt (NaVAP) (Sigma-Aldrich, USA). All drugs were dissolved in 96% ethanol to prepare stock solutions (10.24 mg/ml). From the stocks, dilutions were prepared in RPMI 1640 medium. Final drug concentrations ranged from 64 to 1024 μg/ml for AMD, NaVAP, RDEP and from 4 to 64 μg/ml for CPZ and TPZ. The tested conventional antifungal agents, such as amphotericin B (AMB, Medispec Pharmaceuticals Pvt. Ltd., India), caspofungin (CSP, Sigma-Aldrich, USA), itraconazole (ITC, Sigma-Aldrich, USA), terbinafine (TRB, Sigma-Aldrich, USA) and voriconazole (VRC, Pfizer PGM, France) were provided by the manufacturers as standard powders. The typical solvent of antipsychotic drugs is ethanol; to avoid the possible bias arising from the different mixtures of solvents, stock solutions (at 5 mg/ml) of antifungal drugs were also prepared in ethanol. Further dilutions were prepared in RPMI 1640 medium in a final concentration range of 1–512 μg/ml for each antifungal drug. The possibility of incomplete solubilisation of the drugs (causing higher MIC readings) was ruled out by using *Aspergillus flavus* ATCC 204304 as a reference strain.

The *in vitro* MIC and minimum effective concentration (MEC) values were determined in a 96-well flat-bottom microtiter plate bioassay based on the slightly modified instructions of the Clinical and Laboratory Standards Institute M38-A2 broth microdilution method, in triplicates [32]. The tested isolates sporulated poorly after 7 days; hence, in order to get the sufficient amount of conidia, the CLSI recommended incubation time was extended by another week on MEA slants at 30°C. Conidia were diluted in RPMI 1640 adjusting the concentration to 10^5 conidia/ml. The plates were incubated at 37°C for 72 h; then the absorbance (OD_{620}) was measured with a microtiter plate reader (SPECTROstar Nano, Germany) in well-scanning mode. The absorbance of the untreated control cultures were referred to 100% growth in each case. MIC was defined as the lowest antifungal concentration, which was required for the total growth inhibition of a certain isolate. MEC was defined as the lowest concentration of CSP, which led to abnormally branched, compact hyphal forms.

Interaction tests

Drug interactions between phenothiazines (CPZ and TPZ) and four conventional antifungals (AMB, CSP, TRB, and VRC) were investigated using the checkerboard microdilution method [33]. To define the type of interaction between two compounds, the fractional inhibitory concentration index (FICI) was used [34]. Synergism was defined as FICI≤0.5, indifference as 0.5<FICI≤4 and antagonism was defined when FICI>4 [35]. The final CPZ and TPZ concentrations ranged from 4 to 64 μg/ml. The final AMB concentrations were between 0.125 and 128 μg/ml and the

Table 3. Results of the combination tests of phenothiazines and conventional antifungal drugs against clinical *Scedosporium* and *Pseudallescheria* isolates based on the fractional inhibitory concentration indexes (FICI).

		FICIs/ Interaction between[b]							
Strain number[a]	Species	CPZ-AMB	CPZ-CSP	CPZ-TRB	CPZ-VRC	TPZ-AMB	TPZ-CSP	TPZ-TRB	TPZ-VRC
CBS 136046	*S. aurantiacum*	0.80/NI[c]	0.75/NI	0.50/S	1.25/NI	3.00/NI	1.25/NI	2.00/NI	2.00/NI
CBS 116910	*S. aurantiacum*	1.30/S[c]	1.50/NI	0.50/S	1.25/NI	1.30/NI	2.00/NI	2.50/NI	4.00/NI
CBS 120157	*P. boydii*	0.40/NI	1.50/NI	0.75/NI	2.50/NI	2.30/NI	2.25/NI	3.00/NI	4.00/NI
CBS 117432	*P. boydii*	0.80/NI	1.25/NI	2.25/NI	2.00/NI	1.00/NI	0.75/NI	1.25/NI	1.50/NI

[a]CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
[b]AMB, amphotericin B; CPZ, chlorpromazine hydrochloride; CSP, caspofungin; TPZ, trifluoperazine hydrochloride; TRB, terbinafine; VRC, voriconazole.
[c]NI, no interaction (0.5<FICI≤4); S, synergism (FICI≤0.5).

CSP, TRB and VRC concentrations were between 0.125 and 64 μg/ml. Three replicates were performed.

Results

Antifungal susceptibility tests

Susceptibility of *Scedosporium* and *Pseudallescheria* isolates to the tested antipsychotic drugs and conventional antifungals are summarized in Table 2. Among antipsychotic drugs, CPZ and TPZ showed the lowest MICs: 16–32 μg/ml and 8–32 μg/ml, respectively. Contrarily, AMD, NaVAP, and RDEP, with a few exceptions, displayed generally high MIC values (≥1024 μg/ml). Antifungal agents demonstrated similar antifungal activity against all the tested isolates with high MICs ranged between 8–128 μg/ml, except VRC, which had a relatively lower MIC range of 8–32 μg/ml. The mean MECs of CSP were in a range of <1.0–16.0 μg/ml.

Interaction tests

Compared to the single use, the MIC values of all investigated agents decreased or remained the same in the combination tests (Table 3). To interpret the interaction between phenothiazines and antifungals, FICIs were calculated. Based on these values, in most cases no interactions were revealed between the tested compounds (FICI range: 0.6–4) and antagonism was not detected at all. CPZ was able to act synergistically in combination with AMB (FICI: 0.4) and TRB (FICI: 0.5).

Discussion

Among antipsychotics, antifungal activities of phenothiazines are the most investigated. Despite their low achievable plasma concentration (0.5–1 μg/ml) [20], they have a great potential to treat patients with locally invasive fungal infections. Phenothiazines can accumulate in tissues, and their final level in the brain may be a seventy times higher than their plasma level [20,21]. The moderate *in vitro* antifungal effect of phenothiazines was previously described against different fungal pathogens: *Candida*, *Aspergillus*, Mucorales and *Scedosporium* species [17,20,22,23,25]. The reported MICs of *Scedosporium* isolates in these experiments ranged from 16 to 128 μg/ml. Our results were comparable to these previously reported *in vitro* activities: MICs of CPZ and TPZ were found between 16–32 μg/ml and 8–32 μg/ml, respectively (Table 2). It is noteworthy that these concentrations are in the reachable range in the CNS [20,21]. Afeltra et al. [20] reported that TPZ was more effective against the tested yeasts and moulds than CPZ: the mean MICs of TPZ were between 21.3 and 38.4 μg/ml, while CPZ mean MICs were in the range of 38.9–53.6 μg/ml. In the present study, we also observed that *Scedosporium* spp. were more sensitive to TPZ than CPZ, as the mean MICs were 18.6 and 26.6 μg/ml, respectively.

The antifungal potential of the other three non-antifungals (AMD, NaVAP and RDEP) was recently investigated by our group [17] and a slight antifungal effect (MIC≥512 μg/ml) was observed against Mucorales moulds and *Aspergillus* spp. With only one exception, we also found similarly high MICs (≥256 μg/ml) against *Scedosporium/Pseudallescheria* spp.; *S. aurantiacum* CBS 116910 proved to be more sensitive to NaVAP than the other tested isolates (MIC<64 μg/ml). Apart from the latter case, other concentrations of NaVAP obtained in this study are unreachable in the CNS during the therapy [18,29,30].

Scedosporium spp. reported to be intrinsically resistant to the majority of the current antifungals: their high *in vitro* MIC values seemed to correlate with the poor response to clinical therapy [27,36,37]. The previously determined MICs of AMB, CSP, ITC, TRB and VRC were highly variable and were in the ranges of 0.125–>32 μg/ml, 0.5–>16 μg/ml, 0.03–>32 μg/ml, 1–>32 μg/ml, and ≤0.03–>16 μg/ml, respectively [12,36–46]. Compared to these data, all tested isolates responded poorly to the five

antifungal agents in the present work. Although our MIC results were less variable, they were all in a higher range (8–128 μg/ml), and these concentrations cannot be reached under therapeutical conditions in the CNS [17,47]. However, in agreement with the report of Wiederhold et al. [45], MEC values of CSP were at least 4-fold lower than the MICs.

In vitro combination of antipsychotic drugs and conventional antifungal agents is a less studied field. Previously, we investigated the *in vitro* interactions between these two groups of drugs against *Aspergillus* spp., *Candida* spp. and representatives of the order Mucorales [17,22,23]. The combinations of phenothiazines and amphotericin B were able to act both antagonistically and synergistically against *Candida* strains [22]. Against Mucoralean fungi, TPZ acted synergistically, while CPZ acted antagonistically with AMB [23]. In contrast to these, we did not detect antagonism between the antifungal and non-antifungal agents against *Scedosporium* isolates. In most cases, there were no interactions between the two compounds, but CPZ was able to act synergistically with both AMB and TRB (Table 3). Wood et al. [48] also observed synergism between CPZ and AMB against *Candida* spp.

In conclusion, these results underline the need of further *in vitro* and *in vivo* studies to clarify the mode of action and to prove the possible clinical efficiency of the discussed non-antifungal drugs against *Scedosporium* and *Pseudallescheria* spp.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. O'Brian TA. Pseudallescheriasis in the 21st century. *Expert Rev Anti Infect Ther* 2005; **3**(5): 765–773.
2. Cortez KJ, Roilides E, Quiroz-Telles F et al. Infections caused by *Scedosporium* spp. *Clin Microbiol Rev* 2008; **21**(1): 157–197.
3. Castiglioni B, Sutton DA, Rinaldi MG et al. *Pseudallescheria boydii* (anamorph *Scedosporium apiospermum*) infection in solid organ transplant recipients in a tertiary medical center and review of the literature. *Medicine* 2002; **81**(5): 333–348.
4. Apostolova L, Johnson E, Adams H. Disseminated *Pseudallescheria boydii* infection successfully treated with voriconazole. *J Neurol Neurosurg Psychiatry* 2005; **76**(12): 1741–1742.
5. Albernaz V, Huston B, Castillo M et al. *Pseudallescheria boydii* infection of the brain: imaging with pathologic confirmation. *Am J Neuroradiol* 1996; **17**(3): 589–592.
6. Nguyen BD. Pseudallescheriasis of the lung and central nervous system multimodality imaging. *Am J Roentgenol* 2001; **176**(1): 257–258.
7. Alastruey-Izquierdo A, Mellado E, Peláez T et al. Study Group. Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). *Antimicrob Agents Chemother* 2013; **57**(7): 3380–3387.
8. Eltoukhy NS, Crank CW. Antifungal distribution into cerebrospinal fluid, vitreous humour, bone, and other difficult sites. *Curr Fungal Infect Rep* 2010; **4**(2): 111–119.
9. Scully EP, Baden LR, Katz JT. Fungal brain infections. *Curr Opin Neurol* 2008; **21**(3): 347–352.
10. Kullberg BJ. Fungal infections of the central nervous system. *Advanced Studies in Medicine* 2003; **3**(1A): S10–S11.
11. Laniado-Laborín R, Cabrales-Vargas MN. Amphotericin B: side effects and toxicity. *Rev Iberoam Micol* 2009; **26**(4): 223–227.
12. Alastruey-Izquierdo A, Cuenca-Estrella M, Monzón A et al. Prevalence and susceptibility testing of new species of *Pseudallescheria* and *Scedosporium* in a collection of clinical mold isolates. *Antimicrob Agents Chemother* 2007; **51**(2): 748–751.
13. Gilgado F, Serena C, Cano J et al. Antifungal susceptibilities of the species of the *Pseudallescheria boydii* complex. *Antimicrob Agents Chemother* 2006; **50**(12): 4211–4213.
14. Black KE, Baden LR. Fungal infections of the CNS: treatment strategies for the immunocompromised patient. *CNS Drugs* 2007; **21**(4): 293–318.
15. Braley TJ, Chervin RD. Fatigue in multiple sclerosis: mechanisms, evaluation, and treatment. *Sleep* 2010; **33**(8): 1061–1067.
16. Crosby NJ, Deane K, Clarke CE. Amantadine in Parkinson's disease. *Cochrane Database of Syst Rev* 2003, Issue 1. CD003468.
17. Galgóczy L, Tóth L, Virágh M et al. *In vitro* interactions of amantadine hydrochloride, R-(-)-deprenyl hydrochloride and valproic acid sodium salt with antifungal agent against filamentous fungal species causing central nervous system infection. *Acta Biol Hung* 2012; **63**(4): 490–500.
18. Kornhuber J, Quack G, Danysz W et al. Therapeutic brain concentration of the NMDA receptor antagonist amantadine. *Neuropharmacology* 1995; **34**(7): 713–721.
19. Lafleur MD, Sun L, Lister I et al. Potentiation of azole antifungals by 2-adamantanamine. *Antimicrob Agents Chemother* 2013; **57**(8): 3585–3592.
20. Afeltra J, Vitale RG, Verweij PE. *In vitro* activity of antipsychotic drugs against pathogenic yeasts and molds. In: Afeltra J, ed. *In vitro interaction between antifungals and other agents against pathogenic fungi*. Nijmegen: Quickprint, 2003: 59–75.
21. Amaral L, Kristiansen JE. Phenothiazines: an alternative to conventional therapy for the initial management of suspected

multidrug resistant tuberculosis. A call for studies. *Int J Antimicrob Agents* 2000; **14**(3): 173–176.
22. Galgóczy L, Bácsi A, Homa M et al. *In vitro* antifungal activity of phenothiazines and their combination with amphotericin B against different *Candida* species. *Mycoses* 2011; **54**(6): 737–743.
23. Galgóczy L, Papp T, Kovács L et al. *In vitro* activity of phenothiazines and their combinations with amphotericin B against Zygomycetes causing rhinocerebral zygomycosis. *Med Mycol* 2009; **47**(3): 331–335.
24. Judd WR, Martin CA. Antifungal activity of nontraditional antifungal agents. *Curr Fungal Infect Rep* 2009; **3**(2): 86–95.
25. Siavoshi F, Tavakolian A, Foroumadi A et al. Comparison of the effect of non-antifungal and antifungal agents on *Candida* isolates from the gastrointestinal tract. *Arch Iran Med* 2012; **15**(1): 27–31.
26. Taylor D., ed. *Schizophrenia in Focus*. London: Pharmaceutical Press, 2000: 42.
27. Vitale RG, Afeltra J, Meis JF et al. Activity and post antifungal effect of chlorpormazine and trifluoperazine against *Aspergillus*, *Scedosporium* and zygomycetes. *Mycoses* 2007; **50**(4): 270–276.
28. Fowler JS, Logan J, Volkow ND et al. Evidence that formulations of the selective MAO-B inhibitor, selegiline, which bypass first-pass metabolism, also inhibit MAO-A in the human brain. *Neuropsychopharmacology* 2015; **40**(3): 650–657.
29. Mahmood I. Clinical pharmacokinetics and pharmacodynamics of selegiline. An update. *Clin Pharmacokinet* 1997; **33**(2): 91–102.
30. Bazinet RP, Weis MT, Rapoport SI et al. Valproic acid selectively inhibits conversion of arachidonic acid to arachidonoyl-CoA by brain microsomal long-chain fatty acyl-CoA synthetases: relevance to bipolar disorder. *Psychopharmacology (Berl)* 2006; **184**(1): 122–129.
31. Chiu CT, Wang Z, Hunsberger JG et al. Therapeutic potential of mood stabilizers lithium and valproic acid: beyond bipolar disorder. *Pharmacol Rev* 2013; **65**(1): 105–142.
32. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. In: Approved standard CLSI document M38-A2. Wayne: CLSI. 2008.
33. Eliopoulos GM, Moellering RC. Antimicrobial combinations. In: Lorian V, ed. *Antibiotics In: Laboratory Medicine*, 4th ed. Baltimore: Williams and Wilkins Co., 1996: 330–396.
34. Johnson MD, MacDougall C, Ostrosky-Zeichner L et al. Combination antifungal therapy. *Antimicrob Agents Chemother* 2004; **48**(3): 693–715.
35. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother* 2003; **52**(1): 1.
36. Carrillo AJ, Guarro J. *In vitro* activities of four novel triazoles against *Scedosporium* spp. *Antimicrob Agents Chemother* 2001; **45**(7): 2151–2153.
37. Guarro J, Kantarcioglu AS, Horré R et al. *Scedosporium apiospermum*: changing clinical spectrum of a therapy-refractory opportunist. *Med Mycol* 2006; **44**(4): 295–327.
38. Farina C, Marchesi G, Passera M et al. Comparative study of the *in vitro* activity of various antifungal drugs against *Scedosporium* spp. in aerobic and hyperbaric atmosphere versus normal atmosphere. *J Med Mycol* 2012; **22**(2): 142–148.
39. Heath CH, Slavin MA, Sorrell TC et al. Population-based surveillance for scedosporiosis in Australia: epidemiology, disease manifestations and emergence of *Scedosporium aurantiacum* infection. *Clin Microbiol Infect* 2009; **15**(7): 689–693.
40. Lackner M, de Hoog SG, Verweij PE et al. Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. *Antimicrob Agents Chemother* 2012; **56**(5): 2635–2642.
41. Lewis RE, Wiederhold NP, Klepser ME. *In vitro* pharmacodynamics of amphotericin B, itraconazole, and voriconazole against *Aspergillus*, *Fusarium*, and *Scedosporium* spp. *Antimicrob Agents Chemother* 2005; **49**(3): 945–951.
42. Meletiadis J, Meis JF, Mouton JW et al. *In vitro* activities of new and conventional antifungal agents against clinical *Scedosporium* isolates. *Antimicrob Agents Chemother* 2002; **46**(1): 62–68.
43. Shah KB, Wu TG, Wilhelmus KR et al. Activity of voriconazole against corneal isolates of *Scedosporium apiospermum*. *Cornea* 2003; **22**(1): 33–36.
44. Stripeli F, Pasparakis D, Velegraki A et al. *Scedosporium apiospermum* skeletal infection in an immunocompetent child. *Med Mycol* 2009; **47**(Special Issue): 441–444.
45. Wiederhold NP, Lewis RE. Antifungal activity against *Scedosporium* species and novel assays to assess antifungal pharmacodynamics against filamentous fungi. *Med Mycol* 2009; **47**(Special Issue): 422–432.
46. Zeng J, Kamei K, Zheng Y et al. Susceptibility of *Pseudallescheria boydii* and *Scedosporium apiospermum* to new antifungal agents. *Jpn J Med Mycol* 2004; **45**(2): 101–104.
47. Schwartz S, Milatovic D, Thiel E. Successful treatment of cerebral aspergillosis with a novel triazole (voriconazole) in a patient with acute leukaemia. *Br J Haematol* 1997; **97**(3): 663–665.
48. Wood NC, Nugent KM. Inhibitory effects of chlorpromazine on *Candida* species. *Antimicrob Agents Chemother* 1985; **27**(5): 692–694.