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In vitro activity of colistin as single agent and in combination with antifungals against filamentous fungi occurring in patients with cystic fibrosis

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Summary

Because published reports indicate that the antibiotic colistin (COL) has antifungal properties, this study investigated the antifungal *in vitro* activity of COL as single agent and in combination with the antifungal compounds voriconazole (VRC), caspofungin (CAS) and amphotericin B (AMB) against *Scedosporium/Pseudallescheria* spp., *Exophiala dermatitidis* and *Geosmithia argillacea*. In total, susceptibility was determined for 77 *Scedosporium/Pseudallescheria* spp., 82 *E. dermatitidis* and 17 *G. argillacea* isolates. The minimal inhibitory concentrations (MICs) of COL and the antifungals as single compound and in combination were determined with MIC test strips. Drug interactions were detected by crossing the MIC test strips at a 90° angle. The fractional inhibitory concentration index was used to categorise the drugs' interaction. The MIC_{50} value of COL was 12 μg ml^{-1} for *S. prolificans*, 16 μg ml^{-1} for *P. apiosperma*, 16 μg ml^{-1} for *P. boydii*, 12 μg ml^{-1} for *E. dermatiditis* and 6 μg ml^{-1} for *G. argillacea*. VRC was the most active drug in combination without any antagonism with the exception of few *P. boydii* isolates. COL as single agent and in most combinations with antifungals exhibits *in vitro* antifungal activity against filamentous ascomycetes occurring in cystic fibrosis patients and may offer a novel therapeutic option, especially for multidrug-resistant *S. prolificans*.

Key words: Colistin, susceptibility testing, *Scedosporium/Pseudallescheria*, *Exophiala*, *Geosmithia*, cystic fibrosis.

Introduction

Bacterial colonisation or infection of the respiratory tract is one of the main reasons for increased morbidity and mortality rates among cystic fibrosis (CF) patients.[1] However, the respiratory tract secretions of CF patients often contain filamentous fungi, such as *Aspergillus* spp.[2] *Scedosporium/Pseudallescheria* spp. and *Exophiala dermatitidis* are reported to be the most common non-*Aspergillus* species moulds, whereas *Geosmithia argillacea* was recently described as an emerging pathogen in this patient population.[3–5]

The prevalence of filamentous fungi in the CF community shows great interstudy and geographical variations.[3,6] This is most likely due to differences in the procedures used for mycological examination of sputum samples (conventional vs. selective agar), the methods used for species identification (phenotypic vs. molecular) and environmental differences. In the context of CF, the exact clinical relevance of these fungal infections is still a

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matter of debate. However, invasive and fatal infections with *Scedosporium/Pseudallescheria* spp., *E. dermatitidis* and *G. argillacea*, especially in immunocompromised patients, have been repeatedly reported.[7–14]

Colistin (COL; polymyxin E) belongs to the antibiotic substance class of polymyxins, produced by *Bacillus polymyxa*. The mode of action of this antibiotic is the disruption of the outer membrane of gram-negative bacteria, which results in an increased cell permeability with subsequent leakage of cell content, cell lysis and cell death.[15] Nebulised COL is a frequently used agent for inhalation therapy in CF patients chronically colonised with *Pseudomonas aeruginosa* or other multi-drug-resistant gram-negative rods.[16] Other authors reported that the antibiotic COL shows reasonable antifungal activity against *Candida* spp., *Cryptococcus neoformans* and *Mucorales* spp.[17,18] The aim of this study was to analyse the *in vitro* activity of COL as single agent and in combination with voriconazole (VRC), caspofungin (CAS) and amphotericin B (AMB) against *Pseudallescheria/Scedosporium* spp., *E. dermatitidis* and *G. argillacea*.

Materials and methods

Isolates

In total, we tested 77 *Scedosporium/Pseudallescheria* spp. isolates, 82 *E. dermatitidis* isolates and 17 *G. argillacea* isolates, including 69 (39%) reference strains. The clinical isolates were obtained from the following institutions: Institute of Medical Microbiology, University Hospital Essen, Germany; Consultant Laboratory for Cystic Fibrosis-Bacteriology (Northern Germany), Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany; Institute for Medical Microbiology, Immunology and Hygiene, University Hospital of Cologne, Cologne, Germany; and the Collection of the ECMM-ISHAM Working Group on *Pseudallescheria/Scedosporium* infections.

We tested a total of 19 *S. prolificans* (52% isolated from CF patients, nine reference strains of international collections), 30 *P. apiosperma* (67% from CF, 16 reference strains) and 28 *P. boydii* isolates (71% from CF, 14 reference strains). Of the 82 tested *E. dermatitidis* isolates, 80% (n = 66) were obtained from CF patients and 34% (n = 28) were reference strains from the Centraalbureau voor Schimmelcultures (CBS), including those from CF patients. Of the tested *G. argillacea* strains, two isolates were CBS reference strains and 13 (76%) were obtained from CF patients. In total, 131 of the 176 (74.4%) tested fungal species were obtained from the respiratory tract of CF patients.

Molecular species identification

All clinical isolates were identified by phenotypic characteristics to the genus level. Subsequent species identification was performed using molecular methods. All isolates from Essen, Cologne and Hannover were identified by sequencing the internally transcribed spacer (ITS1-5.8S-ITS2) region of the rDNA, as described previously.[19] Isolates from the Collection of the ECMM-ISHAM Working Group on *Pseudallescheria/Scedosporium* Infections were identified to the species level by amplified fragment length polymorphisms, as reported.[20] Species identification of reference strains were provided by the international collections.

Susceptibility testing

Susceptibility testing for all isolates was performed according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) document M38-A2.[21] However, in accordance with Pfaller *et al.* [22], the CLSI test method was modified from a broth dilution method to a minimal inhibitory concentration (MIC) test strip method. The following drugs were tested as single compounds: COL, VRC, CAS and AMB. In addition, COL was tested in combination with VRC, CAS and AMB (MIC test strips; Liofilchem s.r.l., Roseto degli, Italy).

Before susceptibility testing, isolates were grown on malt extract agar (Oxoid, Hampshire, UK) at 30 °C for 7 days. They were then sub-cultured on potato dextrose agar (Oxoid) and incubated at 35 °C for 7 days so that adequate sporulation could be ensured.

Spore suspensions were prepared in sterile 0.9% NaCl solution, adjusted to a density of 0.5 McFarland standard and inoculated onto RPMI MOPS 2% Glucose Agar (Reactivos Para Diagnostico S.L., Barcelona, Spain). For single testing, the MIC test strip was placed in the middle of the plate. The plates were incubated at 30 °C and read after 72–96 h as recommended by the manufacturer. The MIC reading in single testing was interpreted as the zone diameter of inhibition that intersected with the strip.[22]

Combination testing

In accordance with the method described by Kiraz *et al.* [23], the MIC test strips were placed at a 90 ° angle to each other. The MIC strip was placed so that the crossing of the two strips was at the MIC_{50} found in

single testing for each substance. For combination testing, the COL strip was always placed first on the RPMI agar and the antifungal agent strip was placed above. The plates were incubated at 30 °C and were read after 72–96 h. For combination testing, the MIC was read as each drug concentration at which the inhibition zone between the test strips met.

We determined synergistic, indifferent or antagonistic effects by using the following formula to calculate the fractional inhibitory concentration index (FICI): FICI = [MIC A/MIC A(A + B)] + [MIC B/MIC B(A + B)] where MIC A is the MIC of drug A alone, MIC A(A + B) is the MIC of drug A in combination testing, MIC B is the MIC of drug B alone and MIC B(A + B) is the MIC of drug B in companion testing. The FICI thus obtained was interpreted as follows: synergy, <0.5; indifference, 0.5 to 4.0; and antagonism, >4.0.[24] All tests were performed twice.

Results

Scedosporium/*Pseudallescheria* spp.

The range, geometric mean, MIC_{50} and MIC_{90} of COL and the antifungal compounds against *Scedosporium/Pseudallescheria* spp. isolates (n = 77) are shown in Table 1. The MIC_{50} for COL was 12 μg ml^{-1} for *S. prolificans,* 16 μg ml^{-1} for *P. apiosperma* and 16 μg ml^{-1} for *P. boydii* (Table 1). *S. prolificans* exhibited high MICs (>32 μg ml^{-1}) against all tested antifungals in single testing. The MIC values for VRC and AMB against *P. boydii* and *P. apiosperma* were lower than those for *S. prolificans*. CAS exhibited no activity against any tested isolate (>32 μg ml^{-1}).

The MICs and the FICIs determined by susceptibility testing for COL in combination with VRC, CAS and AMB are shown in Tables 2 and 3. The MIC_{50} of the combination of VRC and COL were decreased for both substances and the combination exerted no antagonistic effects against the tested species with the exception of some *P. boydii* isolates (11%). The interaction of CAS and COL against *P. boydii* and *P. apiosperma* increased the MIC_{50} of COL up to 256 μg ml^{-1}, resulting in a high rate of antagonism (>50%).

The MIC_{50} of AMB in single testing against all tested isolates was >32 μg ml^{-1}. When AMB was tested in combination with COL against *S. prolificans*, no antagonistic effects were detected. This combination resulted in fewer than 25% antagonistic effects against *P. apiosperma*, but in 54% antagonism against *P. boydii* isolates. For these last two species, the MIC_{50} of COL in combination with AMB was as much as six times higher than the MIC_{50} of COL alone.

Table 1 *In vitro* susceptibility of *Scedosporium prolificans, Pseudallescheria apiosperma, P. boydii, Exophiala dermatitidis* and *Geosmithia argillacea* against colistin (COL), voriconazole (VRC), caspofungin (CAS) and amphotericin B (AMB) by MIC strip test.

	COL [μg ml^{-1}]				VRC [μg ml^{-1}]			CAS [μg ml^{-1}]			AMB [μg ml^{-1}]			
Species	Range	GM	MIC_{50}	MIC_{90}	Range	GM	MIC_{50}	MIC_{90}	Range	GM	MIC_{50}	MIC_{90}	Range	GM	MIC_{50}	MIC_{90}
S. prolificans (n = 19)	3–16	8.1	12	12	0.016–>32	30.9	>32	>32	>32	>32	>32	>32	0.5–>32	49.6	>32	>32
P. apiosperma (n = 30)	6–32	14.7	16	32	0.047–0.64	0.1	0.094	0.19	0.19–>32	52.7	>32	>32	2–>32	46.9	>32	>32
P. boydii (n = 28)	4–48	18.5	16	36.8	0.016–12	0.1	0.064	0.208	3–>32	57.4	>32	>32	>32	64	>32	>32
E. dermatitidis (n = 82)	1.5–64	11.7	12	24	0.006–>32	0.1	0.047	0.125	>32	64	>32	>32	0.038–0.5	0.2	0.25	0.38
G. argillacea (n = 17)	4–8	6.2	6	8	>32	64	>32	>32	0.0016–>32	<0.1	0.008	0.392	0.75–>32	28.0	>32	>32

MIC, minimum inhibitory concentration; GM, geometric mean; MIC_{50}, minimal inhibitory concentrations of 50% of the isolates; MIC_{90}, minimal inhibitory concentrations of 90% of isolates; n, number of isolates.

Table 2 MIC_{50} of the *in vitro* interaction of colistin (COL) with voriconazole (VRC), caspofungin (CAS) and amphotericin B (AMB) against *Scedosporium prolificans*, *Pseudallescheria apiosperma*, *P. boydii*, *Exophiala dermatitidis* and *Geosmithia argillacea* by MIC test strip.

	Combination of COL + VRC [MIC_{50}, μg ml^{-1}]		Combination of COL + CAS [MIC_{50}, μg ml^{-1}]		Combination of COL + AMB [MIC_{50}, μg ml^{-1}]	
Species	COL MIC (in combination/ alone)	VRC (in combination/ alone)	COL (in combination/ alone)	CAS (in combination/ alone)	COL (in combination/ alone)	AMB(in combination/ alone
S. prolificans (*n* = 19)	6/12	0.5/>32	6/12	6/>32	6/12	4/>32
P. apiosperma (*n* = 30)	0.22/16	0.047/0.094	14/16	12/>32	54/16	15.5/>32
P. boydii (*n* = 28)	0.315/16	0.094/0.064	>256/16	>32/>32	96/16	>32/>32
E. dermatitidis (*n* = 82)	0.19/12	0.032/0.047	8/12	7/>32	4/12	0.19/0.25
G. argillacea (*n* = 17)	6/6	0.5/>32	8/6	3/0.008	6/6	2/>32

MIC, minimal inhibitory concentration; MIC_{50}, minimal inhibitory concentrations of 50% of the isolates; *n*, number of isolates.

Exophiala dermatitidis

The range, geometric mean, MIC_{50} and MIC_{90} of COL against all tested *E. dermatitidis* isolates ($n = 82$) are shown in Table 1. The combination of COL and VRC was synergistic against 20% of isolates, and no antagonism was observed (Table 3). In single testing, *E. dermatitidis* exhibited high MIC values against CAS (MIC_{50}, 32 μg ml^{-1}). Interestingly, the MIC_{50} of CAS decreased from >32 μg ml^{-1} to 7 μg ml^{-1} when COL and CAS were applied in combination; this decrease resulted in a rate of synergy against 7.3% of the strains. AMB alone exhibited low MICs against all isolates (MIC_{50}, 0.25 μg ml^{-1}). When AMB was tested in combination with COL, the MIC_{50} of both drugs decreased and showed indifference to 95% of the isolates.

Geosmithia argillacea

The range, geometric mean, MIC_{50} and MIC_{90} of COL against all tested *G. argillacea* isolates ($n = 17$) are shown in Table 1. VRC alone and AMB alone did not exert any antifungal activity (MIC_{50}, 32 μg ml^{-1} for both substances). The combination of COL and CAS was antagonistic against 88% of *G. argillacea* isolates. When COL was combined with either AMB or VRC, only indifferent effects were observed, but the MIC_{50} of AMB and VRC decreased from >32 to 2 μg ml^{-1} and to 0.5 μg ml^{-1} respectively.

Discussion

In this study, we could demonstrate that the antibiotic COL has besides its bactericidal activity also antifungal activity against *Scedosporium/Pseudallescheria* spp., *E. dermatitidis* and *G. argillaceae*. In addition, we showed that combining COL with antifungal substances could reduce the MIC values of antifungal agents' species specific. Based on the *in vitro* data, COL might be considered as a novel agent for the antifungal therapy in combination with an antifungal compound.

Infections in CF patients with the emerging fungal pathogen *S. prolificans* can be life-threatening due to the therapy-refractory nature of this fungus.[12] As shown in this study and by others, *S. prolificans* is resistant to nearly all antifungals tested as single agent.[20,25–27] One novelty of our study is the finding that COL exerts *in vitro* antifungal activity against *S. prolificans* (MIC_{90} = 12 μg ml^{-1}). To the best of our knowledge, no other single agent that is approved for clinical use has to date demonstrated such low MIC value and this fact suggests that the efficacy of COL may be superior to that of any other antifungal agent against *S. prolificans*.

The combination of either ravuconazole and CAS or terbinafine and miconazole or VRC and micafungin has been reported to be synergistic against *S. prolificans*.[25,28,29] We could also show that the combination of COL and VRC at low concentrations inhibits the growth of *S. prolificans*. In addition, no antagonism against *S. prolificans* was observed when COL was combined with VRC, CAS or AMB.

In general, clinical practice, COL is administered via inhalation as a common prophylaxis and also as treatment for chronic *P. aeruginosa* colonisation in CF patients. In a pharmacokinetic study, Ratjen and colleagues demonstrated that inhalation of 2 million units of COL produces a sputum peak above 30 μg ml^{-1} after 90 min and reaches sputum levels higher than 12 μg ml^{-1} for 8 h after administration.[30] Thus, inhalation twice daily is recommended for maintaining sufficient sputum concentrations of COL.[30] However, one must consider whether a peak COL concentration >30 μg ml^{-1} can actually exhibit fungicidal effects in

Table 3 Combination testing of colistin (COL) with voriconazole (VRC), caspofungin (CAS) and amphotericin B (AMB) against *Scedosporium prolificans*, *Pseudallescheria apiospermum*, *P. boydii*, *Exophiala dermatitidis* and *Geosmithia argillacea* by MIC test strips.

	COL + VRC				COL + CAS				COL + AMB			
Species	ΣFICI[1] (median)	Synergy[2] (*n*/%)	Indifference[3] (*n*/%)	Antagonism[4] (*n*/%)	ΣFICI (median)	Synergistic (*n*/%)	Indifferent (*n*/%)	Antagonistic (*n*/%)	ΣFICI (median)	Synergistic (*n*/%)	Indifferent (*n*/%)	Antagonistic (*n*/%)
S. prolificans (*n* = 19)	0.8	1/5.3	18/94.7	0/0	1.1	1/5.3	18/94.7	0/0	0.8	3/15.8	16/84.2	0/0
P. apiosperma (*n* = 30)	0.7	4/13.3	26/86.7	0/0	4.3	3/10	14/46.7	13/43.3	1.3	5/16.7	18/60	7/23.3
P. boydii (*n* = 28)	1.3	2/7.1	23/82.1	3/10.7	6.3	4/14.3	8/28.6	16/57.1	4.9	2/7.1	11/39.3	15/53.6
E. dermatitidis (*n* = 82)	0.7	16/19.5	66/80.5	0/0	0.9	6/7.3	74/90.2	2/2.4	1.1	1/1.2	78/95.1	3/3.7
G. argillacea (*n* = 17)	0.9	0/0	17/100	0/0	130.6	0/0	2/11.8	15/88.2	1.2	0/0	17/100	0/0

MIC, Minimal inhibitory concentration; *n*, Number of isolates.

[1]ΣFICI, fractional inhibitory concentration index, resulting from the addition of single-drug FICI.

[2]Synergy: FICI ≤ 0.5.

[3]Indifference: FICI > 0.5–4.0.

[4]Antagonism: FICI > 4.0.

the lungs of CF patients. Nonetheless, our *in vitro* data suggest that COL may exert antifungal therapeutic effects alone or in combination with antifungal agents.

COL was found to have *in vitro* activity against non-*Aspergillus* moulds from the genus *Mucorales* and in combination with AMB also exerts synergistic activity against *Rhizopus oryzae*.[18] More importantly, the results of *in vivo* experiments have demonstrated that the prophylactic intranasal administration of colistimethate to intranasally infected immunosuppressed mice harbouring *R. oryzae* spores resulted in a higher survival rate and a lower fungal burden than in untreated control mice.[18] The authors suggested that preclinical and clinical studies evaluating the use of COL for the treatment of mucormycosis should be conducted promptly.

The activity of polymyxin B in combination with fluconazole against *C. neoformans*, *C. albicans* and non-albicans *Candida* species has been previously assessed.[17] This study showed that polymyxin B plus fluconazole was potent at low concentrations against *C. neoformans* isolates. The authors postulated that this drug combination could potentially be an effective treatment option for cryptococcal meningitis.

Because *A. fumigatus* is the mould most frequently isolated in samples from CF patients, we also tested several isolates of this species. In agreement with the findings of a previous study,[18] we found that COL as single agent exerted no antifungal activity (MIC > 2 μg ml^{-1}) against *A. fumigatus* isolates (n = 10) from CF patients (data not shown).

As shown by Gilgado *et al.* [31] and by Lackner *et al.* [20], *P. boydii* exhibits high MIC values for AMB, but VRC shows good antifungal activity *in vitro*. In our study, the highest MICs for COL were also recorded among *P. boydii* isolates (MIC_{90} = 36.8 μg ml^{-1}). Furthermore, the combination of COL with either CAS or AMB exerted antagonistic effects of more than 50% against *P. boydii*. These findings underline the importance of correct identification and determination of the clade origin of the isolate, because the *Scedosporium/Pseudallescheria* spp. are known to have heterogenous antifungal susceptibilities.[20,31] As pointed out recently, susceptibility testing for targeted therapy against *Scedosporium/Pseudallescheria* species is recommended because of a wide MIC distribution for most substances.[20] Our findings confirm these observations: the activity of COL alone and in combination greatly differs among the various species of this genus.

Single testing of COL against *E. dermatitidis* yielded results that were not superior to those of VRC or AMB, for which low MIC values were recorded. However, the

combination of COL with the less-effective drug CAS resulted in a fourfold MIC_{50} of CAS. Moreover, the combination of COL and VRC exhibited synergistic effects in up to 20% of isolates. Consequently, COL seems to have no major disadvantages in combination with antifungal agents against *E. dermatitidis.*

Like in previous studies, we also found high MICs for VRC and low MICs for CAS in *G. argillacea* isolates from CF patients.[4,5] The MICs of AMB were more heterogenous (range 0.75 to >32 μg ml^{-1}), as previously reported.[5] COL exerted antifungal activity against *G. argillacea* with only indifferent effects when it was combined with VRC or AMB. However, COL plus CAS exhibited nearly 90% antagonistic effects. These *in vitro* findings may indicate that concomitant administration of COL and CAS is less effective in treating *G. argillacea* infections.

Most studies analysing antifungal susceptibility of these fungal species have used broth dilution method (BDM), as recommended by CLSI. However, our MIC strip test results for MIC determination of *Scedosporium/Pseudallescheria* spp. are mostly in agreement with the results of a recent study that used BDM.[20] A previous study used Etest to test *E. dermatitidis* and found that isolates were susceptible to CAS.[32] Other studies using BDM found results similar to ours.[33,34] In agreement with Pfaller *et al.* [24], we observed that in case of discrepancies, the MIC values for VRC tend to be lower with a strip test than with BDM. In our study, we used MIC test strips to determine the activity of combinations of agents against filamentous fungi. A previous study demonstrated that Etest could be used successfully to test antifungal interactions for *Candida* spp.[25] However, one limitation of our study is that we did not compare the results of MIC strip interaction testing with the results achieved by BDM.

To the best of our knowledge, no previous studies have tested the activity of COL as single agent or in combination with other antifungal agents against fungi of the genera *Scedosporium/Pseudallescheria*, *Exophiala* and *Geosmithia.* COL is the first agent to demonstrate activity against multidrug-resistant *S. prolificans*. Interaction testing with three antifungal agents demonstrated that VRC appears to be the best combination partner for COL, because almost no antagonism was observed for most species.

In conclusion, we have provided new insights into the antifungal *in vitro* activity of COL. Although the mode of action and cellular target of COL in fungi remains unclear, this substance shows antifungal activity against different filamentous ascomycetes. As our evidence is based on *in vitro* data only, our future perspective is to verify the results by *in vivo* animal models. Furthermore, clinical trials are needed to study the antifungal activity of COL in humans.

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Conflict of interest

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References

1 LiPuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010; **23**: 299–323.
2 Bakare N, Rickerts V, Bargon J, Just-Nübling G. Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. *Mycoses* 2003; **46**: 19–23.
3 Pihet M, Carrere J, Cimon B *et al.* Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis – a review. *Med Mycol* 2009; **47**: 387–97.
4 Barton RC, Borman AM, Johnson EM *et al.* Isolation of the fungus *Geosmithia argillacea* in sputum of people with cystic fibrosis. *J Clin Microbiol* 2010; **48**: 2615–7.
5 Giraud S, Pihet M, Razafimandimby B *et al. Geosmithia argillacea*: an emerging pathogen in patients with cystic fibrosis. *J Clin Microbiol* 2010; **48**: 2381–6.
6 Borman AM, Palmer MD, Delhaes L *et al.* Lack of standardization in the procedures for mycological examination of sputum samples from CF patients: a possible cause for variations in the prevalence of filamentous fungi. *Med Mycol* 2010; **48**(Suppl. 1): 88–97.
7 Cimon B, Carrère J, Vinatier JF, Chazalette JP, Chabasse D, Bouchara JP. Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 53–6.
8 Cortez KJ, Roilides E, Quiroz-Telles F *et al.* Infections caused by *Scedosporium* spp. *Clin Microbiol Rev* 2008; **21**: 157–97.
9 De Ravin SS, Challipalli M, Anderson V *et al. Geosmithia argillacea*: an emerging cause of invasive mycosis in human chronic granulomatous disease. *Clin Infect Dis* 2011; **52**: e136–43.
10 Kusenbach G, Skopnik H, Haase G, Friedrichs F, Döhmen H. *Exophiala dermatitidis* pneumonia in cystic fibrosis. *Eur J Pediatr* 1992; **151**: 344–6.
11 Morio F, Horeau-Langlard D, Gay-Andrieu F *et al.* Disseminated *Scedosporium/Pseudallescheria* infection after double-lung transplantation in patients with cystic fibrosis. *J Clin Microbiol* 2010; **48**: 1978–82.
12 Rodriguez-Tudela JL, Berenguer J, Guarro J *et al.* Epidemiology and outcome of *Scedosporium prolificans* infection, a review of 162 cases. *Med Mycol* 2009; **47**: 359–70.
13 Sahi H, Avery RK, Minai OA *et al. Scedosporium apiospermum* (*Pseudoallescheria boydii*) infection in lung transplant recipients. *J Heart Lung Transplant* 2007; **26**: 350–6.

14 Symoens F, Knoop C, Schrooyen M *et al.* Disseminated *Scedosporium apiospermum* infection in a cystic fibrosis patient after double-lung transplantation. *J Heart Lung Transplant* 2006; **25**: 603–7.
15 Falagas ME, Kasiakou SK, Tsiodras S, Michalopoulos A. The use of intravenous and aerosolized polymyxins for the treatment of infections in critically ill patients: a review of the recent literature. *Clin Med Res* 2006; **4**: 138–46.
16 Elborn JS, Hodson M, Bertram C. Implementation of European standards of care for cystic fibrosis – control and treatment of infection. *J Cyst Fibros* 2009; **8**: 211–7.
17 Zhai B, Zhou H, Yang L *et al.* Polymyxin B, in combination with fluconazole, exerts a potent fungicidal effect. *J Antimicrob Chemother* 2010; **65**: 931–8.
18 Ben-Ami R, Lewis RE, Tarrand J, Leventakos K, Kontoyiannis DP. Antifungal activity of colistin against mucorales species in vitro and in a murine model of *Rhizopus oryzae* pulmonary infection. *Antimicrob Agents Chemother* 2010; **54**: 484–90.
19 Steinmann J, Schmidt D, Buer J, Rath PM. Discrimination of *Scedosporium prolificans* against *Pseudallescheria boydii* and *Scedosporium apiospermum* by semiautomated repetitive sequence-based PCR. *Med Mycol* 2011; **49**: 475–83.
20 Lackner M, de Hoog GS, Verweij PE *et al.* Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. *Antimicrob Agents Chemother* 2012; **56**: 2635–42.
21 CLSI. *Reference Method for Broth Microdilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard*, 2nd edn. CLSI document M38-A2. Wayne, PA: CLSI, 2008.
22 Pfaller JB, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. In vitro susceptibility testing of *Aspergillus* spp.: comparison of Etest and reference microdilution methods for determining voriconazole and itraconazole MICs. *J Clin Microbiol* 2003; **41**: 1126–9.
23 Kiraz N, Dag I, Yamac M, Kiremitci A, Kasifoglu N, Oz Y. Synergistic activities of three triazoles with caspofungin against *Candida glabrata* isolates determined by time-kill, Etest, and disk diffusion methods. *Antimicrob Agents Chemother* 2010; **54**: 2244–7.
24 Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother* 2003; **52**: 1.
25 Cuenca-Estrella M, Alastruey-Izquierdo A, Alcazar-Fuoli L *et al.* In vitro activities of 35 double combinations of antifungal agents against *Scedosporium apiospermum* and *Scedosporium prolificans*. *Antimicrob Agents Chemother* 2008; **52**: 1136–9.
26 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**: 62–8.
27 Tintelnot K, Just-Nübling G, Horré R *et al.* A review of German *Scedosporium prolificans* cases from 1993 to 2007. *Med Mycol* 2009; **47**: 351–8.
28 Meletiadis J, Mouton JW, Meis JF, Verweij PE. *In vitro* drug interaction modeling of combinations of azoles with terbinafine against clinical *Scedosporium prolificans* isolates. *Antimicrob Agents Chemother* 2003; **47**: 106–17.
29 Heyn K, Tredup A, Salvenmoser S, Müller FM. Effect of voriconazole combined with micafungin against *Candida*, *Aspergillus*, and *Scedosporium* spp. and *Fusarium solani*. *Antimicrob Agents Chemother* 2005; **49**: 5157–9.
30 Ratjen F, Rietschel E, Kasel D *et al.* Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. *J Antimicrob Chemother* 2006; **57**: 306–11.
31 Gilgado F, Serena C, Cano J, Gené J, Guarro J. Antifungal susceptibilities of the species of the *Pseudallescheria boydii* complex. *Antimicrob Agents Chemother* 2006; **50**: 4211–3.
32 Kondori N, Gilljam M, Lindblad A, Jönsson B, Moore ER, Wennerås C. High rate of *Exophiala dermatitidis* recovery in the airways of patients with cystic fibrosis is associated with pancreatic insufficiency. *J Clin Microbiol* 2011; **49**: 1004–9.
33 Badali H, de Hoog GS, Sudhadham M, Meis JF. Microdilution in vitro antifungal susceptibility of *Exophiala dermatitidis*, a systemic opportunist. *Med Mycol* 2011; **49**: 819–24.
34 Sun Y, Liu W, Wan Z, Wang X, Li R. Antifungal activity of antifungal drugs, as well as drug combinations against *Exophiala dermatitidis*. *Mycopathologia* 2011; **171**: 111–7.