Interaction between amorolfine and voriconazole against Fusarium species

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Abstract

Background: *Fusarium species* are environmentally ubiquitous fungi capable of causing diverse superficial, locally invasive, or disseminated infections, making them important pathogens. Compared with antibacterial drugs, the types of antifungal drugs are still limited, and the adverse reactions are significant. Therefore, novel treatments against *Fusarium* spp*.* are an urgent need.

Results: Here we investigated the interaction of amorolfine combined with voriconazole on *Fusarium* spp. Our study demonstrated that amorolfine in combination with voriconazole could inhibited the *Fusarium* spp. significantly*. Galleria mellonella* was also used as a model to show the interaction of the two-drug combination *in vivo* against *Fusarium* spp.; larval survival rates were significantly higher after treatment with the amorolfine-voriconazole combination compared to the monotherapy group.

Conclusions: This study is the first to demonstrate that voriconazole combined with amorolfine has a synergistic effect against *Fusarium* spp*.* infection and may be an effective method for antifungal therapy.

Background

Fusarium species are environmentally ubiquitous fungi [1], and they are capable of inducing a range of diverse superficial, locally invasive, or disseminated infections [2–21]. Fusariosis primarily occurs in humans as a consequence of inhaling or contacting materials that contain *Fusarium* conidia. Subsequently, when a suitable environment is provided, conidia germinate and develop into filamentous structures capable of invading local tissues [21]. There has been no international consensus on the prescriptive treatment of *Fusarium* spp. infecion. The therapeutic effects of all kinds of antifungal drugs against *Fusarium* spp. were not significant. Currently, voriconazole or liposomal amphotericin B are the two primary drugs used to treat invasive fusariosis, and they are the most effective drugs for *Fusarium* infections [22–24]. However, patient outcomes are often poor and primarily depend on a restoration of immune function in affected hosts, with neutropenia in particular being associated with poor outcomes [25]. Additionally, it has been known that patients treated with voriconazole or liposomal amphotericin B are prone to abnormal renal function and other adverse reactions. Compared with antibacterial drugs, the types of antifungal drugs are still lmited, and the adverse reactions are significant. As few novel antifungal drugs are available or in development, combination-based therapeutic approaches instead represent the most promising approach to treat serious fungal infections.

Amorolfine is a derivative of morpholine and a new kind of broad-spectrum antifungal agent that that has been used to treat fungal infections in humans, such as dermatophytes, yeast, and a few condition pathogenic fungi [26].

Herein, we first demonstrated the *in vitro* antifungal activity of amorolfine in combination with voriconazole on *Fusarium* spp. by the checkerboard microdilution method. Moreover, we also investigated the *in vivo* therapeutic effects of the two-drug combination against *Fusarium* spp. infection in the *Galleria mellonella* model by survival analysis.

Results

MIC of amorolfine and voriconazole against Fusarium spp.

For all of the strains, the minimal inhibitory concentration (MIC) ranges for individual agents when used to treat *Fusarium* isolates were 4 to 16 μg/mL for amorolfine, and 2 to 8 μg/ml for voriconazole (Table 1). A combination of amorolfine with voriconazole showed synergistic antifungal effects against 11 (73.3%) strains of *Fusarium* spp. and the effective MIC ranges of amorolfine and voriconazole were primarily in the 1–2 μg/ml and 0.5–2 μg/ml ranges, respectively. These effects were also indicated *in vitro* by the FICI: the FICIs for most *F. solani* (Jzfs1, Jzfs2, Jzfs3, Jzfs4, Jzfs6, Jzfs7, Jzfs8, Jzfs10) and *F.oxysporum* (Jzfo2, Jzfo3, Jzfo5) strains were all ≤ 0.5 (Table 1); nevertheless, the FICIs were > 0.5 for some of *Fusarium* strains including Jzfs5, Jzfs9, Jzfo1and Jzfo4 strains, confirming that the two-drug combination has no interaction on these strains.

Table 1
MIC and FICI results with combination of AMO and VOR against *Fusarium* spp.

	Strains	MICs (μg/ml)				
		AMO	VOR	AMO/VOR	FICI	IN
F. Solani						
	Jzfs1	4	4	1/0.5	0.375	SYN
	Jzfs2	8	4	1/0.5	0.25	SYN
	Jzfs3	4	2	1/0.5	0.5	SYN
	Jzfs4	8	4	2/1	0.5	SYN
	Jzfs5	4	2	2/0.5	0.75	NI
	Jzfs6	16	8	1/1	0.1875	SYN
	Jzfs7	8	4	1/0.5	0.25	SYN
	Jzfs8	8	2	1/0.5	0.375	SYN
	Jzfs9	8	2	1/1	0.625	NI
	Jzfs10	16	8	2/2	0.375	SYN
F. oxysporum						
	Jzfo1	8	2	1/1	0.625	NI
	Jzfo2	16	4	1/0.5	0.1875	SYN
	Jzfo3	8	4	2/0.5	0.375	SYN
	Jzfo4	8	2	2/1	0.75	NI
	Jzfo5	8	2	1/0.5	0.375	SYN

AMO Amorolfine; *VOR* Voriconazole; *MICs* Minimal inhibitory concentrations; *FICI*, fractional inhibitory concentration index; *NI* no interaction; *IN* interaction; *SYN* synergism; *NI* no interaction

Efficacy of voriconazole and amorolfine in Fusarium**-infected** G. mellonella

We investigated the *in vivo* antifungal activity of voriconazole (100 μg/mL), amorolfine (100 μg/mL), or the combination thereof using *G. mellonella* infected with *Fusarium* isolate (jzfs1). Our study illustrated that after 2 days of infection, larvae treated with the two-drug combination had the highest survival rate(68%) compared to monotherapy (voriconazole, 51%; amorolfine, 43%) and conidial alone groups (28%) ($P < 0.05$). At 4 days after infection, larvae treated with the combination group exhibited survival rates that were 3-fold higher than those of the conidial alone group. At 6 days after infection, *G.*

mellonella with monotherapy exhibited lower survival rate (voriconazole, 15%; amorolfine, 12%) than *G. mellonella* with combination therapy (30%) with 3% of *G. mellonella* in the conidial group ($P < 0.05$). However, larvae treated with amorolfine alone exhibited slightly decreased survival rates compared to larvae treated with voriconazole alone (Fig. 1).

Histopathology Study

At 2 days of infection, we conducted histopathologic staining of *G. mellonella* larvae infected with *F. solani* (jzfs1) and treated with different drugs. Histopathologic staining revealed the differences in the presence and abundunce of fungi and cytoplasmic staining. We found that plenty of mycelia and spores existed in infected larvae. In different groups, the number of spores decreased significantly in the group treated with a combination of voriconazole (100 μg/mL) and amorolfine (100 μg/mL) (Fig. 2d). In addition, the number of yeast cells in the voriconazole alone group (Fig. 2c) was lower than that in the amorolfine alone group (Fig. 2b). Mean infected area size in the combination group was smaller relative to the voriconazole group. The conidial (treatment with the conidia alone) group (Fig. 2a) had higher infection levels than other assessed groups.

Discussion

Fusariosis can be treated effectively using voriconazole or amphotericin B monotherapeutic approaches[21, 27]. However,the above two treatments have major side effects and higher costs. Therapeutic approaches, such as the application of novel compounds or existing drugs in combination with azoles, may represent an efficacious approach for fungal infections, as they may enhance antifungal efficacy, enable a wider antifungal spectrum, and reduce side effects. Previous studies have demonstrated that licofelone can synergistically function with fluconazole when used to treat conidia of fluconazole- resistant *C. albicans* [28]. And pyrvinium pamoate was also a synergist of azoles against *Exophilal dermatitidis* [29]. Kathrin H et al. [30]reported that the combination of voriconazole and micafungin was synergistic *in vitro* against *F.solani*. Amorolfine, as broad- spectrum antifungal agent, has been found to have antifungal activity against dermatophytes, yeast, and a few condition pathogenic fungi [26]. We then evaluated the interaction between voriconazole and amorolfine against *Fusarium* spp. In vitro, amorolfine alone has a moderate effect on *Fusarium* spp.: the MICs of amorolfine alone against *Fusarium* spp. were 4, 8 and 16 μg/mL. In addition, amorolfine can synergistically function with voriconazole; the MIC of voriconazole fell from 8, 4,2 to 2,1 and 0.5 μg/mL when combined with amorolfine (1,2 μg/mL), whereas the combination had little impact on the *F. solani* (jzfs 5,9)and *F.oxysporum* strains (jzfo 1,4) (Table 1). Therefore, it is reasonable to speculate that the combination of amorolfine and voriconazole could improve the susceptibility of *Fusarium* spp. by decreasing the MICs of the above two drugs. Based on FICIs, we found that voriconazole could synergistically work with amorolfine against *Fusarium* spp. (Table 1).

To further verify the interaction between amorolfine and voriconazole in our study, we detected the combined antifungal effect of voriconazole-amorolfine against *Fusarium* spp. at different time points *in*

vivo. For monotherapy, voriconazole or amorolfine was used. For combination therapy, voriconazole with an amorolfine formulation was administered. We note that, compared to the conidia alone group, monotherapy with voriconazole or amorolfine improved the survival of *G.mellonella*, and the survival rate of vo riconazole monotherapy-treated larva was slightly higher than that of amorolfine monotherapy-treated larvae, potentially because this improvement in survival may be attributed to the availability of antifungal agents, and the antifungal activity of voriconazole was stronger than that of amorolfine. In addition, combination therapy in *G. mellonella* was better than voriconazole or amorolfine monotherapy ($P < 0.05$), suggesting that amorolofine could potentiate the activity of voriconazole and showed favorable synergistic effect with voriconazole against *Fusarium* spp.

Furthermore, the fungal virulence and antifungal drug activity has recently been investigated in *G. mellonella* model on account of a similar immune response as mammals and other advantages including lack of ethical concerns, low cost, and easy manipulation[28, 31, 32]. As illustrated in our study, the survival rate of larvae in voriconazole- amorolfine combination group was significantly improved compared to the monotherapy groups (including voriconazole or amorolfine-treated group) ($P < 0.05$). Moreover, we observed larva after infection microscopically (Fig. 2). In the histological tissue of *G. mellonella*, our results showed that the combined group with high survival rate contained fewer *Fusarium* spp. than the conidia group and monotherapy group. This suggested that amorolfine can further potentiate the therapeutic effect of voriconazole, and voriconazole combined with amorolfine showed a synergistic effect against *Fusarium* spp. infection.

Conclusion

Taken together, what we have identified in this study suggest that amorolfine could be an effective synergist with voriconazole against *Fusarium* spp. and the combination of the two drugs may represent a viable therapeutic strategy for antifungal treatment to significantly relieve the clinical symptoms of *Fusarium* spp.-related diseases. However, to some extent, the fact that amorolfine has no injection and oral preparation limites the possible application of the drug to invasive system infections. With ongoing development in pharmaceutical manufacturing technologies, we can look forward to innovative formulations of amorolfine enabling their broader application. More studies on the in-depth mechanistic basis for the antifungal properties of amorolfine may also guide new antifungal strategies.

Methods

Strains and Media

In the present study, Fifteen clinical isolates were stored in Department of Dermatology, Zhongnan hospital of Wuhan University in China, including ten *Fusarium solani* strains and five *Fusarium oxysporum* strains. *Aspergillus flavus* strain (ATCC 204304) was used for quality control. Clinical isolates were collected from patients that had provided written informed consent, and the Research Ethical Committee of Zhongnan Hospital of Wuhan University approved the present study.The susceptibility

assay preparation for *Fusarium* spp. were performed in accordence to Clinical and Laboratory Standards Institute document M38-A2 [33]. The *Fumarium* spp. conidia were obtained from cultures grown on Potato Dextrose Agar(PDA) solid medium for 2 weeks at 28°C. Conidia were collected by washing the surface of these plates using PBS, after which a hemocytometer was used to count them. In the current study, we first determined the minimal inhibitory concentration (MIC) of voriconazole and amorolfine in single use before performing a large number of experiments.The MIC was defined as the lowest concentration of the drug that inhibited fungal growth by 100% compared with the growth of the control.

MIC Determination by Broth Microdilution Assays

Herein, the impact of amorolfine alone and combined with voriconazole was investigated against a total of 15 strains of *Fusarium* isolates (10 *F. solani* strains and 5 *F.oxysporum* strains). *Aspergillus flavus* strain (ATCC 204304) was included for quality control. All *Fusarium* isolates were identified by microscopic morphology and internal transcribed spacer (ITS) ribosomal DNA (rDNA) sequencing[34]. For the checkerboard assays [33], dimethyl sulfoxide (DMSO) was used to prepare stocks of each antifungal agent (3,200 μg/ml). Working solutions were prepared using sterile RPMI-1640 medium to serially dilute voriconazole (0.25 to 16 μg/ml; Shanghai Selleck Co., Ltd., China) and amorolfine (0.5 to 32 μg/ml; Shanghai Selleck Co., Ltd., China). For broth microdilution checkerboard analyses, the M38-A2 technique was adapted as per protocols developed by the Clinical and Laboratory Standards Institute, with MICs corresponding to

the lowest concentrations that completely inhibited growth (100% inhibition)[33].

Interactions between amorolfine and voriconazole were assessed based upon the fractional inhibitory concentration index (FICI), which was determined as follows: FICI= (Ac/Aa) +(Bc/Ba), wherein Ac and Bc correspond to antifungal MICs for drugs in combination and Aa and Ba correspond to MICs for drugs A and B as single agents. A FICI of ≤ 0.5 represents synergistic, while a FICI of > 0.5 to ≤ 4 indicates a lack of any interaction (indifference), and a FICI of > 4 represents an antagonism interaction[35]. Analyses were conducted in triplicate.

Efficacy of Voriconazole and Amorolfine in G. mellonella **infected with** Fusarium **strains**

As previously described [28, 36, 37], *Galleria mellonella* larval were used and divided into six groups: untreated group, growth control group (10 μl saline only), conidial group, voriconazole (100 μg/mL)-treated group, amorolfine (100 μg/mL)-treated group, and voriconazole (100 μg/mL) with amorolfine (100 μg/mL)-treated group. And each experimental group contained 20 larvae (0.3– 0.4 g in weight). *Fusarium* isolate (jzfs1) conidia, obtained from PDA cultures grown at 28 °C for 1 week, had been collected via washing the agar surface using PBS and counted via hemocytometer (10^7CFU/ml). Ten microlitres of conidia suspension was injected into *Galleria mellonella* of different groups except untreated group and growth control group (10 μl saline only). The infected larvae were stored at 37°C for 2 h and then treated with antifungal drugs (5 μl). Finally, the larvae was incubated 37°C and monitored for survival rate every 24 h for 6 days. And the experiment was repeated thrice.

Histological Study

To detect the *Fusarium* isolate in the tissue of *G. mellonella*, we collected larvae separately from different groups, including conidial alone group, voriconazole- treated group, amorolfine-treated group, and voriconazole with amorolfine-treated group, at day 2 after infection. According to previously described [28], the larvae infected with 1×10^5 cells/larva *F. solani* (jzfs1) were processed for ultrathin tissue sections, which were subsequently stained with hematoxylin and eosin (HE) and Periodic Acid-Schiff (PAS).

Statistical Analysis

GraphPad Prism 5.0 was used to prepare Figures, while SPSS 16.0 was used for all statistical analysis. Data are given as means ± SEM, and were compared using ANOVAs. Experiments were repeated in triplicate, with $P < 0.05$ as the significance threshold.

Abbreviations

F. solani: Fusarium solani; *F.oxysporum*:*Fusarium oxysporum*; AMO:Amorolfine; VOR:Voriconazole; MICs:Minimal inhibitory concentrations; FICI:Fractional inhibitory concentration index; NI:No interaction; IN:Interaction; SYN:Synergism; NI:No interaction

Declarations

Ethics approval and consent to participate

Research Ethical Committee of Zhongnan Hospital of Wuhan University granted permission to access the clinical samples used. And ethical approval was given by the Research Ethical Committee of Zhongnan Hospital of Wuhan University. Written consent was given by the patients for the use of clinical strains.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. The data used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PL, QL contributed to the conception of the project, data analysis, interpretation of results, creation of figures, writing and editing the manuscript. JS performed sample collection and fungal culture. The authors have read and approve of the manuscript.

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References

1. Nelson PE, Dignani MC, Anaissie EJ. Taxonomy, biology, and clinical aspects of Fusarium species. Clin Microbiol Rev. 1994;7(4):479–504.
2. Bourguignon RL, Walsh AF, Flynn JC, Baro C, Spinos E. Fusarium species osteomyelitis. Case report. J Bone Joint Surg Am. 1976;58(5):722–3.
3. Chang DC, Grant GB, O'Donnell K, Wannemuehler KA, Noble-Wang J, Rao CY, Jacobson LM, Crowell CS, Sneed RS, Lewis FM, Schaffzin JK, Kainer MA, Genese CA, Alfonso EC, Jones DB, Srinivasan A, Fridkin SK, Park BJ. Multistate outbreak of Fusarium keratitis associated with use of a contact lens solution. JAMA. 2006;296(8):953–63.
4. Flynn JT, Meislich D, Kaiser BA, Polinsky MS, Baluarte HJ. Fusarium peritonitis in a child on peritoneal dialysis: case report and review of the literature. Perit Dial Int. 1996;16(1):52–7.
5. Gabriele P, Hutchins RK. Fusarium endophthalmitis in an intravenous drug abuser. Am J Ophthalmol. 1996;122(1):119–21.
6. Grant GB, Fridkin S, Chang DC, Park BJ. Postrecall surveillance following a multistate fusarium keratitis outbreak, 2004 through 2006. JAMA. 2007;298(24):2867–8.
7. Hilmioglu-Polat S, Metin DY, Inci R, Dereli T, Kilinc I, Tumbay E. Non-dermatophytic molds as agents of onychomycosis in Izmir, TurkeyVa prospective study. Mycopathologia. 2005;160(2):125–8.
8. Jakle C, Leek JC, Olson DA, Robbins DL. Septic arthritis due to Fusarium solani. J Rheumatol. 1983;10(1):151–3.

9. Kerr CM, Perfect JR, Craven PC, Jorgensen JH, Drutz DJ, Shelburne JD, Gallis HA, Gutman RA. Fungal peritonitis in patients on continuous ambulatory peritoneal dialysis. Ann Intern Med. 1983;99(3):334–6.
10. Kurien M, Anandi V, Raman R, Brahmadathan KN. Maxillary sinus fusariosis in immunocompetent hosts. J Laryngol Otol. 1992;106(8):733–6.
11. Madhavan M, Ratnakar C, Veliath AJ, Kanungo R, Smile SR, Bhat S. Primary disseminated fusarial infection. Postgrad Med J. 1992;68(796):143–4.
12. Murray CK, Beckius ML, McAllister K. Fusarium proliferatum superficial suppurative thrombophlebitis. Mil Med. 2003;168(5):426–7.
13. Nelson PE, Dignani MC, Anaissie EJ. Taxonomy, biology, and clinical aspects of Fusarium species. Clin Microbiol Rev. 1994;7(4):479–504.
14. Nucci M, Anaissie E. Cutaneous infection by Fusarium species in healthy and immunocompromised hosts: implications for diagnosis and management. Clin Infect Dis. 2002;35(8):909–20.
15. Nucci M, Anaissie E. Fusarium infections in immunocompromised patients. Clin Microbiol Rev. 2007;20(4):695–704.
16. Pflugfelder SC, Flynn HW Jr, Zwickey TA, Forster RK, Tsiligianni A, Culbertson WW, Mandelbaum S. Exogenous fungal endophthalmitis. Ophthalmology. 1988;95(1):19–30.
17. Rippon JW, Larson RA, Rosenthal DM, Clayman J. Disseminated cutaneous and peritoneal hyalohyphomycosis caused by Fusarium species: three cases and review of the literature. Mycopathologia. 1988;101(2):105–11.
18. Sander A, Beyer U, Amberg R. Systemic Fusarium oxysporum infection in an immunocompetent patient with an adult respiratory distress syndrome (ARDS) and extracorporal membrane oxygenation (ECMO). Mycoses. 1998;41(3–4):109–11.
19. Saw SM, Ooi PL, Tan DT, Khor WB, Fong CW, Lim J, Cajucom-Uy HY, Heng D, Chew SK, Aung T, Tan AL, Chan CL, Ting S, Tambyah PA, Wong TY. Risk factors for contact lens-related fusarium keratitis:a case-control study in Singapore. Arch Ophthalmol. 2007;125(5):611–7.
20. Sturm AW, Grave W, Kwee WS. Disseminated Fusarium oxysporum infection in patient with heatstroke. Lancet. 1989;1(8644):968.
21. Muhammed M, Anagnostou T, Desalermos A, Kourkoumpetis TK, Carneiro HA, Glavis- Bloom J, Coleman JJ, Mylonakis E. Fusarium Infection Report of 26 Cases and Review of 97 Cases From the Literature. Medicine. 2013;92(6):305–16.
22. Horn DL, Freifeld AG,Schuster MG, Azie NE, Franks B, Kauffman CA. Treatment and outcomes of invasive fusariosis: review of 65 cases from the PATH Alliance registry. Mycoses. 2014;57(11):652–8.
23. Rosanova MT, Brizuela M, Villasboas M, Guarracino F, Alvarez V, Santos P, Finquelievich J. Fusarium spp. infections in a pediatric burn unit: nine years of experience. Braz J Infect Dis. 2016;20(4):389–92.

24. Tortorano AM, Richardson M, Roilides E, van Diepeningen A, Caira M, Munoz P, Johnson E, Meletiadis J, Pana ZD, Lackner M, Verweij P, Freiberger T, Cornely OA, Arikan-Akdagli S, Groll DE, Lagrou AH, Chakrabarti K, Lanternier A, Pagano F, Skiada L, ,Akova A, Arendrup M, Boekhout MC, Chowdhary T, Cuenca-Estrella A, Guinea M, Guarro J, de Hoog J, Hope S, Kathuria W, Lortholary S, Meis O, Ullmann JF, Petrikkos AJ. G, Lass-Flörl C. ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: Fusarium spp., Scedosporium spp. and others. Clin Microbiol Infect. 2014;20(Suppl 3):27–46.
25. Nucci F, Nouér SA, Capone D, Anaissie E, Nucci M. Fusariosis Semin Respir Crit Care Med. 2015;36(5):706–14.
26. Wang JW. Therapeutic effect of Itraconazole combined with amorolfine cream on Malassezia-associated skin diseases. Medical Information Jan. 2019;32(1):157–9.
27. Perfect JR. Treatment of non-Aspergillus moulds in immunocompromised patients, with amphotericin B lipid complex. Clin Infect Dis. 2005;40(Suppl 6):401-8.
28. Liu XN, Li T, Wang DC, Yang YL, Sun WW, Liu JQ, Sun SJ. Synergist antifungal effect of fluconazole combined with licofelone against resistant Candida albicans. Front Microbiol. 2017;8:2101.
29. Gao LJ. Sun Y, He CY. Zeng TX, Li M. Synergy between pyrvinium pamoate and azoles against Exophiala dermatitidis. ANTIMICROB AGENTS CH 2018; 62(4):1–3.
30. Kathrin H, Antje T, Stefanie S, Frank-Michael CM. Effect of voriconazole combined with micafungin against Candida, Aspergillus, and Acedosporium spp. and Fusarium solan. ANTIMICROB AGENTS CH. 2005;49(12):5157–9.
31. Vilcinskas A. **Insects emerge as valuable model hosts to explore viru***lence*. Virulence *2011*; 2(5), 376–8.
32. Favre-Godal Q, Dorsaz S, Queiroz EF, Conan C, Marcourt L, Wardojo BP, Voinesco F, Buchwalder A, Gindro K, Sanglard D, Wolfender J. Comprehensive approach for the detection of antifungal compounds using a susceptible strain o*f Candida albicans* and confirmation of in vivo activity with the *Galleria mellonella* model. Phytochemistry. 2014;105:68–78.
33. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testingof filamentous fungi. Approved standard, 2nd ed CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
34. Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol. 1995;61:1323–30.
35. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother. 2003;52(1):1.
36. Mylonakis E, Moreno R, El Khoury JB, Idnurm A, Heitman J, Calderwood SB, Ausubel FM, Diener A. Galleria mellonella as a model system to study Cryptococcus neoformans pathogenesis. Infect Immun. 2005;73(7):3842–50.
37. Amorim-Vaz S, Delarze E, Ischer F, Sanglard D, Coste AT. Examining the virulence of Candida albicans transcription factor mutants using Galleria mellonella and mouse infection models. Front Microbiol.

2015;6:367.

Figures

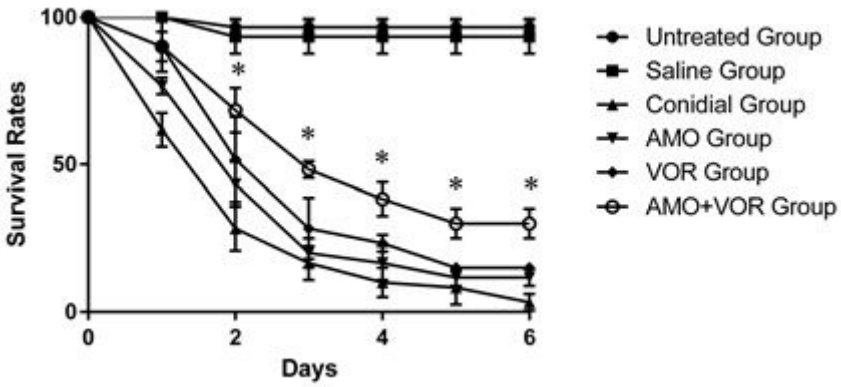

Figure 1

Survival rate of G. mellonella infected with Fusarium isolate and administered different treatments. The curves consisted of the untreated group, saline group, conidial alone group, voriconazole (100μg/mL) group, amorolfine (100 μg/mL) group and amorolfine combined with fluconazole (100 μg/mL) group. ∗P < 0.05 vs. monotherapy (voriconazole; amorolfine) and conidial alone groups. Triplicate analyses were performed.

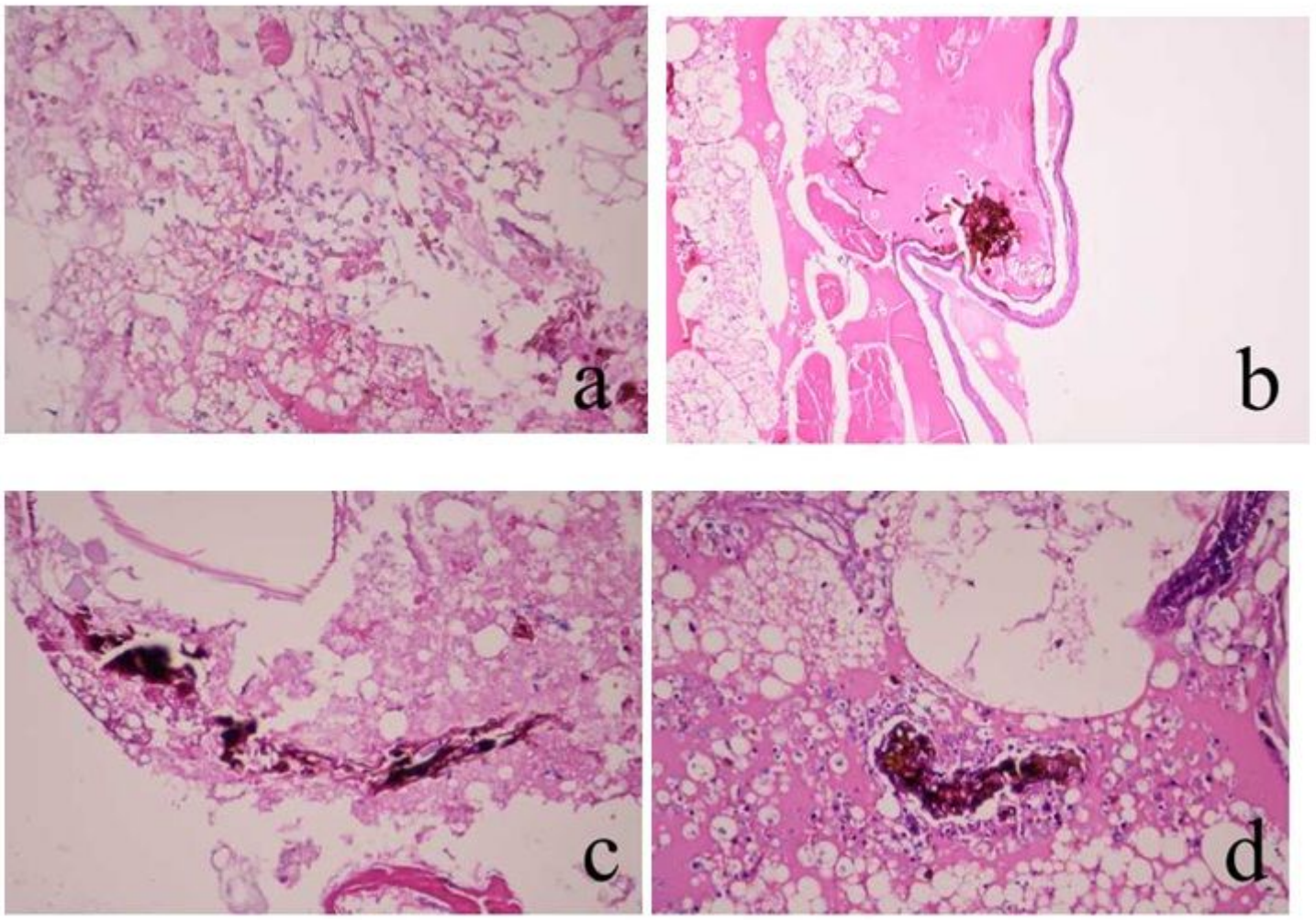

Figure 2

Histopathology detection of infected G. mellonella treated with different drugs. (a). Conidia alone group; (b). Larvae treated with amorolfine group; (c). Larvae treated with voriconazole group; (d). Larvae treated with fluconazole- amorolfine combination group. Triplicate analyses were performed.