In-vitro Efficacy Of Antifungals Combined With Recrystallised Calcium Sulfate

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Background:

Fungal osteomyelitis is a challenging and often overlooked health problem, compounded by emerging resistance and the limited range of antifungal drugs available.[1] The use of antibiotic loaded, recrystallised calcium sulfate* (RCS) has been documented, however, little data has been published on the combination and release of antifungals in this way.[2] This is despite growing concerns of resistance and the more limited range of antifungals available. Antifungals have been reported to be combined with PMMA beads with good clinical outcomes,[3] however, unlike RCS, these had to be surgically removed at 12 weeks post-op. This study aimed to investigate the efficacy of 7 antifungals in combination with RCS, through Zone Of Inhibition (ZOI) testing. Five fungal pathogens known to be associated with orthopaedic complications or diabetic foot infections were selected for investigation.

Materials/methods:

6mm diameter hemispherical beads were prepared with 5cc packs of RCS unloaded or in combination with either amphotericin B, caspofungin, voriconazole, anidulafungin, isavuconazole, micafungin or posaconazole. For the posaconazole, this was in liquid form therefore 3ml was used in place of the mixing solution provided. All antifungals except amphotericin B and voriconazole mixed and set easily. The mixing technique was modified to achieve set beads for these two antifungals. Antifungal concentrations were based on a maximum 24 hour systemic dose for an average weight adult. Beads of each type were then placed onto Mueller-Hinton agar plates inoculated with actively growing colonies of the following fungi: *A. fumigatus, A. niger, C. parapsilosis, C. albicans or T. rubrum.* Plates were then incubated aerobically at 30°C and examined daily for growth and areas of inhibition. Contact times were 48h for yeasts and 5 days for moulds. Zones were measured and photographed. Testing was repeated in triplicate.

Results:

Mixing protocols were established to enable formation of set beads; all setting within 30 minutes. Amphotericin B and voriconazole required pre-mixing the RCS with saline rather than the mixing solution provided, prior to adding the antifungal powder. For ZOI testing, three combinations were known to be resistant and no zone was recorded. For the remaining samples, loaded beads produced ZOIs against the fungal organisms tested, except for 4 combinations – anidulafungin and caspofungin against *T. rubrum,* isavuconazole against *C. parapsilosis* and posaconazole against *C. albicans.* **(Table 1)**

Bead type	Antifungal Concentration in 5cc pack RCS	Average Zone Diameter (mm) Against Selected Fungal Pathogens				
		Candida albicans (NCPF 3179)	*Candida parapsilosis* (ATCC 90018)	*Aspergillus niger* (ATCC 16888)	*Aspergillus Fumigatus* (NCPF 2140)	*Trichophyton rubrum* (NCPF 0419)
Amphotericin B	25mg	23.67	24.33	Resistant	13.67	19.00
Anidulafungin	100mg	30.67	31.67	39.67	18.00	No Zone
Caspofungin	35mg	27.33	23.67	30.50	31.67	No Zone
Isavuconazole	200mg	15.00	No Zone	49.67	51.67	77.67
Micafungin	500mg	26.00	25.33	32.33	6.00	37.50
Posaconazole	54mg (3ml)	No Zone	35.67	45.00	43.33	53.55
Voriconazole	100mg	Resistant	Resistant	51.33	58.00	76.70

Table 1. ZOI results. Red - No zone, Yellow - Zone <30mm, Green - Zone >30mm

The smallest ZOIs were recorded for *A. fumigatus* with micafungin (6mm) and amphotericin B (13.67mm).

The largest ZOIs were recorded for *T. rubrum* with isavuconazole (77.67mm) and voriconazole (76.7mm). **(Figures 1 and 2)**

For all other combinations, mean ZOIs ranged between 15mm and 51.7mm. Unloaded beads did not inhibit growth. **(Figure 3)**

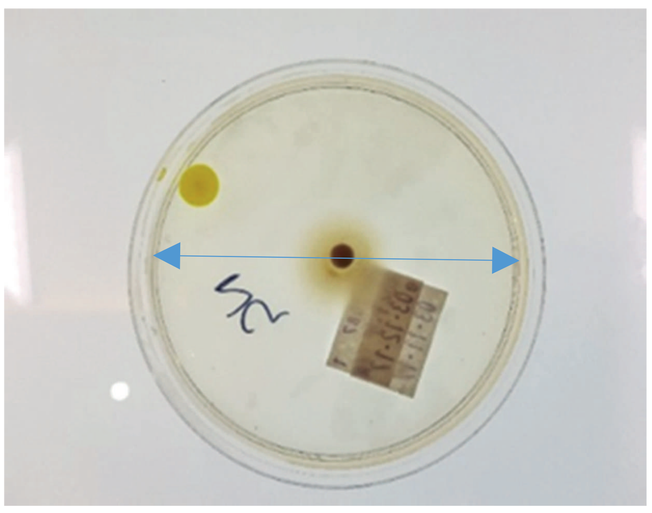

Fig. 1. Isavuconazole bead against *T. rubrum*

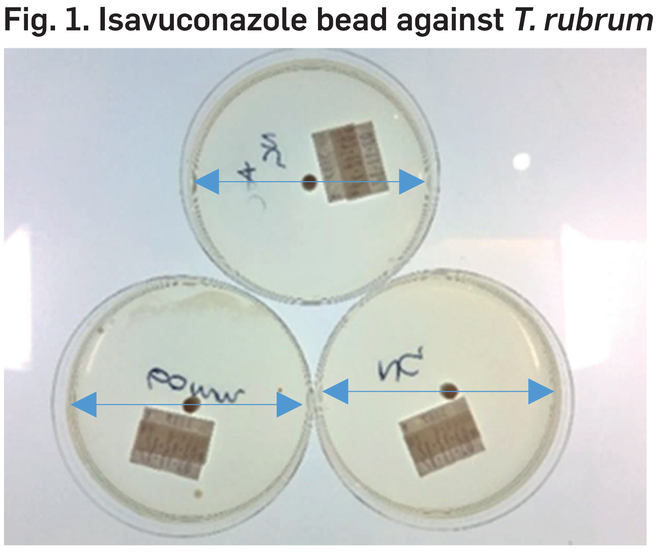

Fig. 2. Voriconazole beads against *T. rubrum*

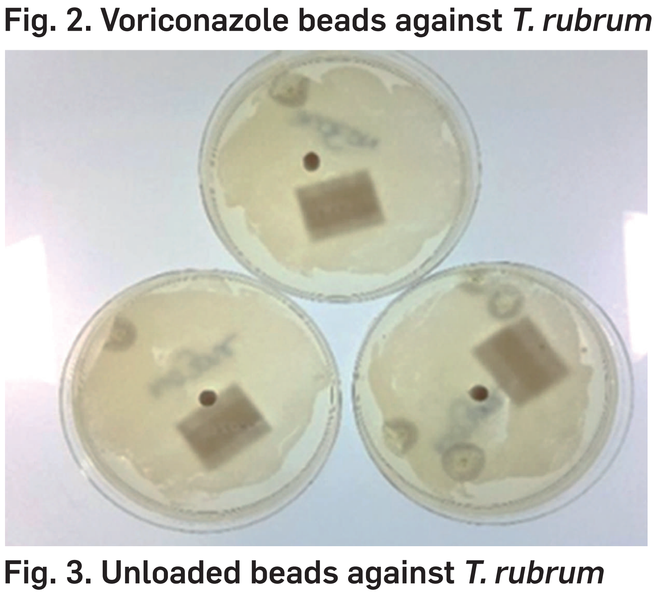

Fig. 3. Unloaded beads against *T. rubrum*

Conclusions:

All antifungals were successfully combined and set with recrystallised calcium sulfate. A modified mixing technique was required for amphotericin B and voriconazole to achieve set beads. Against susceptible strains, the eluted antifungals demonstrated *in-vitro* efficacy in this study. For the 4 combinations where no zones were observed, further investigation is warranted. Local release of antifungals from RCS may have a potential role in the management of difficult to treat fungal infections. *In-vitro* results do not necessarily correlate *in-vivo.*

Reference:

1. Kontoyiannis DP. Antifungal Resistance: An Emerging Reality and A Global Challenge. J Infect Dis. 2017 Aug 15; 216 (suppl_3): S431-S435.
2. Narayana Rao VV, Anil Babu P. Amphotericin mixed Biocomposite bone cement along with surgical debridement in treatment of Fungal osteomyelitis of Calcaneum. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2015; 14(2): 5-7.
3. Arockiaraj J, *et al.* Amphotericin B Cement Beads: A Good Adjunctive Treatment for Musculoskeletal Mucormycosis Indian Journal of Orthopaedics. 46.3 (2012): 369-372.

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