Elution and Efficacy of Colistin when Combined with a Synthetic Calcium Sulfate

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Aim

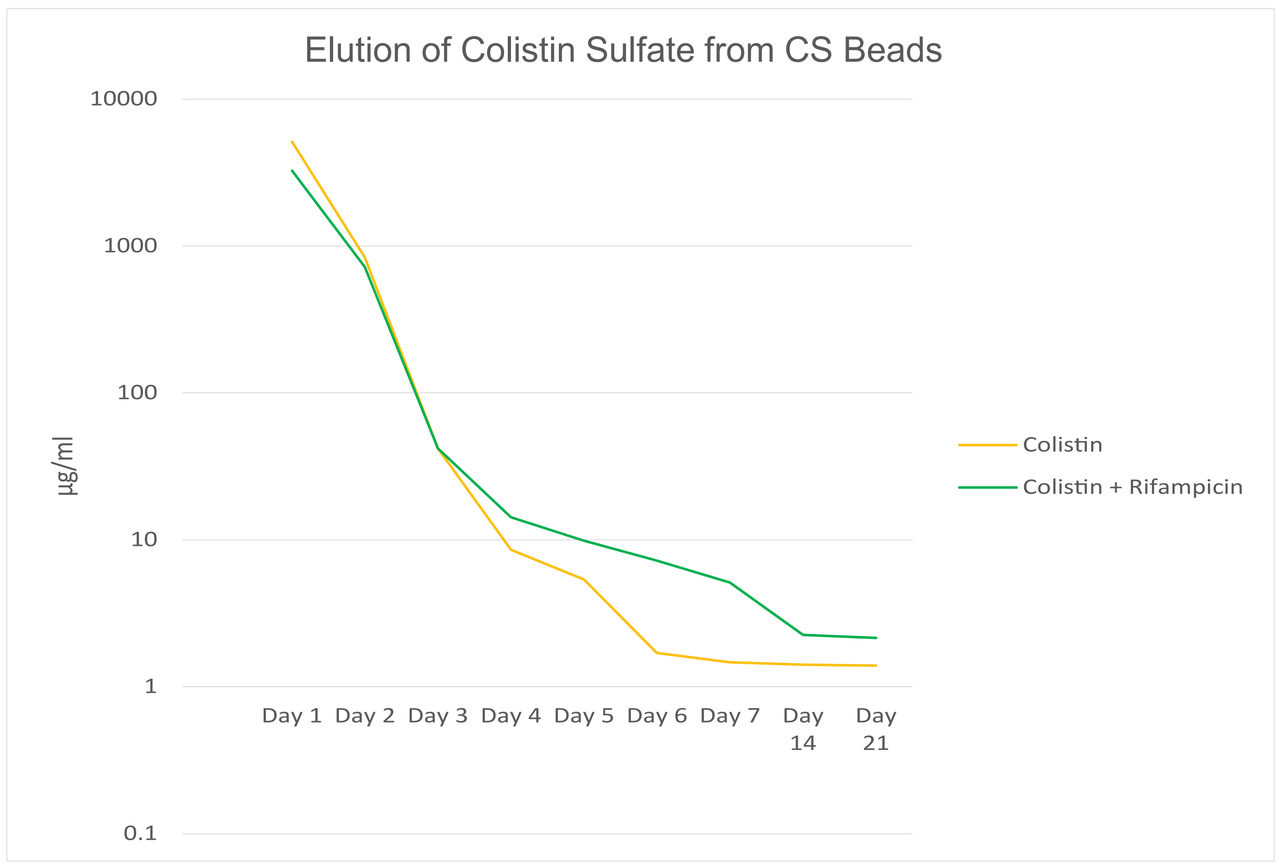
The emergence of multi-drug resistant Gram-negative bacteria is a serious threat to health. The polymyxin antibiotic colistin may be the last line of defence against these pathogens in the 2Ist century[1]. Local release of antibiotics from calcium sulfate to the site of infection, enables high concentrations many times MIC, while systemic levels and associated side effects remain low. The potential of a synthetic calcium sulfate to mix with, set and elute colistin in bactericidal concentrations was established.

Method

Colistin sulphate containing beads were prepared using a commercially available high purity calcium sulfate hemihydrate (CSH) - $CaSO_4$ ½H_2O (Stimulan Rapid Cure®, Biocomposites Ltd., UK). 400mg of colistin sulfate powder (Sigma Aldrich, UK) was homogeneously blended with 20g CSH powder, prior to addition of 6ml of sterile water. When thoroughly mixed, the resultant paste was pressed into 6mm diameter hemispherical cavities in a flexible rubber mould where it was allowed to hydrate and set. lg of beads were put into vials containing 2ml phosphate buffered saline (PBS) at 37°C. Total exchange of the PBS containing eluted antibiotic was performed daily, out to 2l days, and replenished with fresh PBS. Mixing and setting characteristics were measured and colistin elution levels quantified by LC-MS.

Zone of inhibition (ZOI) testing of the antibiotic loaded beads was carried out against *Pseudomonas aeruginosa* NCTC I3437 and *Acinetobacter baumannii* NCTC I3424 using Tryptone soya agar (TSA) plates seeded with an actively growing suspension of the relevant organism (0.2ml).

The plates were transferred to an incubator operating at 35 ± 2°C for 30 minutes. The plates were then removed from the incubator and beads placed on the surface. The plates were then incubated at 35 ± 2 °C for 24 hours, after which time they were removed from the incubator and examined for any clear zones around the beads. Any zones were measured across 3 diameters and images were taken of the plates. For comparison, 50µg colistin antimicrobial susceptibility discs (Oxoid, UK 6mm diameter) were tested against both bacteria types.



Results

The CSH mixed and set with colistin sulfate (20gm + 400mg) within I0 minutes. Levels of colistin in the eluate samples peaked at 5I09µg/ml on day I but had dropped to I.4µg/ml by day 7. Eluate levels remained above MICs for I00% of 56I *P. aeruginosa* isolates out to 3 days[2]. For *A. baumannii*, eluate levels were above MICs for I00% of 3I isolates out to 3 days and 74.2% out to 7 days[2]. Elution profiles as shown in fig I. Both microorganisms tested for ZOI showed susceptibility to colistin eluted from CS beads. ZOIs of I9mm diameter were observed for *P. aeruginosa* and I8 mm diameter for *A. baumannii*. Higher sustained colistin elution levels were achieved when 600mg rifampicin was added to the 20g CSH powder as a second antibiotic, achieving levels above MIC out to 2I days for 9I.6% of 56I *P. aeruginosa* isolates and 93.5% of 3I *A. baumannii* isolates[2]. The ZOIs for colistin eluted from CS beads against *P. aeruginosa* and *A. baumannii* are shown in Figures 2a and 3a respectively. The comparative results for the antimicrobial susceptibility discs are shown in Figures 2b and 3b respectively. The zone diameters are shown in Table I.

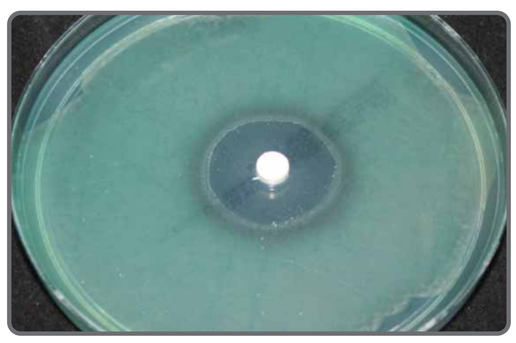


Figure 2a. Stimulan result against *P. aeruginosa*

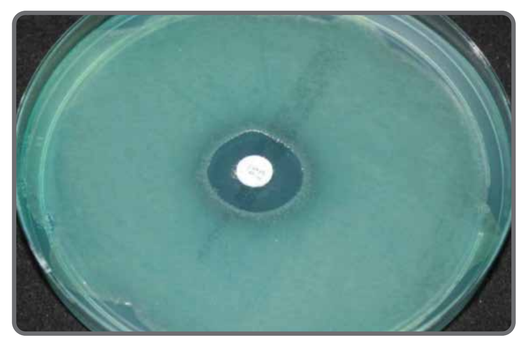


Figure 2b. Colistin disc result against *P. aeruginosa*

Figure 3a. Stimulan result against *A. baumannii*

Figure 3b. Colistin disc result against *A. baumannii*

Zone Diameters (mm)		
	(*Pseudomonas aeruginosa*) NCTC I3437	(*Acinetobacter baumannii*) NCTC I3424
CS/Colistin beads	22	I5.5
50mcg Colistin discs	I7	I4.5

Table I.

Conclusion

Colistin sulfate will mix, set and elute from synthetic high purity calcium sulfate at bactericidal levels. Literature has demonstrated successful clinical outcomes when colistin is administered in this way both alone and synergistically with other antibiotics[3,4]. Correlating *in-vitro* elution data to clinical performance is difficult. In the study protocol adopted here, total fluid exchange was performed daily. This would correlate with a highly dynamic and fluid *in-vivo* environment for the implanted beads which would result in a relatively rapid release profile of antibiotic. A partial exchange of eluant performed daily would more closely mimic the *in-vivo* situation where the antibiotic loaded beads were in a more protected site and this would have resulted in a significantly more prolonged release profile. Further work to investigate elution characteristics of colistin/CS beads combined with other antibiotics is warranted.

Acknowledgement

ZOI testing was performed by The Surgical Materials Testing Laboratory, Bridgend, South Wales. www.smtl.co.uk

References

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