ESI for:

Encapsulation of erythromycin and bacitracin antibiotics into natural sporopollenin microcapsules: Antibacterial, cytotoxicity, *in vitro* and *in vivo* release studies for enhanced bioavailability

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Experimental details

- **Extraction of LCS from the raw *Lycopodium clavatum* pollens**

50 g of raw *Lycopodium clavatum* pollen powder were mixed with around 400 mL acetone under reflux for 4 hours then filtered and 400 mL of 6 wt.% KOH solution was added under reflux for another 6 hours. The pollens were filtered through 20-25 μm filter paper then washed with hot water and ethanol several times. The collected pollens were then mixed with 400 mL of 85 wt.% H3PO4 acid under reflux until all the cytoplasmic and other materials are removed. Then, the LCS are filtered and washed with water, acetone and finally with pure ethanol and then dried at 60 °C until constant weight.

- **labelling antibiotic with eosin yellow for CLSM analysis**

A 125 mg of EM Were transferred into a 5 ml glass vial and dissolved in a 1:1 v/v water/ethanol solution. 0.5 wt.% of the eosin yellow was added to the EM solution were mixed well. Then, 0.4 M acetate buffer was added to adjust the pH = 3. The mixture was then stirred for another 10 minutes to allow the formation of the EM-eosin Y binary complex.

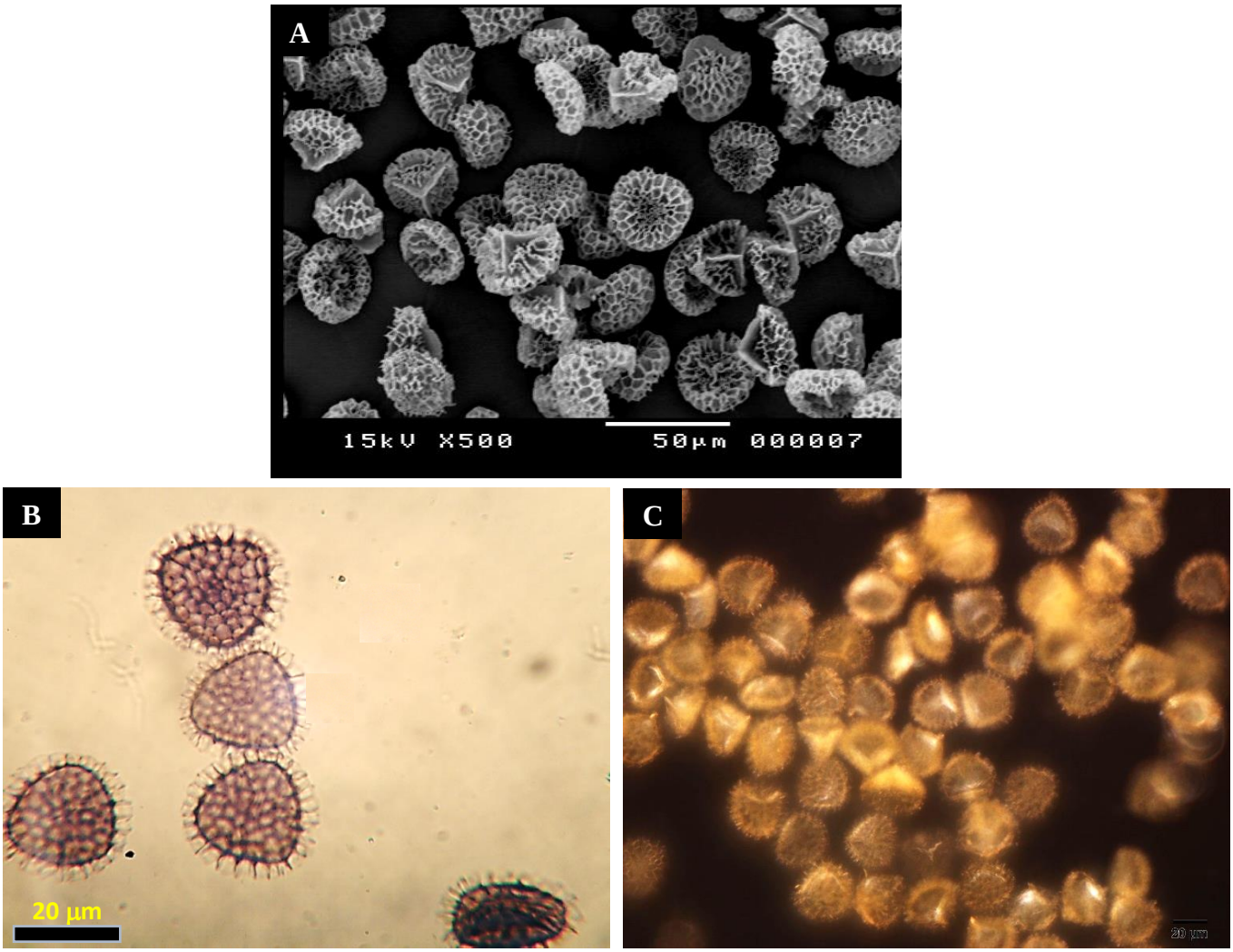


Figure S1. (A) an SEM image of dry raw *Lycopodium clavatum L.* spores. (B, C) optical images of the spore in pure water and in dry condition, respectively.

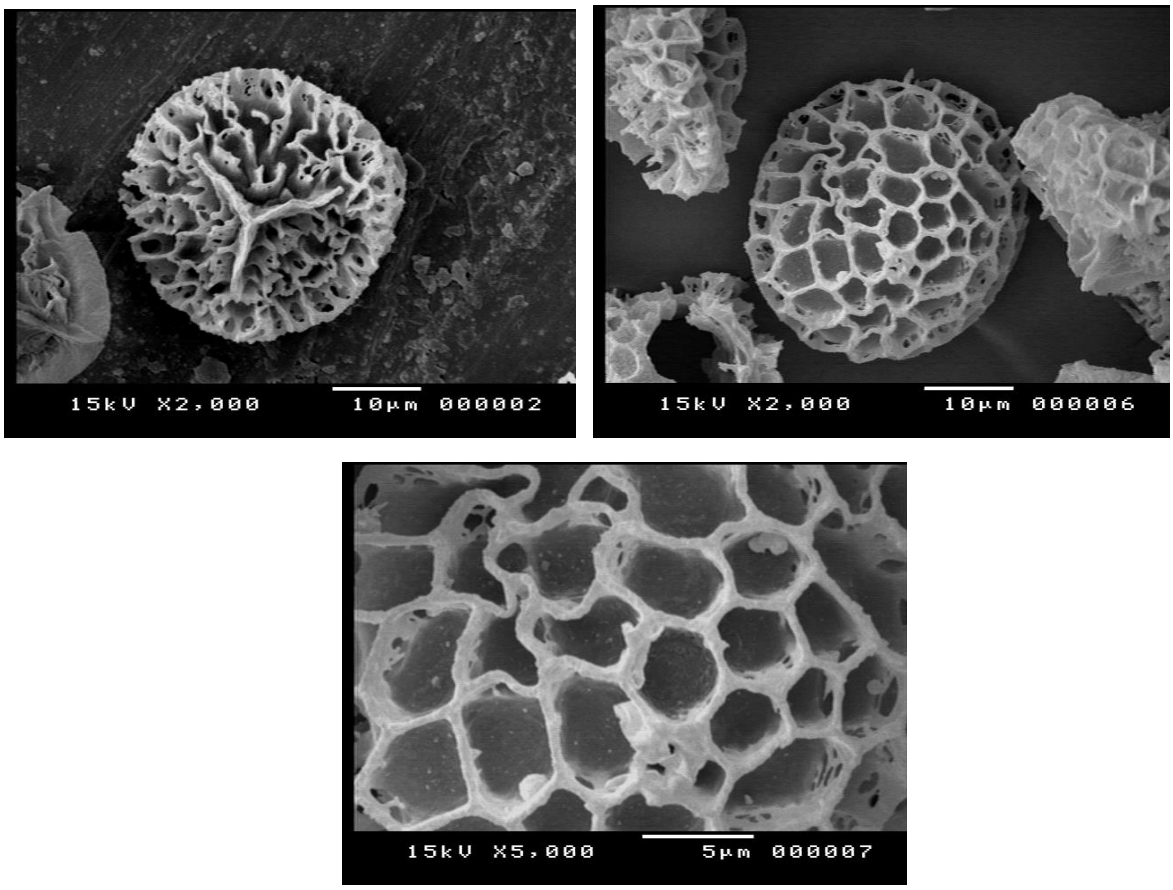


Figure S2. SEM of extracted empty LCS sporopollenin before loading process.

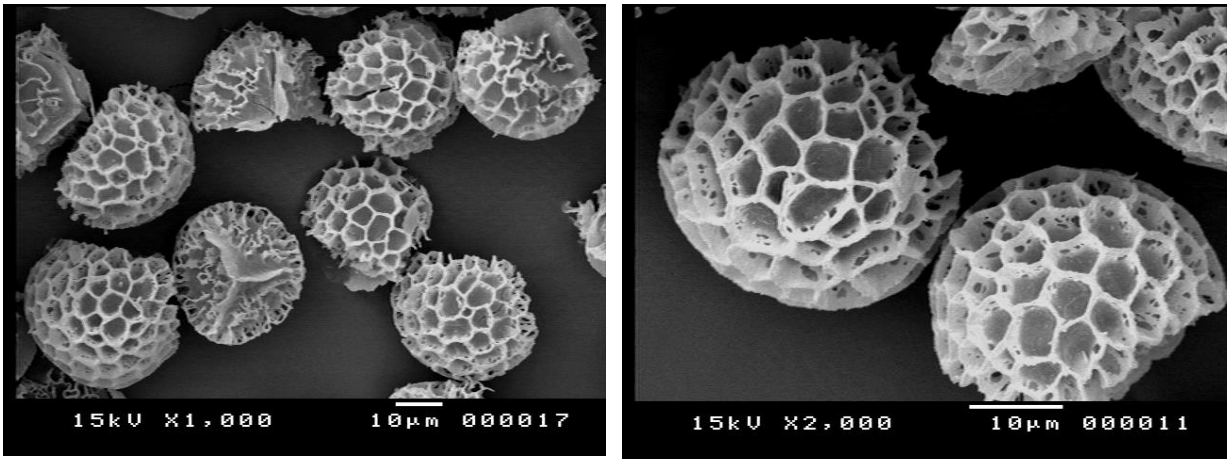


Figure S3. SEM of EM-loaded LCS microcapsules after passive-vacuum protocol.

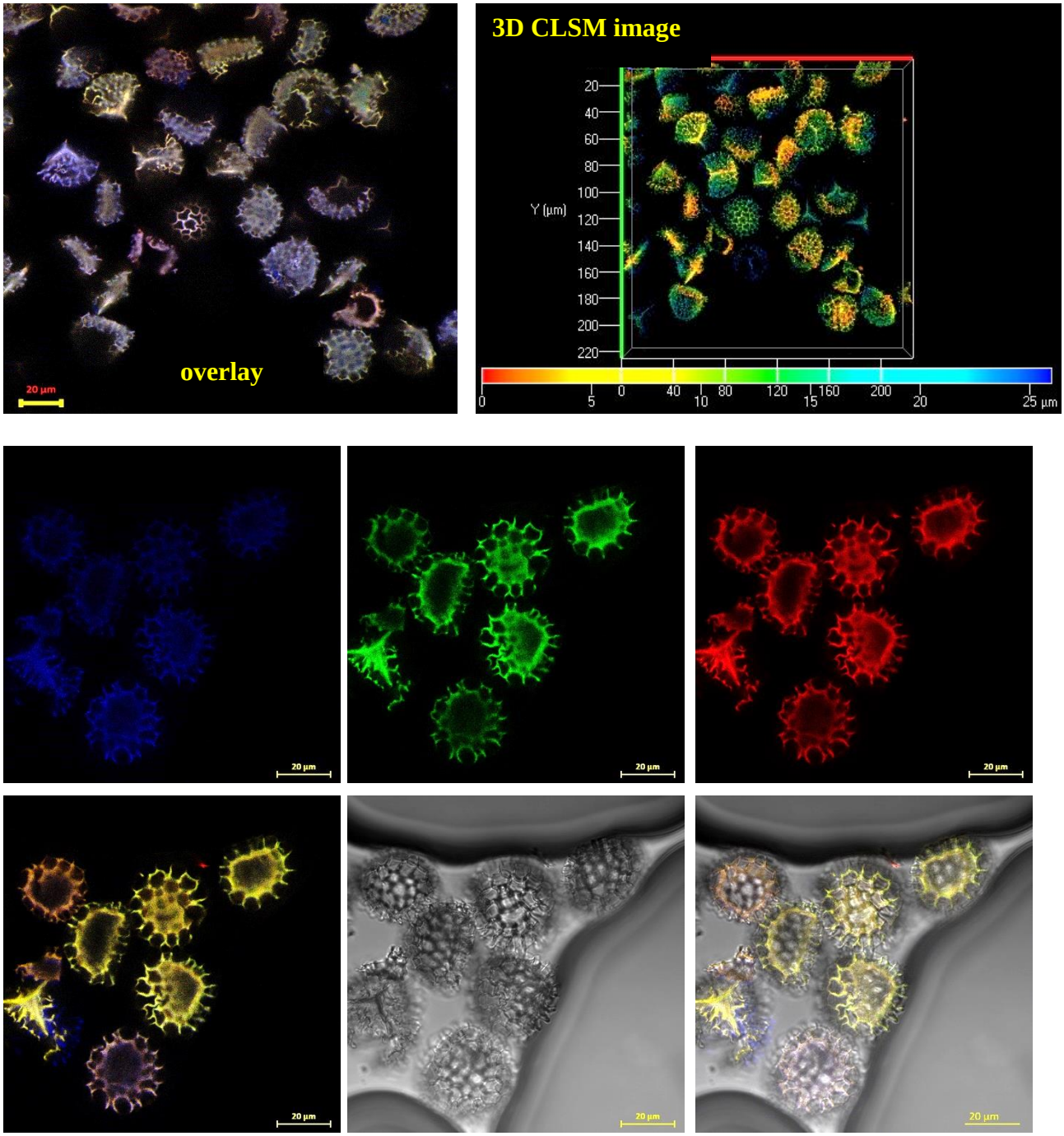


Figure S4. 2D and 3D CLSM images of empty LCS microparticle before the loading process. The microparticle was scanned with three laser excitation wavelengths represented by the blue, green and red channels. Images were captured at a random slice of the microparticles.

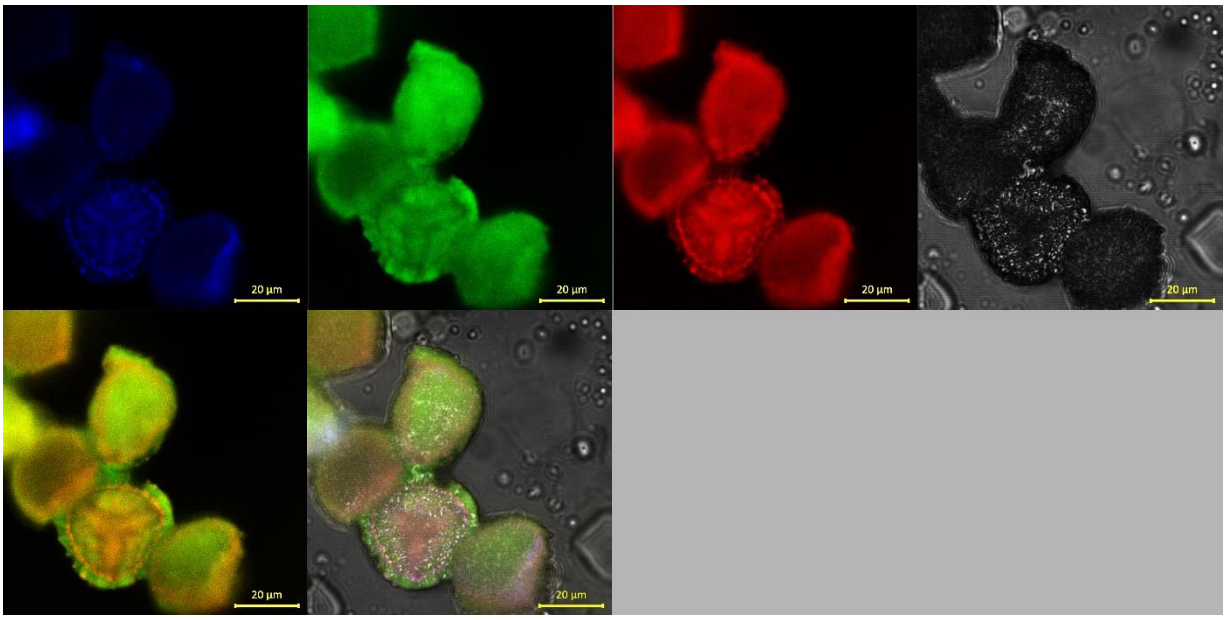


Figure S5. CLSM images of LCS microparticles loaded with EM-eosin Y binary complex via passive-vacuum technique at different channels showing the successful loading of the EM antibiotic. Images were captured at the middle slice of the microparticles.

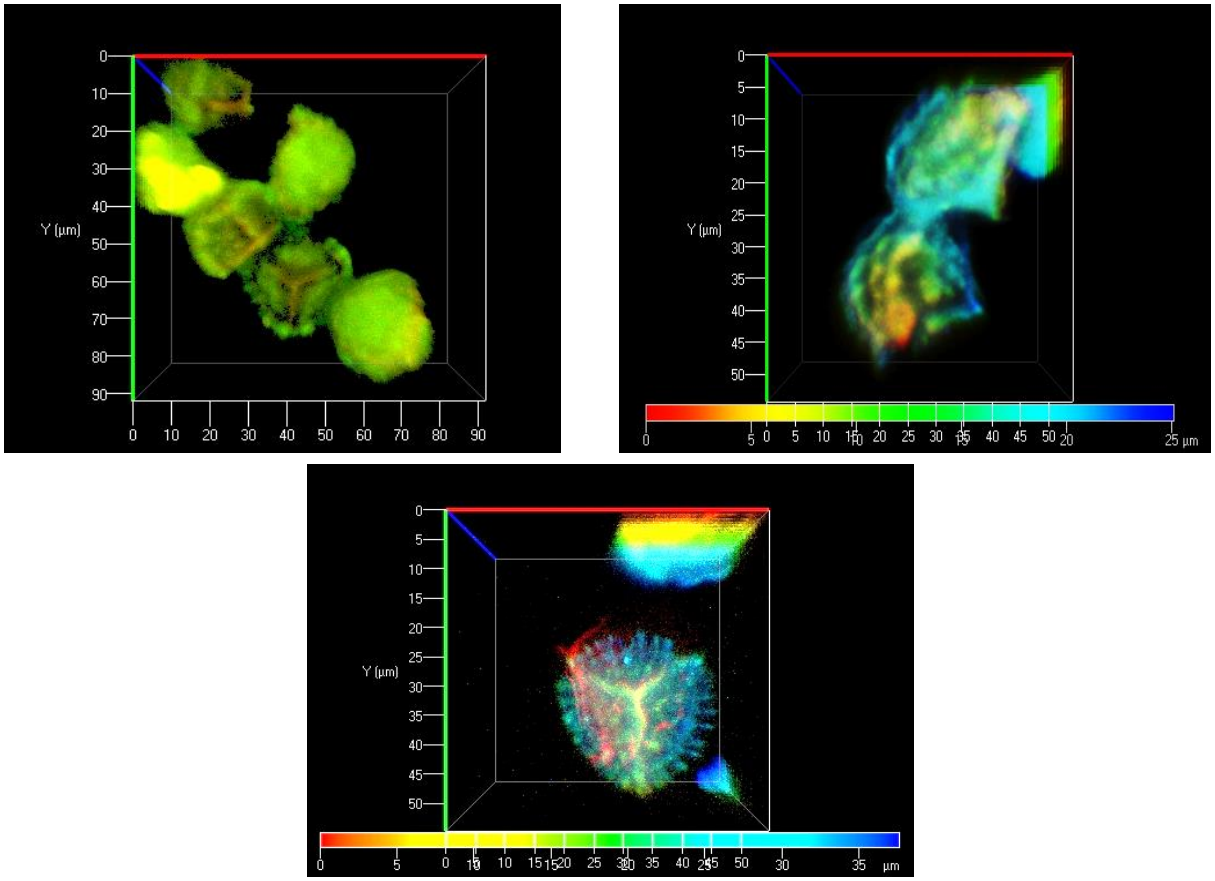


Figure S6. 3D overlay CLSM images of selected LCS microparticles loaded with EM-eosin Y binary complex via passive-vacuum technique showing the successful loading of the EM antibiotic. Images were captured at the middle slice of the microparticles.

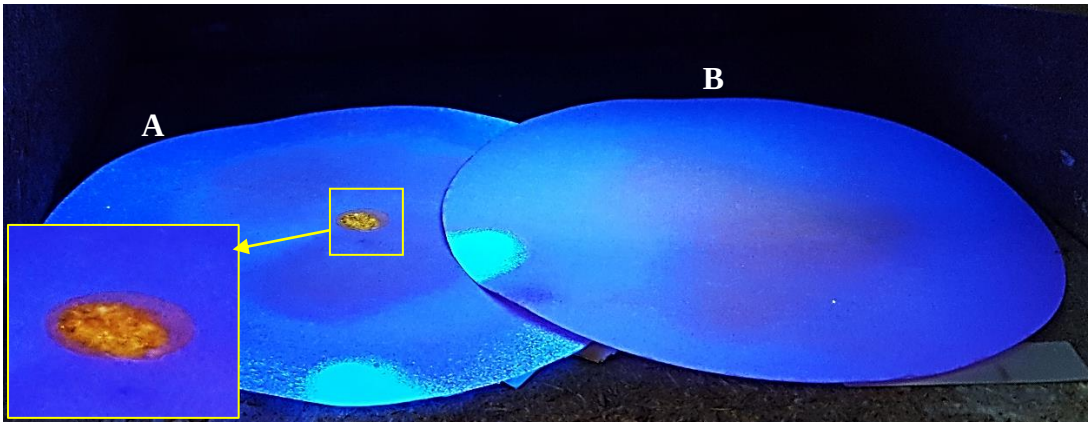


Figure S7. L. Filter papers sprayed with Eosin yellow aqueous solution (1 wt.%) under a UV lamp. (A) a paper with spot of EM solution in the middle showing the fluorescein from Esoin Y-labeled EM. (B) paper sprayed with the dye only without the EM. The inset is a close-up image of the labeled antibiotic spot.

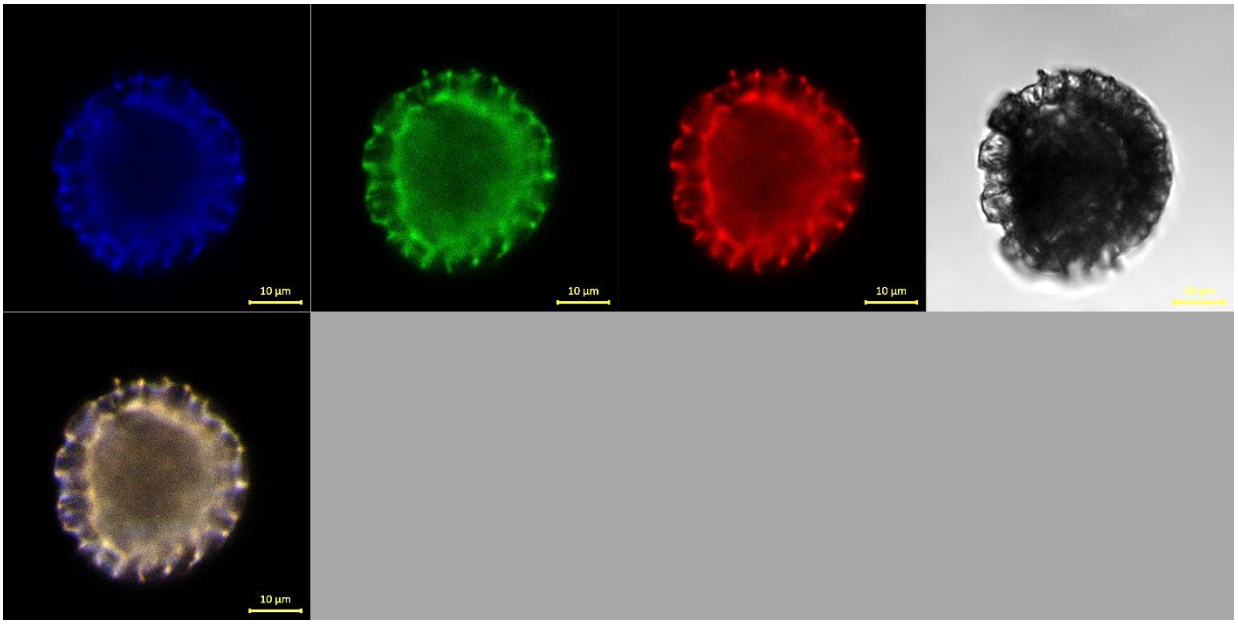
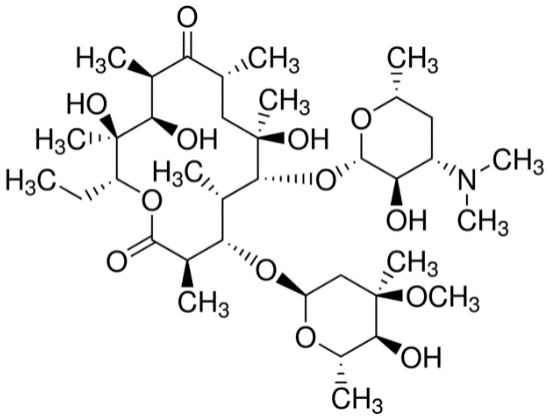
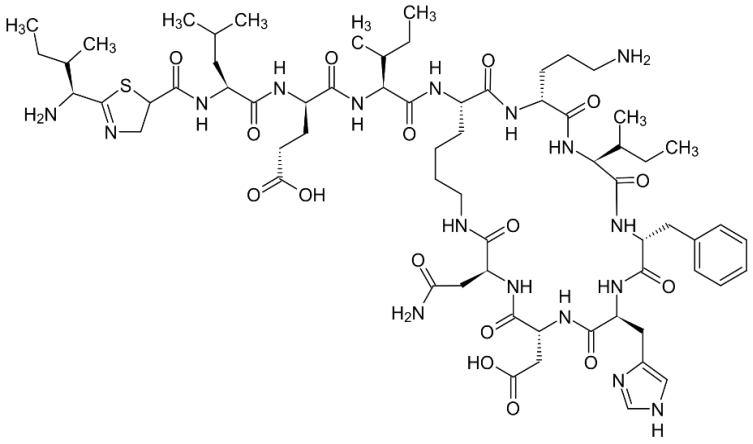


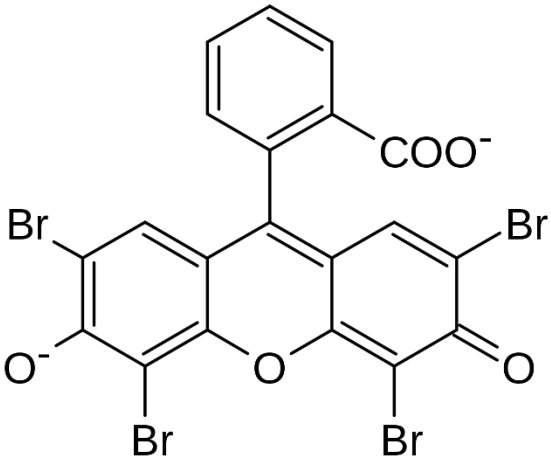
Figure S8. CLSM images of LCS microparticles loaded with BAC via passive-vacuum technique at different channels showing the successful loading of the BAC antibiotic. Images were captured at the middle slice of the microparticles.



Erythromycin standard



Bacitracin



Eosin yellow (eosin Y)

Schem S1. Structures of EM, BAC antibiotics and Eosin yellow dye.

Table S1: Antibacterial and synergistic activity of LCS loaded with antibiotics.

	Antibacterial activity (mm)				Increase in fold area (%)	
	Antibiotics		(LCS + Antibiotics)			
Bacterial strains	EM	BAC	LCS+EM	LCS+BAC	LCS+EM	LCS+BAC
Staphylococcus aureus	7.0 ±0.2	6.0 ±0.04	15.0±0.6	14.0 ±0.40	114.2±0.06	133.3 ±0.12
Pseudomonas aeruginosa	9.0 ±0.4	8.5±0.6	19.5±0.6	18.0 ±0.40	116.6±0.04	111.7±0.2
Klebsiella pneumonia	8.5 ±0.4	9.0 ±0.2	18.0±0.4	19.0±0.40	111.7±0.12	111.1±0.04

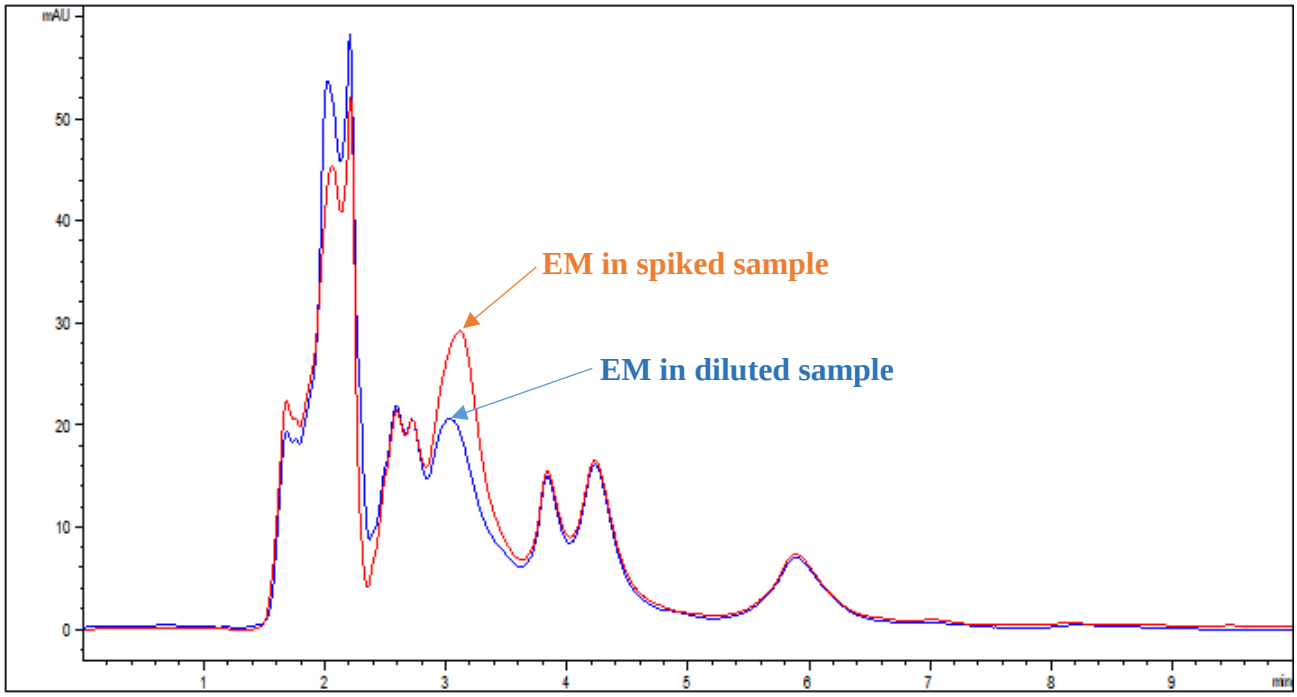


Figure S9. Overlay chromatograms represent matrix spike of a plasma sample diluted (1:1) at 250 mg/L of erythromycin standard (EM).