

# Complete Killing of Agar Lawn Biofilms by Systematic Spacing of Antibiotic-Loaded Calcium Sulfate Beads

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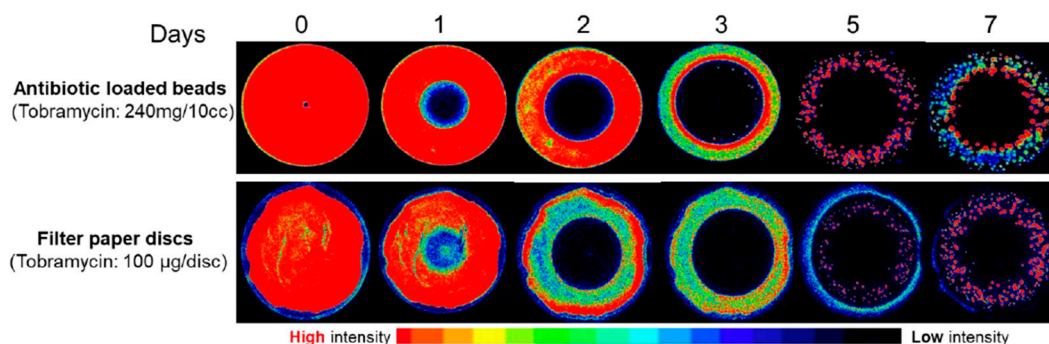
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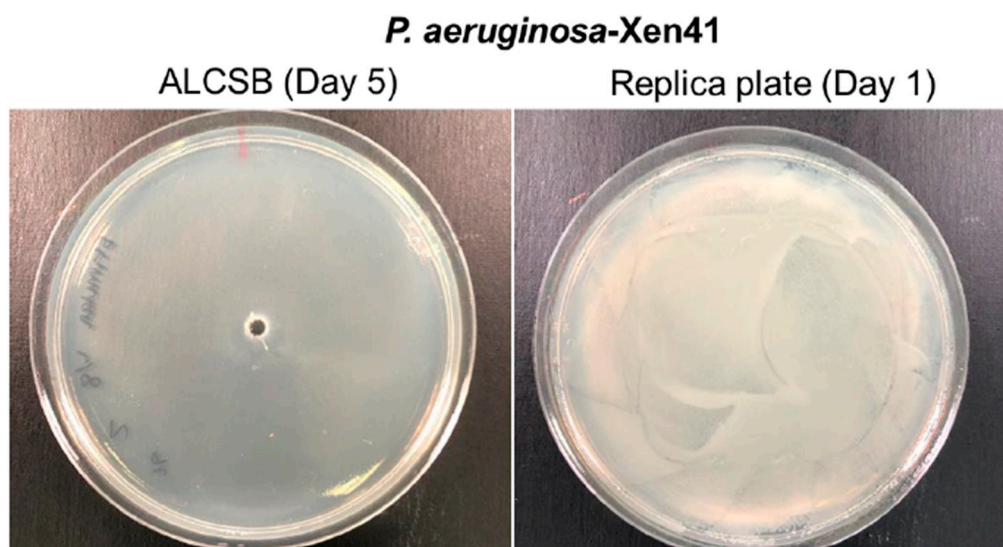
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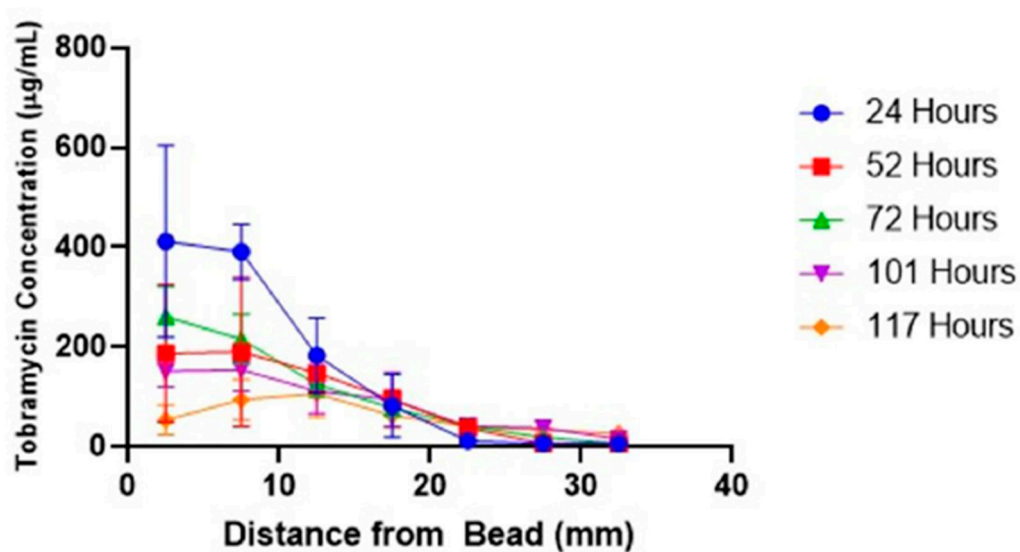
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**Figure S1.** Killing of lawn biofilms of *P. aeruginosa* (PA-Xen41) with antibiotic-loaded calcium sulfate beads (ALCSB) and paper discs loaded with tobramycin. The ALCSB loaded with 240 mg/10 cc tobramycin and filter paper discs with 100 µg/disc tobramycin were placed in the center on 24 h lawn biofilms. Zone of biofilm killing (ZOB-K) and resistant colonies were observed with both ALCSB and paper discs loaded with tobramycin.



**Figure S2.** Carryover of antibiotic during replica plating. ALCSB containing tobramycin placed on 24 h grown lawn biofilms at day five was replica plated onto fresh tryptic soy agar (TSA) plates and spread with PA-Xen41. No inhibition of growth of PA-Xen41 was observed, suggesting no carryover of tobramycin during replica plating.



**Figure S3.** Tobramycin concentration remains above MIC during resistant phenotype development. Tobramycin-loaded bead was placed in sterile TSA, and at various time points, agar plugs were extracted at various radii to examine the concentration of tobramycin in them by plating for MIC ( $n = 3$ ). MIC zones were compared to a standard curve to calculate the tobramycin concentrations in the agar plugs. Data is reported as mean  $\pm$  SD.