

1 **Supplementary Information for**

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3 **Differential protection from tobramycin by extracellular polymeric**
4 **substances from *Acinetobacter baumannii* and *Staphylococcus aureus***
5 **biofilms**

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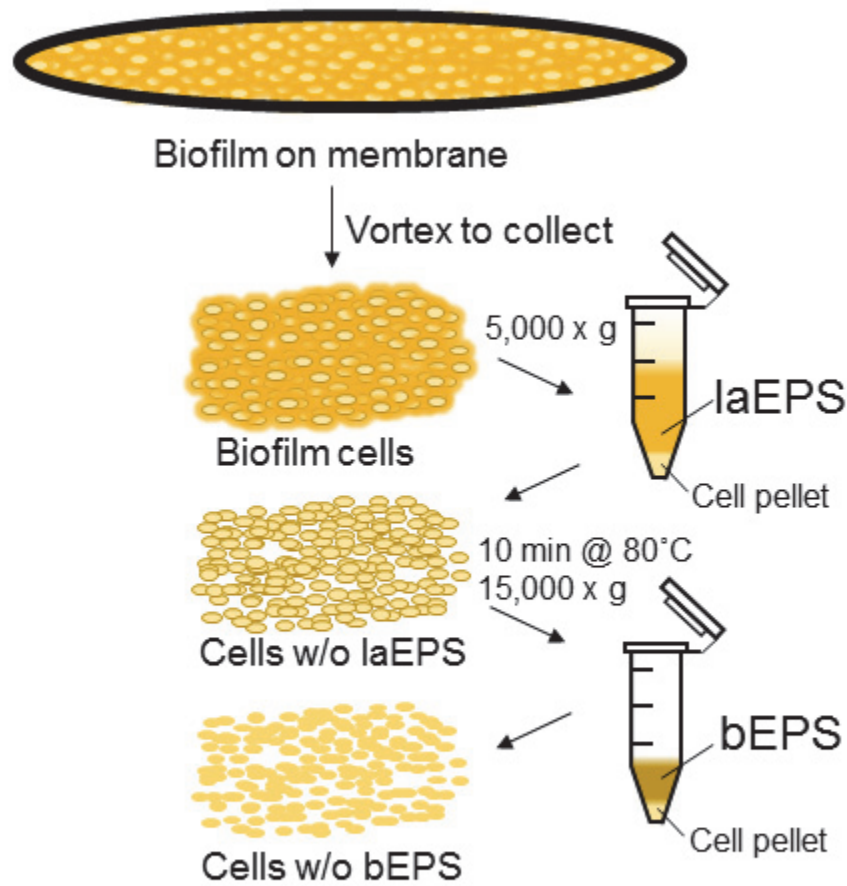
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14 Running head: Extracellular polymeric substance protects cells from tobramycin

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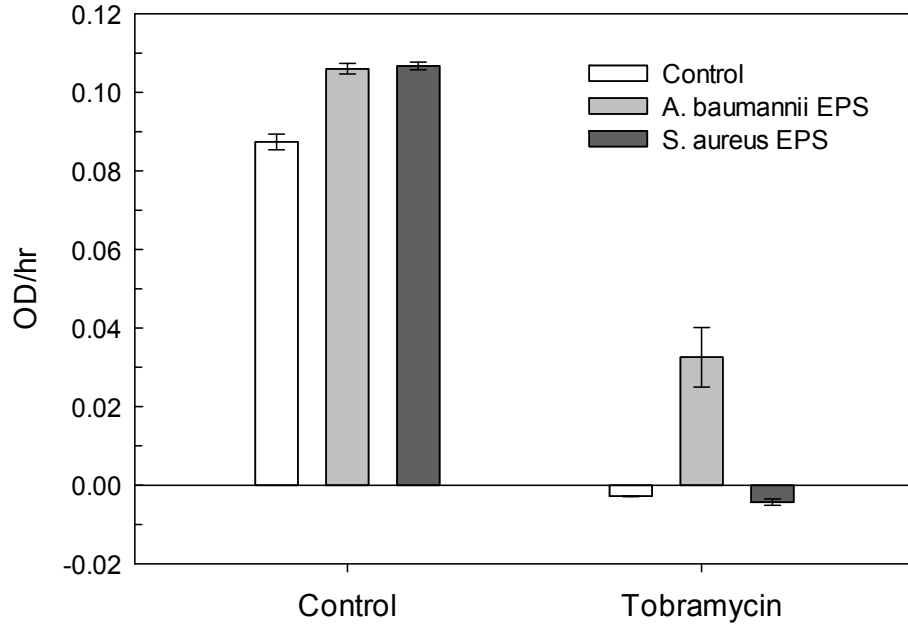


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20 Figure S1. EPS harvesting method. Membrane from the membrane bioreactor is vortexed to
 21 collect biofilm. Cells are then centrifuged at 5,000 $\times g$ to collect laEPS. The cell pellet is
 22 resuspended in 0.9% NaCl solution, heated for 10 min at 80°C, and centrifuged at 15,000 $\times g$ to
 23 collect bEPS.

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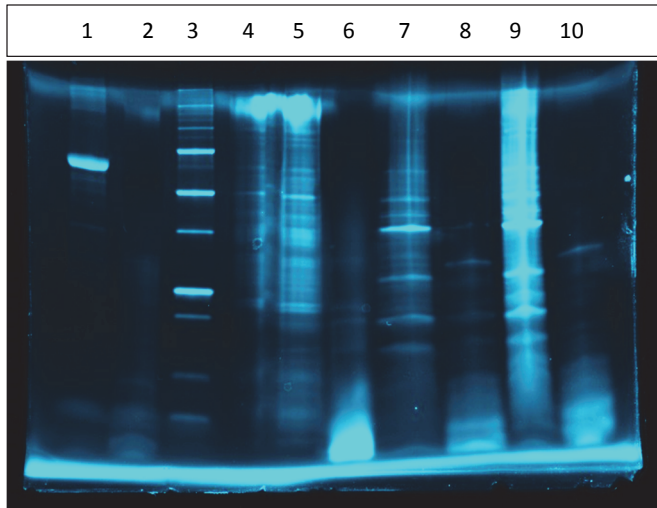
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27 Figure S2. EPS from both *A. baumannii* and *S. aureus* were added with tobramycin to *E. coli* K-
28 12 cultures. When tobramycin was added, only cultures containing *A. baumannii* EPS showed
29 growth. EPS from *S. aureus* did not have any effect on *E. coli* growth when tobramycin was
30 present. Error bars represent one standard deviation.

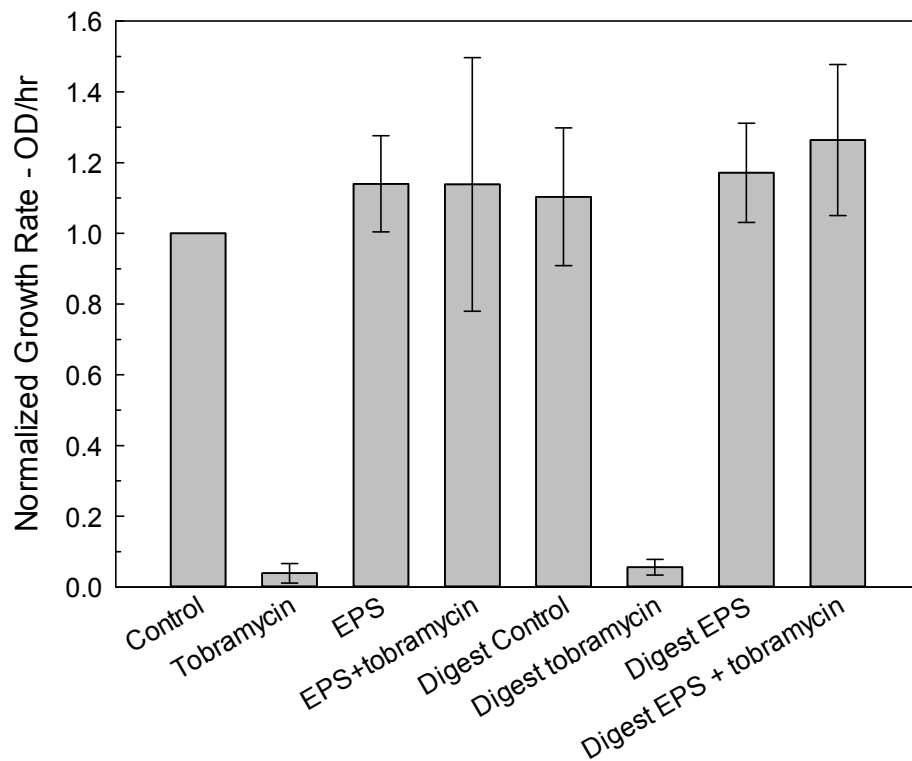
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33 Figure S3. SDS-PAGE of *A. baumannii* EPS digestion by proteinase K. Lane 1: bovine serum
34 albumin (BSA) control; lane 2: BSA digest; lane 3: ladder; lane 4: empty; lane 5: EPS control;
35 lane 6: EPS digest; lane 7: laEPS control; lane 8: laEPS digest; lane 9: bEPS control; lane 10:
36 bEPS digest.

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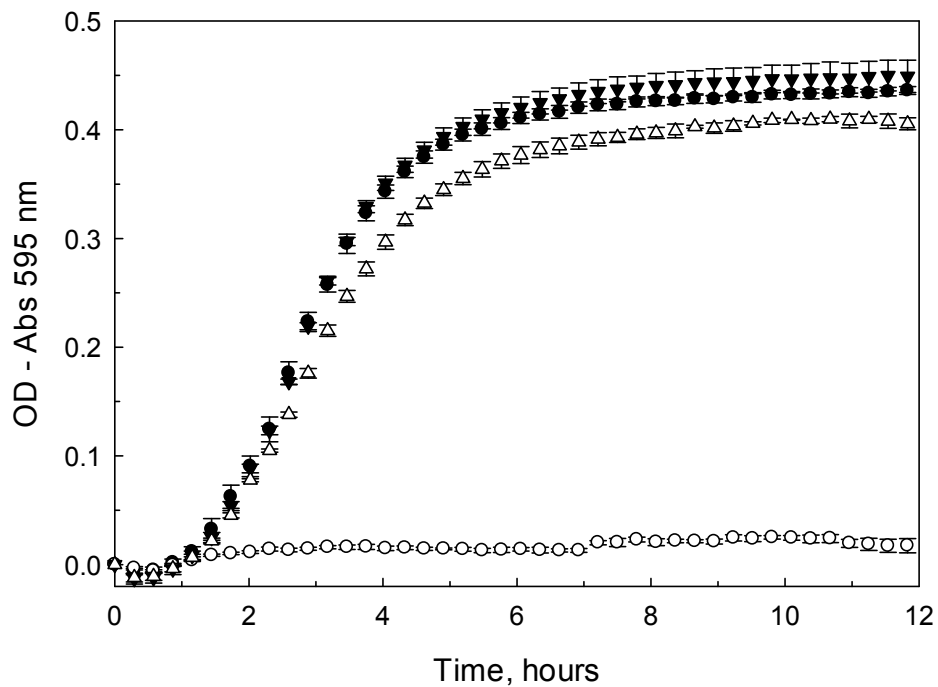
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39 Figure S4. Effects of an overnight proteinase K digest on *A. baumannii* EPS. Digested EPS did
 40 not show a significant difference in protection from tobramycin from the untreated EPS samples.

41 Error bars represent one standard deviation.

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 45 Figure S5. Loosely associated EPS used as EPS fraction in *A. baumannii* cultures. Growth curves
 46 for *A. baumannii* that was cultured in 0.33× TSB alone (●), with 1 μg/mL tobramycin (○), with
 47 50% (v/v) *A. baumannii* laEPS (▼), or with both tobramycin and laEPS (Δ). Error bars represent
 48 one standard deviation and **may not be visible behind the symbol.**