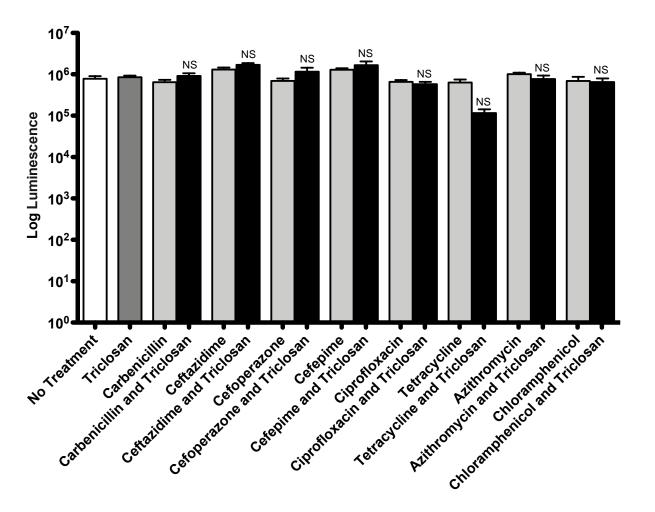


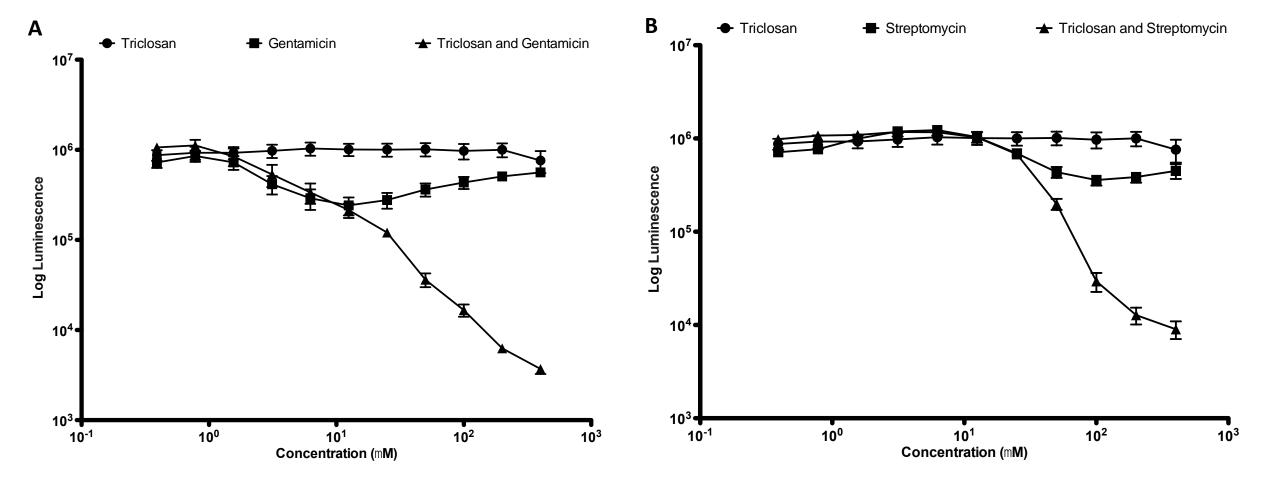
# Figure S1: Log Luminescence units correlate to CFUs.

Serial dilutions of cells were plated in triplicate and BacTiter-Glo<sup>TM</sup> was added to determine cell number. Aliquots were also taken at each dilution to enumerate colony forming units. Log luminescence units versus CFUs/mL were then plotted. The results represent means plus the SEM. A linear regression was performed to determine goodness of fit (coefficient of determination r<sup>2</sup>=0.9884).



# Figure S2: Triclosan and non-aminoglycoside antibiotics do not synergyize.

24-hr old biofilms grown on MBEC plates were treated for 6-hrs with 100 µM of triclosan and 100 µM of each antibiotic alone and in combination. Number of cells within the biofilms were quantified by BacTiter-Glo<sup>TM</sup>. The assay was performed at least two times in triplicate. The results represent means plus the SEM. A one-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to determine statistical significance compared to each antipseudomonal alone (NS, not significant).



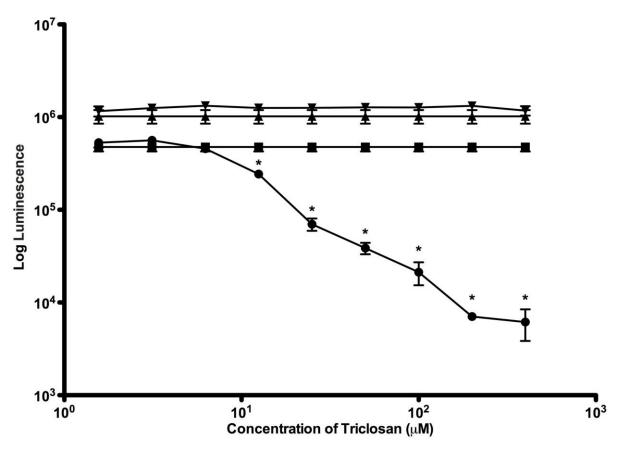
#### Figure S3: Triclosan and gentamicin or streptomycin synergize at multiple concentrations.

24-hr old biofilms grown on MBEC plates were treated for 6-hrs with 2-fold dilutions of equal concentrations of triclosan combined with gentamicin or streptomycin. The number of viable cells within the biofilms were quantified by BacTiter-Glo<sup>™</sup>. The assay was performed at least three times in triplicate. The results represent means plus the SEM.

Triclosan Tobramycin	100 µM	50 µM	25 µM	12.5 μM	0 µM
534 µM	<b>1.2E</b> <sup>4</sup>	<b>2.4E</b> <sup>4</sup>	<b>8.8E</b> <sup>5</sup>	<b>3.5E⁵</b>	<b>3.5E<sup>5</sup></b>
	(3.1E <sup>3</sup> )	(1.2E <sup>3</sup> )	(5.6E <sup>4</sup> )	(1.8E⁵)	(3.1E <sup>5</sup> )
267 µM	<b>1.4E</b> <sup>4</sup>	<b>2.7E</b> <sup>4</sup>	<b>8.04E</b> <sup>5</sup>	<b>3.9E</b> ⁵	<b>3.6E</b> <sup>5</sup>
	(6.4E <sup>3</sup> )	(1.6E <sup>4</sup> )	(3.3E <sup>4</sup> )	(1.6E⁵)	(3.3E <sup>5</sup> )
133 µM	<b>1.5E</b> ⁴ (6.1E³)	<b>3.9E</b> <sup>4</sup> (9.8E <sup>4</sup> )	<b>3.4E<sup>5</sup></b> (4.0E <sup>5</sup> )	<b>4.2E</b> <sup>5</sup> (1.2E <sup>4</sup> )	<b>7.2E<sup>5</sup></b> (3.9E <sup>5</sup> )
66 µM	<b>2.1E</b> ⁴ (1.5E⁴)	<b>5.1E</b> <sup>4</sup> (2.1E <sup>4</sup> )	<b>4.7E<sup>5</sup></b> (5.0E <sup>5</sup> )	<b>5.5E</b> <sup>5</sup> (2.0E <sup>5</sup> )	<b>8.8E</b> <sup>5</sup> (4.8E <sup>5</sup> )
0 µM	<b>1.2E</b> <sup>6</sup>	<b>1.2E</b> <sup>6</sup>	<b>1.3E</b> <sup>6</sup>	<b>1.2E</b> <sup>6</sup>	<b>1.4E<sup>6</sup></b>
	(6.7E <sup>5</sup> )	(4.8E <sup>5</sup> )	(4.1E <sup>5</sup> )	(3.8E <sup>5</sup> )	(1.8E <sup>5</sup> )

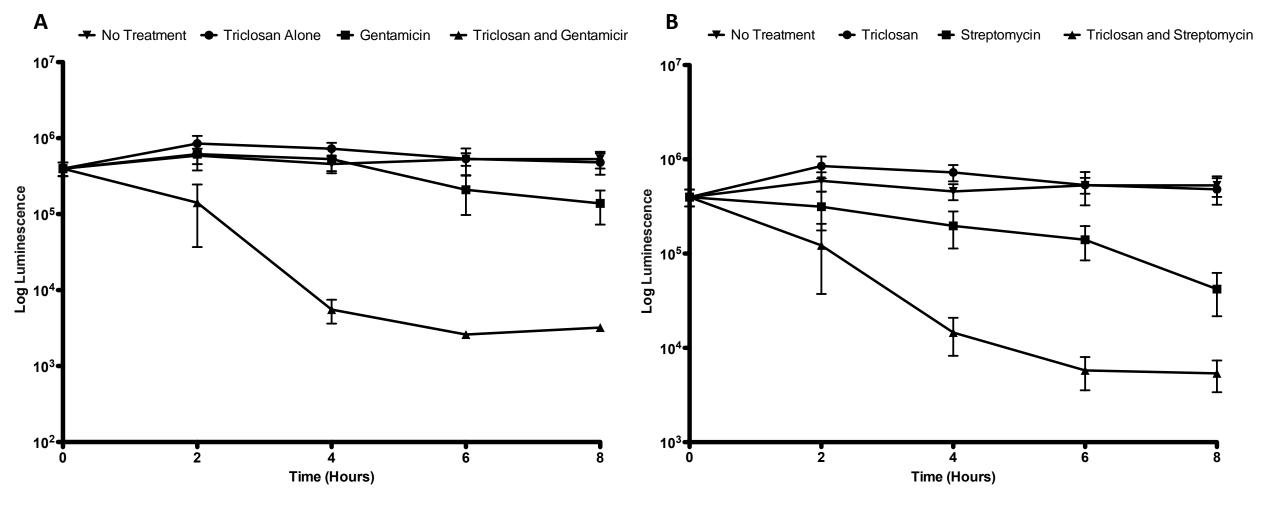
#### Figure S4. Triclosan enhances low concentrations of tobramycin.

24-hr old biofilms grown on MBEC plates were treated for 6-hrs with checkerboard dilutions of triclosan combined with tobramycin. Number of viable cells within the biofilms were quantified by BacTiter-Glo<sup>TM</sup>. The assay was performed at least three times in triplicate. The results represent means plus the Standard Error Deviation. A two-way ANOVA followed by Bonferroni's posttests was used to determine statistical significance compared to tobramycin treatment alone. Shaded cells indicate significance (p<0.05).



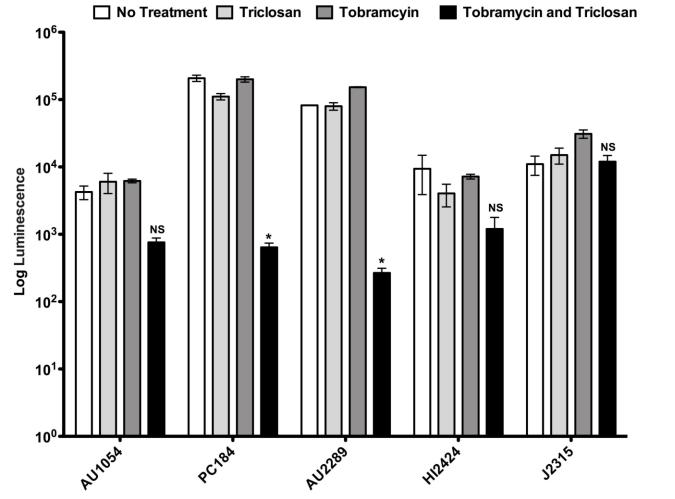
# Figure S5. Triclosan enhances low concentrations of tobramycin.

24-hr old biofilms grown on MBEC plates were treated for 6-hrs with dilutions of triclosan combined with tobramycin at a fixed concentration of 66  $\mu$ M. Number of viable cells within the biofilms were quantified by BacTiter-Glo<sup>TM</sup>. The assay was performed in triplicate. The results represent means plus the SEM. A two-way ANOVA followed by Bonferroni's posttests was used to determine statistical significance compared to tobramycin alone. (\*, p<0.05).



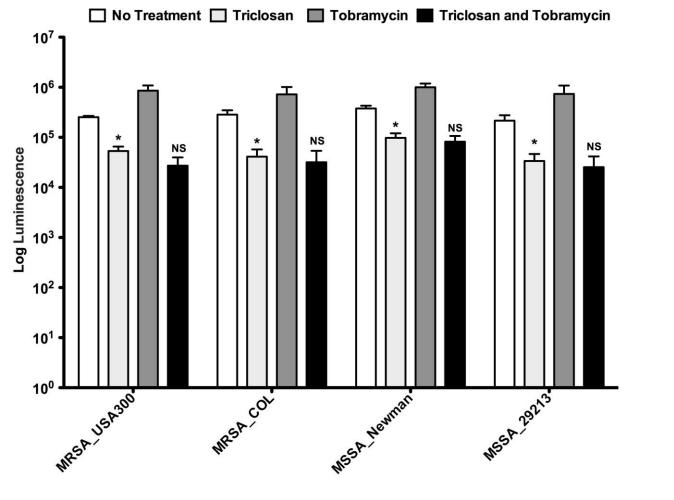
# Figure S6: Gentamicin and streptomycin have a shorter onset of action and enhanced killing when combined with triclosan.

24-hr old biofilms grown on MBEC plates were treated with triclosan 100 µM triclosan, gentamicin, or streptomycin alone and in combination for 8-hrs. At 0, 2, 4, 6, and 8-hrs the number of cells within the biofilms were determined using BacTiter-Glo<sup>™</sup>. The assay was performed at least three times in triplicate. The results represent means plus the SEM.



#### Figure S7. Tobramycin and triclosan are effective against Burkholderia cenocepacia.

24-hr old biofilms grown on MBEC plates were treated with triclosan (100  $\mu$ M) or tobramycin (500  $\mu$ M) alone and in combination for 6-hrs. The number of viable cells within the biofilms were quantified by BacTiter-Glo<sup>TM</sup>. The assay was performed in triplicate. The results represent means plus the SEM. A two-way ANOVA followed by Bonferroni's posttests was used to determine statistical significance compared to tobramycin alone (\*, p<0.05, NS, not significant).



#### Figure S8. Triclosan alone is effective against *Staphylococcus aureus*.

24-hr old biofilms grown on MBEC plates were treated with 100  $\mu$ M triclosan or tobramycin alone and in combination for 6-hrs. The number of viable cells within the biofilms were quantified by BacTiter-Glo<sup>TM</sup>. The assay was performed at least three times in triplicate. The results represent means plus the SEM. A two-way ANOVA followed by Bonferroni's posttests was used to determine statistical significance compared to no treatment and triclosan alone (\*, p<0.05, NS, not significant).