Supplementary Information for:

# Evaluation of anti-biofilm activity of acidic amino acids and synergy with ciprofloxacin on Staphylococcus aureus biofilms

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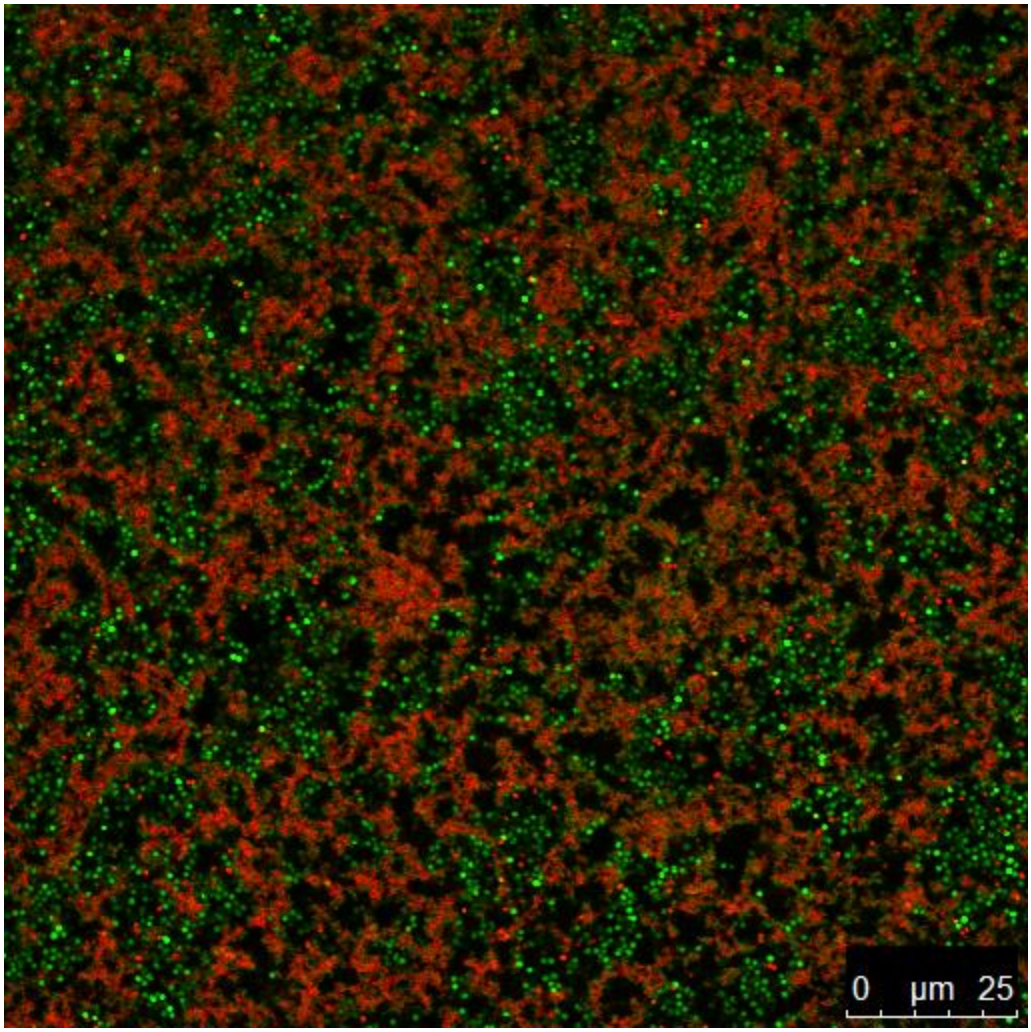
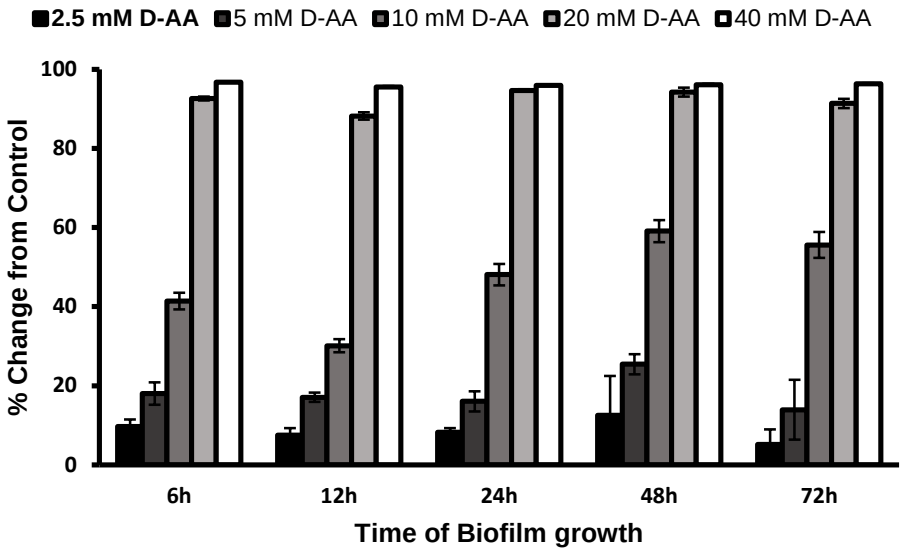
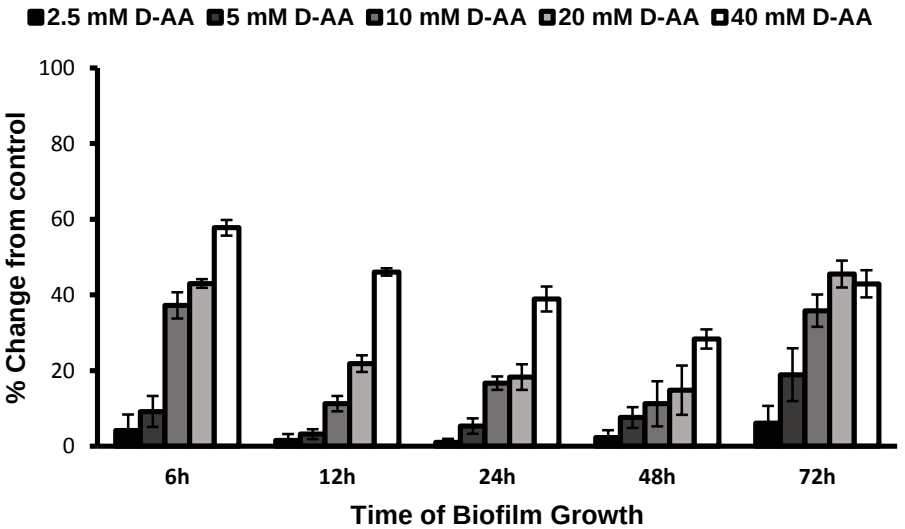


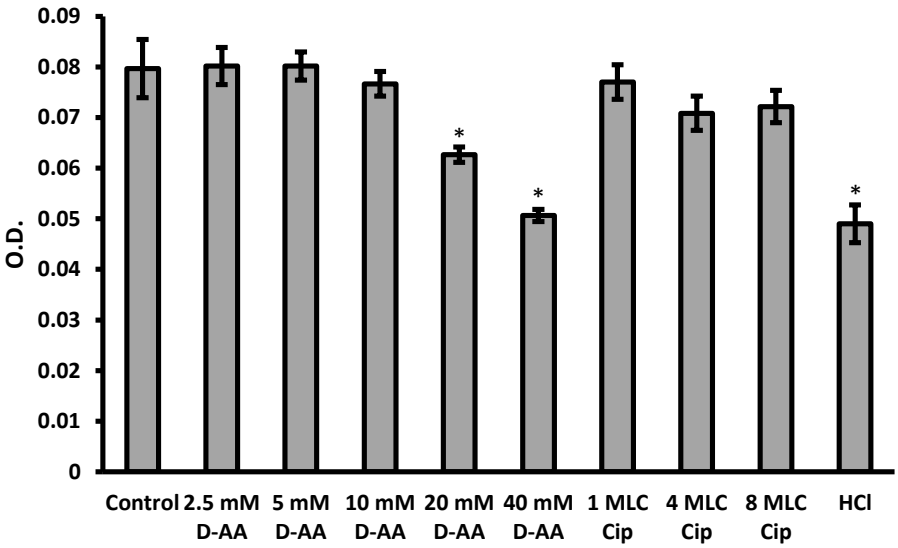

**Supplementary Figure 1:** Confocal Image of biofilm dispersed with Cip alone. eDNA is present as seemingly well fortified boundaries, enclosing persisting *S. aureus cells*.



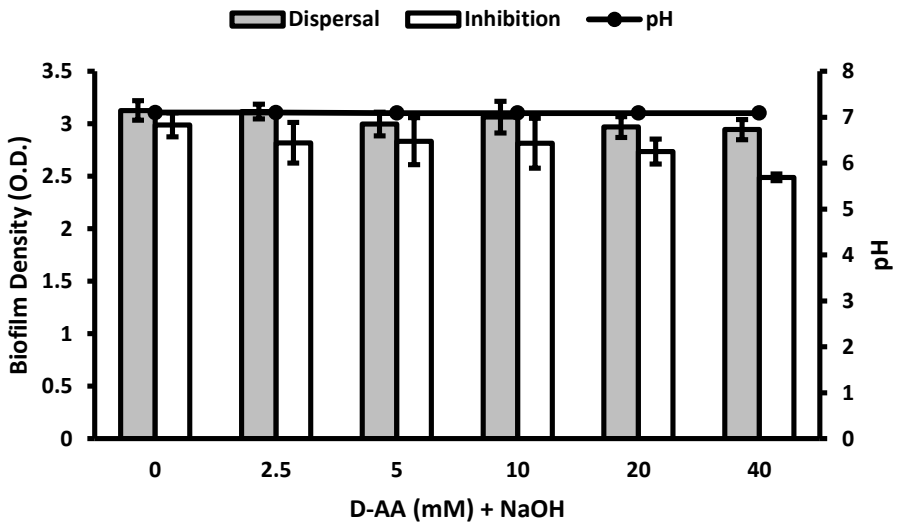

**Supplementary Figure 2:** Percentage change from control upon biofilm inhibition; n=3.




**Supplementary Figure 3:** Percentage change from control upon biofilm dispersal; n=3.




**Supplementary Figure 4:** Effect of D-AA at concentrations of 2.5, 5, 10, 20 and 40mM, and Cip at concentrations of 1 MLC, 4 MLC and 8 MLC on the Optical density of planktonic S. aureus growth after 30 minutes of exposure. One-way ANOVA showed an overall significant difference within the data and was followed by a post-hoc t-test with Bonferroni correction to see which concentration significantly ($p<0.005$ was taken as significant; indicated by * ) killed or effected planktonic growth of S. aureus compared to the control. Significant effect on planktonic growth of S. aureus was observed with 20mM and 40mM D-AA; n=6.




**Supplementary Figure 5:** Effect of NaOH on the anti-biofilm activity of D-AA. Neutralising acidic amino acids using NaOH resulted in the anti-biofilm activity of these agents to be halted; n=4.

**Supplementary Table 1:** Comparison of percent change in biofilm density with percent change in viable cells for dispersed biofilms. There is a greater reduction in number of viable cells as compared to the reduction in total biomass. This pattern is more prominent in dispersed biofilms than inhibited.

| Dispersing agent | Percent reduction in biofilm density | Percent reduction in cell viability |
|---|---|---|
| **40mM D-AA** | 30.16 | 97.59 |
| **40mM L-AA** | 44.92 | 98.55 |
| **40mM D-AA + 8 MLC** | 32.00 | 96.09 |

**Supplementary Table 2:** Comparison of percent change in biofilm density with percent change in viable cells for inhibited biofilms. There is a greater reduction in number of viable cells as compared to the reduction in total biomass. This pattern is more prominent in dispersed biofilms than inhibited.

| Inhibiting agent | Percent reduction in biofilm density | Percent reduction in cell viability |
|---|---|---|
| **40mM D-AA** | 92.78 | 99.98 |
| **40mM L-AA** | 97.01 | 99.98 |
| **40mM D-AA + 8 MLC** | 93.58 | 99.99 |

**Supplementary Table 3:** Percentage of eDNA present in AA and Cip treated biofilms as compared to treated biofilms was quantified using ImageJ. Table also presents percentage reduction in eDNA compared to control.

| | Inhibiton | | Dispersal | |
|---|---|---|---|---|
| | % of eDNA compared to control | % reduction in eDNA compared to control | % of eDNA compared to control | % reduction in eDNA compared to control |
| **Control** | 100 | 0 | 100 | 0 |
| **AA** | 1.96 ± 0.67 | 98.04 ± 0.67 | 4.24 ± 1.66 | 95.76 ± 1.66 |
| **Cip** | 61.47 ± 19.27 | 38.53 ± 19.27 | 127.41 ± 43.28 | -27.41 ± 43.28 |