**Introduction**

*S. aureus* causes a wide range of clinical manifestations, commonly infecting the skin. Staph infections can also be life threatening, causing endocarditis and osteomyelitis. Strains of *S. aureus* have proven very successful at developing (multi) drug resistance, including Methicillin-resistant *S. aureus* (MRSA). Rapid emergence of antimicrobial resistance is now a great threat to the modern health care system and agriculture. Antimicrobial peptides (AMPs) are potential therapeutic agents for multidrug resistant organisms and are naturally produced by microbces in competitive environments. The AMP Lynronne-1 was identified through metagenomic mining of the rumen microbiome. It was then modified, substituting the N and C-terminal L amino acid residues with the D-forms, producing Lynronne-1D (D-Leu-PRRNWSKIVWKVTVF-D-Ser-NH2). This modified AMP showed greatly improved stability in the presence of trypsin. [2]

**Methods**

Cell permeabilization: cell impermeable dye, propidium iodide was used to test if Lynronne-1D induced membrane degradation.

**Time Kill assay:** Oliva et al., 2003 [3] methodology was adapted for these experiments, Lynronne-1D at 24μg/ml was incubated with *S. aureus* in MH broth, various time intervals were sampled and plated onto MH agar plates then incubated and colonies counted.

**Biofilm assay:** crystal violet was used to stain biofilms, AMP was added at time 0 to *S. aureus* to test Lynronne-1D biofilm attachment prevention ability and AMP was also added to established biofilms (established over 24 hours)

**Live/Dead microscopy:** NucBlue® Live reagent stained live cell nuclei. The cell-impermeable NucGreen® Dead reagent stained dead cell nuclei. All experiments were performed in triplicate with the exception of the time kill experiments that contained 12 replicates.

**Results**

![Time Kill Assay](image1)

**Figure 1:** Time dependent kill data for *S. aureus* with Lynronne-1D and comparator antibiotics at 24μg/ml (3X Lynronne-1D MIC value), over a 6-hour period. Error bars represent the standard error of the mean.

![Biofilm Assay: Lynronne-1D at 4XMIC](image2)

**Figure 3:** Comparing absorbance (biofilm) of untreated *S. aureus* samples and Lynronne-1D treated samples at 4XMIC, for both attachment and established biofilm assays (established for 24 hours) . Error bars represent standard deviation.

![Live/Dead microscopy](image3)

**Figure 2:** Live/Dead microscopy images of *S. aureus* produced using NIS Elements Viewer software. Blue stain- alive cells (A-C, DAPI filter) Green Stain- dead cells (D+B- FITC/GFP filter set)

![Changes in cell permeabilization at 32μg/ml treatments](image4)

**Figure 4:** Shows how the cell permeabilization changed over time, with samples taken every 5 minutes over 30-minute time period. AMP at 4XMIC. Error bars of standard deviation.

**Summary**

- Lynronne-1D has an MIC of 8μl against *S. aureus* and induced only 10.27% haemolysis of mammalian erythrocytes at 32xMIC
- At 3XMIC Lynronne-1D showed fast bactericidal activity, causing a ≥ 5log CFU/ml reduction in viable *S. aureus* cells within 50 minutes of treatment, inducing cell death through membrane destruction/degradation
- The AMP was able to prevent *S. aureus* from forming biofilms

With fast acting bactericidal activity, low mammalian cell toxicity and increased serum stability Lynronne-1D has shown potential as a therapeutic agent.

**References**
