Host defense peptide interactions with *Klebsiella pneumoniae* extracellular polysaccharides drive capsular loss and biofilm collapse

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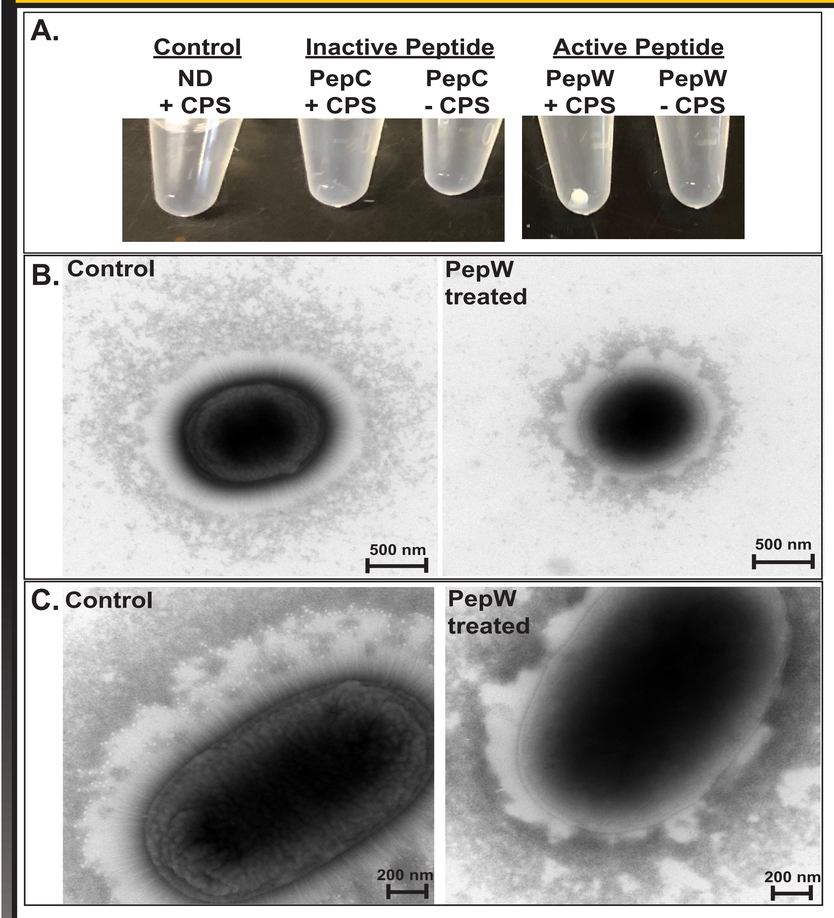
Abstract

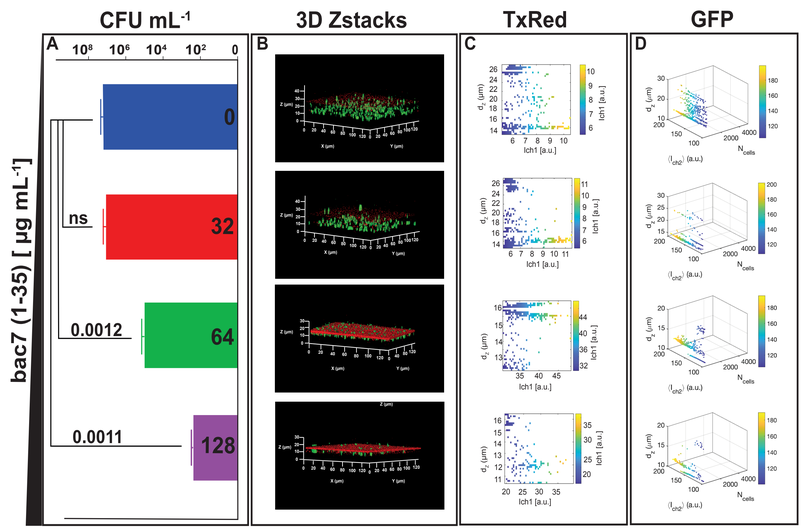
- *Klebsiella pneumoniae* is a dangerous pathogen that has gained much notoriety due to its extreme rate of resistance development.
- Hypervirulent *K. pneumoniae* have increased capsular polysaccharide to evade the host immune and spread within the community of healthy individuals.
- We have shown that host defense peptides can decrease the capsulation of hypervirulent *K. pneumoniae* and disrupt the biofilms formed by these species.
- Polyproline bac7 (1-35) analog assessment revealed antimicrobial activity is independent from the polysaccharide interactions causing biofilm collapse and polysaccharide interactions with host defense peptides drive both capsular loss and biofilm collapse.
- Our mass spectrometry analysis identified the interaction region of bac7 (1-35) with polysaccharides and suggests the N-terminal region becomes disordered causing polysaccharide aggregation and biofilm collapse.
- We show the potential of bac7 (1-35) as a topical therapy to treat a wound infected with hypervirulent *K. pneumoniae*.

Introduction

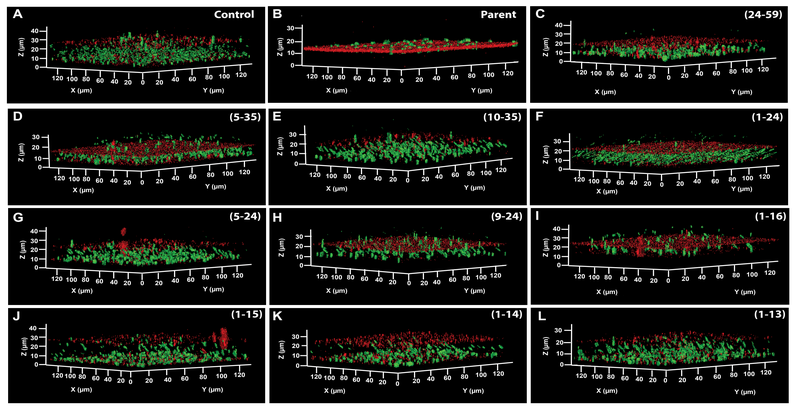


Peptide mediated removal of *K. pneumoniae* capsule protective layer. The images show capsule aggregation with peptides and transmission electron microscopy (TEM) of hypervirulent *K. pneumoniae* stained with 1% phosphotungstic acid as a negative stain to reveal the capsule as a halo around the bacteria. **Figure A** shows extracted capsule aggregates only with the active peptide PepW and not with the inactive PepC or alone with no peptide (ND). The TEM images are shown at two magnifications **Figure B** (2kv mag) and **C** (5kv mag). Both magnifications show the cells with no treatement (Control) or with 64 μg mL^{-1} of PepW (PepW treated). We are currently investigating the mechanism of capsule loss associated with peptide treatment. Adapted from *Fleeman et al. PNAS 2020*.

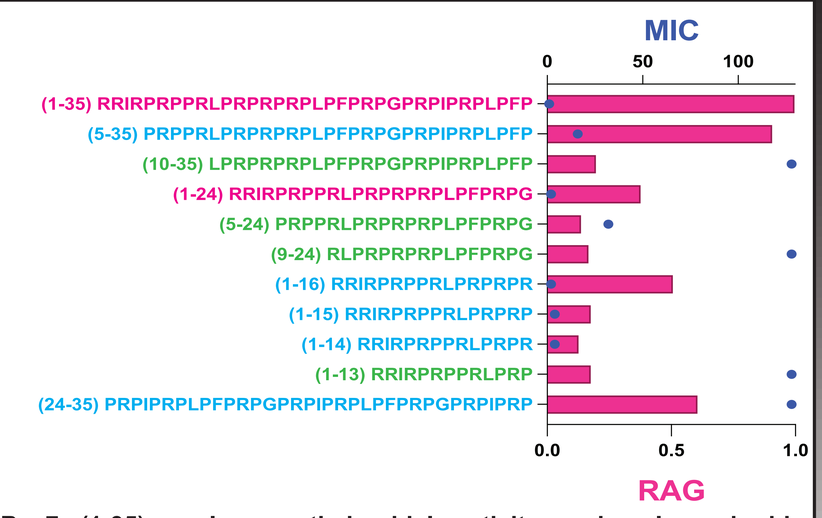
Results



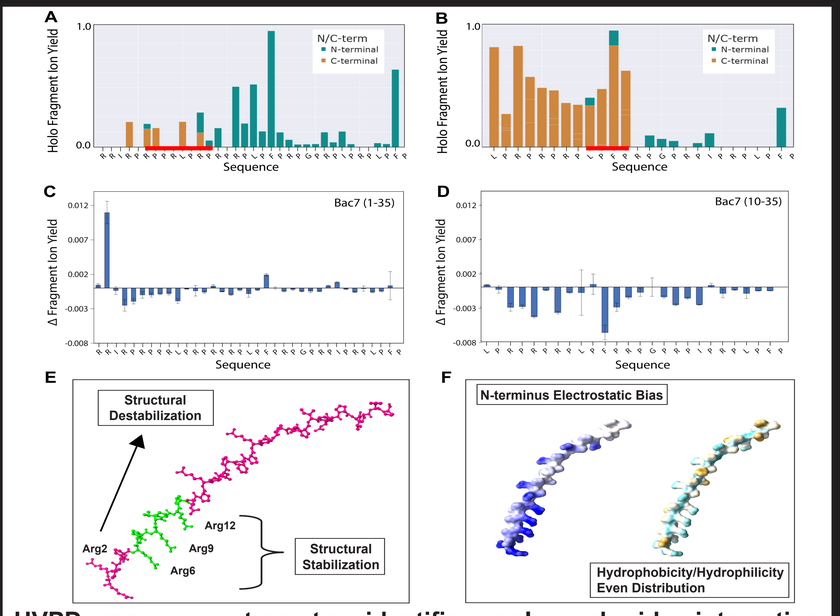
Bac7 (1-35) treatment induces matrix polysaccharide collapse and release of biofilm associated cells. The bacterial viability assessment (**A**) is shown next to 3D rendering of z-stacks images (**B**) and BiofilmQ analysis (**C** and **D**) of biofilms formed by hypervirulent *K. pneumoniae* NTUH K2044 constitutively expressing GFP and stained with Texas Red conjugated concanavalin A.



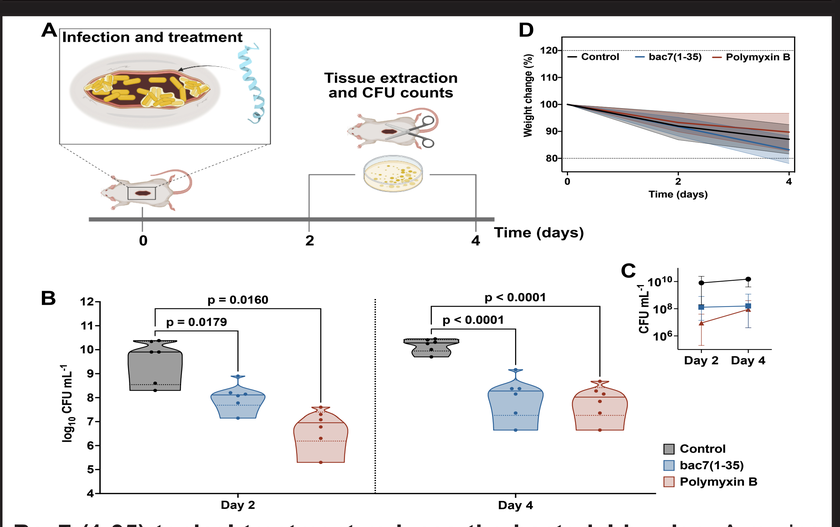
Bac7 (1-35) analogs biofilm polysaccharide analysis. The figure shows the 3D rendering of the confocal z-stack images taken following parent and truncated analog treatment of hypervirulent *K. pneumoniae* NTUH K2044 constitutively expressing GFP and stained with Texas Red conjugated concanavalin A. Figure panels **C** (bac7 24-59), **D** (bac7 5-35), **F** (bac7 1-24), and **I** (bac7 1-16) show analogs with potential to modulate the polysaccharide matrix although not as strongly as the parent (**B**).



Bac7 (1-35) analogs antimicrobial activity and polysaccharide aggregation potential. The figure shows the minimal inhibitory concentrations (**MIC**) of the truncated analogs and their polysaccharide relative aggregation (**RAG**) compared to the parental peptide. The peptide color coding indicates peptide net charge: **> +9** ; **+7 - +8** ; and **< +6**.



UVPD mass spectrometry identifies polysaccharide interaction region. Holo fragment ions graphs are shown for bac7 (1-35) (**A**) and bac7(10-35) (**B**). The graphs in **C** and **D** display fragmentation difference plots of peptides alone or with polysaccharides. Suppression of fragmentation (negative values) correlates with increased secondary structure (more stabilized regions), whereas enhancement of fragmentation (positive values) corresponds to a less stabilized region. Alpha fold generated images of bac7 (1-35) with the interaction region of the peptide highlighted in green (**E**) and the distribution of the electrostatic (Left) and the hydrophobicity/hydrophilicity (Right) (**F**).



Bac7 (1-35) topical treatment reduces the bacterial burden. A murine skin abscess infection model was used to assess a single dose (**A**). Scarified skin areas were enumerated at day 2 and day 4 (**B**). A direct comparsion of the bacterial load between day 2 and 4 are shown (**C**), and the weight change over the infection is shown as as estimate of the overall health of the mice (**D**). Significance determined using One-way Anova (n=6) with Dunnetts correction to obtain p-values.

Conclusions

- Host defense peptides can interact with capsule and disrupt the protective layer of hypervirulent *K. pneumoniae*.
- We are currently investigating the mechanism of capsule loss.
- Bac7 (1-35) polysaccharide interaction causing biofilm matrix collapse is independent of the antimicrobial properties.
- Mass Spec revealed the N-terminal peptide interaction with polyssacharide drives the loss in peptide structure.

Acknowledgements

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