Killing of Persisters, Stationary Phase or Starved Cells of MRSA and CoNS by Anti-Staphylococcal Protein P128

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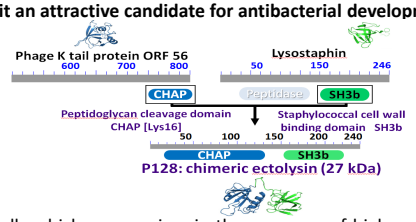
Aim: To assess the bactericidal potential of P128 on persisters, stationary phase or starved cells of MRSA and Coagulase negative staphylococci (CoNS)

Introduction

P128: an engineered Ectolysin with unique properties

- **P128 is a chimeric recombinant ectolysin (phage lysin involved in cleaving the peptidoglycan from outside the bacterium during DNA injection) derived from the tail of *Staphylococcus* phage K.**
- P128 shows rapid bactericidal activity on sensitive and resistant strains of *S. aureus* and CoNS.
- P128 has demonstrated potent antibiofilm activity on MRSA and CoNS strains in various *in vitro* models including those involving clinically relevant mixed bacterial species.
- P128 is highly synergistic with standard-of-care (SoC) drugs in inhibiting *S. aureus* and CoNS cells especially those embedded in biofilms.
- P128 specifically kills *Staphylococcus* sp. and shows no inhibition of other bacterial species or eukaryotic cells even at concentrations as high as 1 mg/ml.
- **These properties make it an attractive candidate for antibacterial development.**



What are persisters ?

- Persisters are bacterial cells which can survive in the presence of high concentrations of antibiotics and are poorly metabolizing bacterial cells which do not show growth or cell division.
- The percentage of persisters is higher in stationary phase cultures or in cells growing in the form of biofilms. Persisters are thought to be responsible for poor treatment outcomes and recurrence/ relapse of infection. Majority of the antibiotics require metabolic activity to show optimum inhibition or killing and thus do not work on non-growing or poorly metabolizing cells.

Material and Methods

Strains used in this study: *S. aureus* BK18 (MRSA), *S. aureus* MW2 (MRSA), *S. aureus* USA300 (MRSA), *S. epidermidis* HM-905 (MRSE), *S. lugdunensis* NR-46406 (MSSL), *S. haemolyticus* HM-1164 (MRSH).

Activity of P128 on non-growing or poorly metabolizing cells

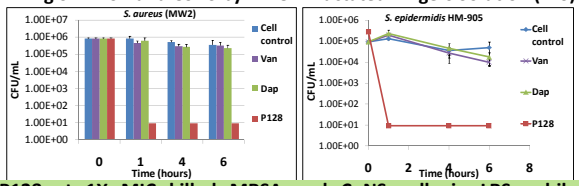
- Spent media was prepared by growing the test strains upto late stationary phase (48 h).
- The cultures were centrifuged, supernatant filtered through a 0.2 µm filter and supplemented with 0.1 % BSA.
- The stationary phase cells were generated by growing the cultures overnight in LB.
- The culture was centrifuged, washed and resuspended in spent media or Lactated Ringers solution (LRS) LRS to obtain 20 ml of ~1×10^5 CFU/ml culture suspension.
- The culture suspensions were aliquoted and treated with 1X MIC of P128, daptomycin or vancomycin for 1, 4 and 6 h and CFU were quantified at each time point.

Activity of P128 on persisters

- Bacterial cultures grown to OD_{600} = 0.5 to 1.0 (2 to 5 x 10^8 CFU/ml) in MHB.
- Treated with 50X or 100X MIC of Vancomycin or daptomycin at 37°C and CFU quantification done at 4, 8 and 24 h. **(A rapid decrease in CFUs followed by stable CFU values up to 24 h indicated the presence of antibiotic tolerant persisters).**
- The antibiotic tolerant persisters were treated with 1X MIC of P128 at 37°C for 1 and 6 h along with control samples with antibiotics only. The treated and control samples were subjected to CFU quantification.
- To rule out any genetic mutations in persister population, the antibiotic persister culture was pelleted, washed in saline and resuspended into fresh LB media and allowed to grow till it reached OD_{600} = 1.0.
- The culture was again subjected to persister genetration as described above.

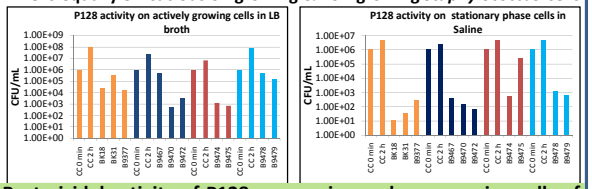
Results

Killing of MRSA and CoNS by P128 in Lactated Ringers Solution (LRS)



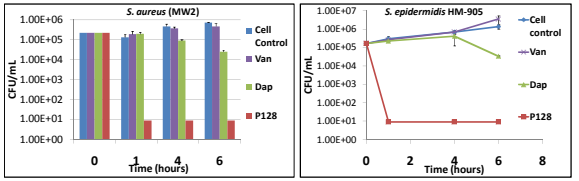
P128 at 1X MIC killed MRSA and CoNS cells in LRS, while daptomycin and vancomycin showed negligible activity.

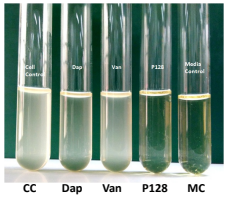
P128 is equally efficacious on growing & non-growing *Staphylococcus* cells



Bactericidal activity of P128 on growing and non-growing cells of MRSA and CoNS in saline is equivalent or better than in LB broth.

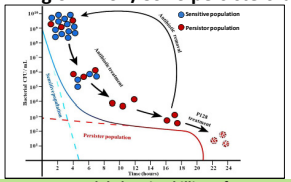
Killing of stationary phase cells of MRSA and CoNS by P128 in spent media



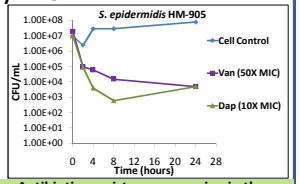


P128 at 1X MIC effectively killed MRSA and CoNS cells in spent media. Daptomycin showed marginal activity while vancomycin showed negligible activity.

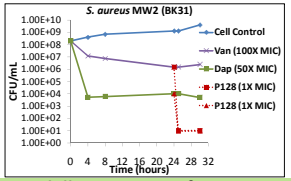
Killing of MRSA/CoNS persisters by P128.

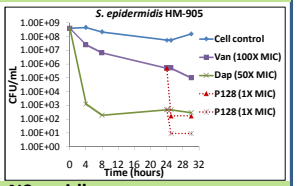


A model showing killing of antibiotic persisters by P128



Antibiotic persisters can survive in the presence of high concentrations of antibiotics





P128 kills persisters of MRSA or CoNS rapidly.

Conclusions

- **Daptomycin or vancomycin treatment killed MRSA / CoNS cell population only partially.**
- **The remaining drug tolerant cell population, reverted to sensitive phenotype once the drug was removed.**
- **P128 at 1X MIC killed vancomycin and daptomycin tolerant persisters efficiently.**
- **P128 killed MRSA / CoNS cells in LRS and spent media as efficiently as in broth.**
- **The potent bactericidal activity of P128 on persisters and non-growing or poorly metabolizing cells makes P128 a good candidate for development as antibacterial for treating recurrent *Staphylococcus* infections.**

References

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Acknowledgements: We thank BEI resources, USA, for providing the CoNS strains. We thank all members of team GangaGen for their support, suggestions and reviewing the presentation.