Reversal of drug resistant phenotype of *Staphylococcus* clinical strains by synergistic strategies of P128 and antibiotics

**Abstract**

Infections caused by drug resistant strains of *S. aureus* are a cause of significant morbidity and mortality all over the world. To overcome the challenge of drug resistance, various approaches are being followed to discover new therapeutics or to rediscover old ones that could potentially enhance the efficacy of existing drugs. P128 is a chimeric recombinant bacteriophage-derived endolysin (plage lysin) involved in clearing the pathogen from the bloodstream during *S. aureus* infection. A recent study found potential bactericidal activity on *S. aureus* and therefore, the aim of the present study was to investigate the potential of P128 to be synergistic on planktonic cells and biofilm of *S. aureus* causing MRSA. To determine whether this synergistic action could result in enhanced activity against drug resistant MRSA, P128 was tested in combination with oxacillin, vancomycin or imipenem by checkerboard assays using strains individually resistant to one of these drugs. On MRSA strains isolated from clinical cases of MRSA, MIC of P128 and oxacillin (0.12–0.5 mg/mL) and 0.5 mg of vancomycin resulted in bacterial growth, indicating a strong synergy. Similar results were seen with other drug combinations of sub-MIC of P128 and oxacillin clinically sensitive concentrations of vancomycin, linezolid, or daptomycin, or Snyder et al. could inhibit the growth of clinical *S. aureus* strains which were individually resistant to these drugs. It was also shown that P128 can be a viable model of biofilms using the USA300 MRSA strain, a combination of 0.12 mg/mL of P128 single-drug treatment led to 99.99% reduction in biomass. These studies show a significant role of P128 in the eradication of drug-resistant isolates of *S. aureus*.

**Methods and Results**

**A** Synergistic effect of combinations of P128 and antibiotics on planktonic cells of drug resistant *Staphylococcus* isolates – *Time Kill Kinetics (TKK)*

- **Time kill kinetics assay with P128 and antibiotics was performed as per modified CGL guidelines.**
- **A > 2 log reduction in viability between the combination and most active single drug was considered as the cut off value to score for synergy.**

**B** Synergistic effect of combinations of P128 and antibiotics on planktonic cells of drug resistant Staphylococcus – Checkerboard assay

- **Checkerboard assay with P128 and antibiotics was performed as per modified CGL guidelines with combination of P128 and antibiotics.**
- **The combination effectiveness was quantified by MTT assay and corresponding FIC indices were calculated.**
- **The combination was considered synergistic when the FIC is ≤ 0.5, indifferent when > 1 and antagonistic when the FIC is ≥ 2.0.**

**C** Synergistic effect of combinations of P128 and antibiotics on biofilm of drug resistant staphylococcal isolates – Checkerboard assay

- **Biofilms were grown in 96 well microtitre plate (Bhat et al., 2014).**
- **A checkerboard assay was performed as per standard guidelines (4 dose point combinations) with combination of P128 and antibiotics.**
- **The combination effectiveness was quantified by MTI assay and corresponding FICI values were calculated.**
- **The combination was considered synergistic when the FICI ≤ 0.5, indifferent when > 1 and antagonistic when the FICI is ≥ 2.0.**

**Conclusion**

- P128, an endolysin in *E. coli* with unique properties.
- P128 is a chimeric recombinant endolysin that is being developed to treat systemic and topical *Staphylococcus* infections.
- P128 shows rapid bactericidal activity on antibiotic sensitive and resistant strains of *S. aureus* and its use in the treatment of Gram-positive infections (CoNS).
- P128 has demonstrated potent anti-biofilm 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 strains especially those embedded in biofilm.
- P128 specifically kills *S. aureus* biofilms and shows no inhibition of other bacterial species or eukaryotic cells even at concentrations as high as 1 mg/mL.

**Staphylococcus** are commensal or otherwise non-pathogenic natural flora in some settings and yet sometimes threatens our life as a tenacious pathogen.

- In addition to its ability to outdo our immune system, its multi-drug resistance phenotype makes it one of the most intractable pathogens in the history of antibiotic chemotherapy. It has conquered most of the antibiotics that have been developed since 1940.
- The overall burden of staphylococcal disease, particularly caused by *S. aureus* is increasing in many countries in both healthcare and community settings.
- Due to the emergence of resistance to conventional therapeutics, efforts to develop non-molecule antibacterial therapeutics have been increasing.
- Rapid killing, low rates of resistance and profound anti-biofilm activity is a few key properties of plankage lysins, which can be exploited to develop clinically useful therapeutics.

This study investigates the in vitro and in vivo efficacy of P128 in combination with SoC antibiotics on respective drug resistant strains of *Staphylococcus*.

**References**