Lactobacillus GG Secrets Small Peptides That Inhibit The Growth of Antibiotic-Resistant Bacteria

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Background

- Probiotics are non-pathogenic bacteria that are claimed to prevent and/or treat certain diseases. For example, diarrhea, gastroenteritis, inflammatory bowel disease, genitourinary tract infections, cancer, respiratory infections and others.
- Lactobacillus GG (LGG) is one of the most important probiotics, which was isolated in 1983 from a human being's intestine and patented in 1985. Silva M, *et al* reported that LGG secretes small molecular antimicrobial substances. Manley KJ *et al* reported that probiotics can be used to treat vancomycin-resistant enterococci.
- Several hypotheses have been proposed to explain the antibacterial effects of probiotics, including their capability to compete for nutrients, the establishment of a microenvironment in which pathogenic microorganisms are not able to survive, or the elaboration of toxins lethal for pathogenic bacteria.

  However, their mechanism of action has not yet been completely established.

AIMS

- To establish whether LGG elaborate anti-bacterial peptides.
- To study the anti-bacterial spectrum of these peptides
- To perform preliminary structure-function analysis of these peptides to enhance their anti-bacterial effect.

Materials and Methods

Starting Material: Lactobacillus GG Conditional Media (LGGCM)

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Concentrated and Filtered

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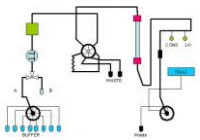
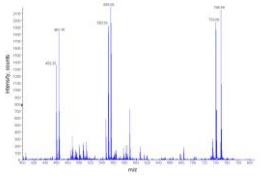
Liquid Chromatography  **-MS/MS** 

Search against database  **identified 7 small peptides**

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Peptides synthesis and Purification 

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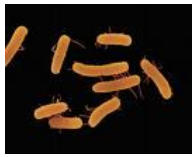
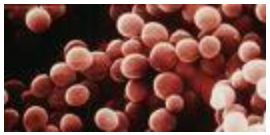
Peptides activity assay (bacteria inhibition assay):

(including Plate culture and A_{600} measurement)

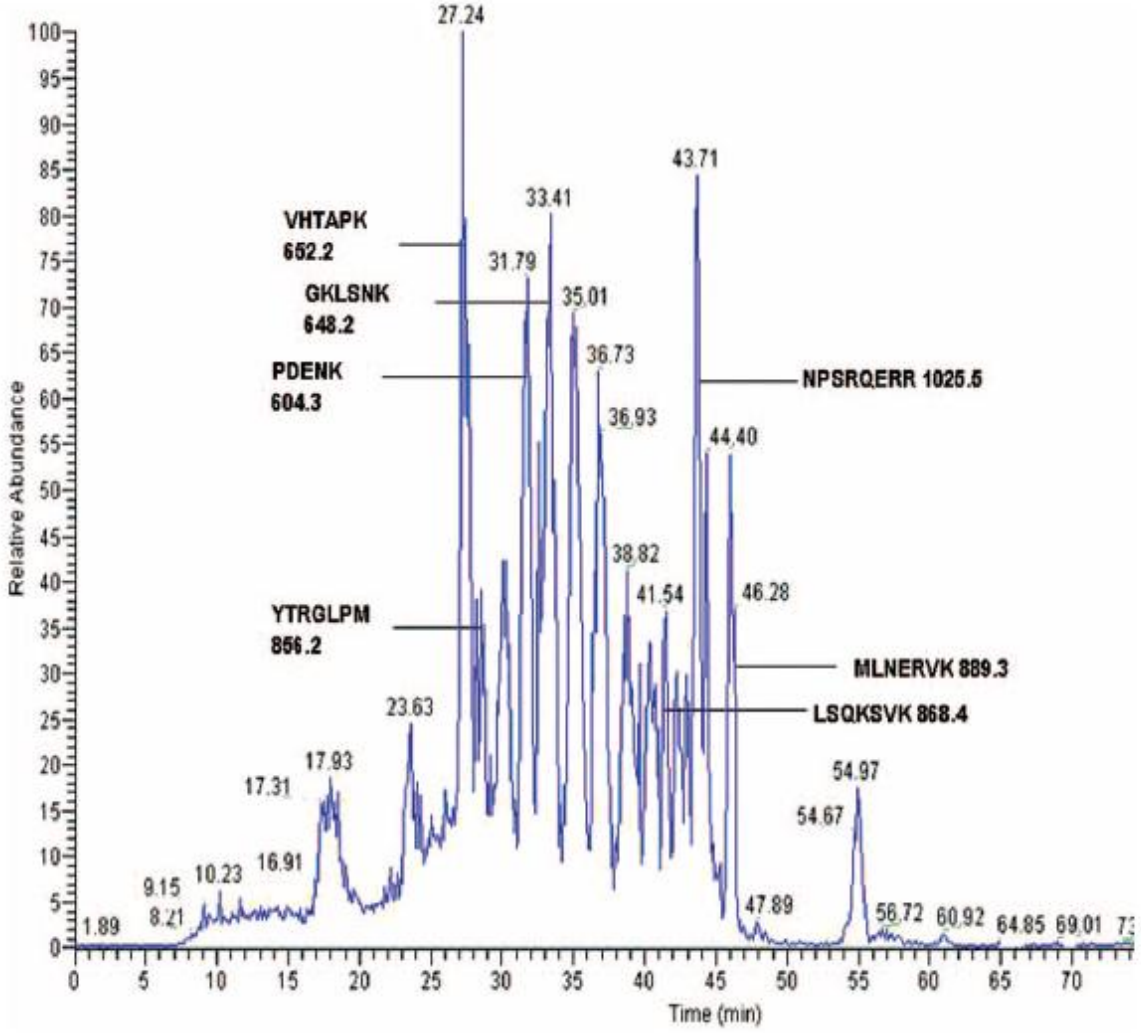



Gram Negative Bacteria: E. coli (EAEC 042); Salmonella typhi
Gram Positive Bacteria: Staphylococcus aureus
Antibiotics Resistant Bacteria:
Kanamycine-resistant E. coli SM10 λpir
Tetracycline-resistant E. coli TOPO10
MRSA – Methicillin-resistant Staphylococcus aureus or Multiple-resistant Staphylococcus aureus



Results

Identification of antibacterial peptides from LGG CM by LC-MS/MS analysis



Seven peptides:

VHTAPK
GKLSNK
MLNERVK
PDENK
LSQKSVK
YTRGLPM
NPSRQERR

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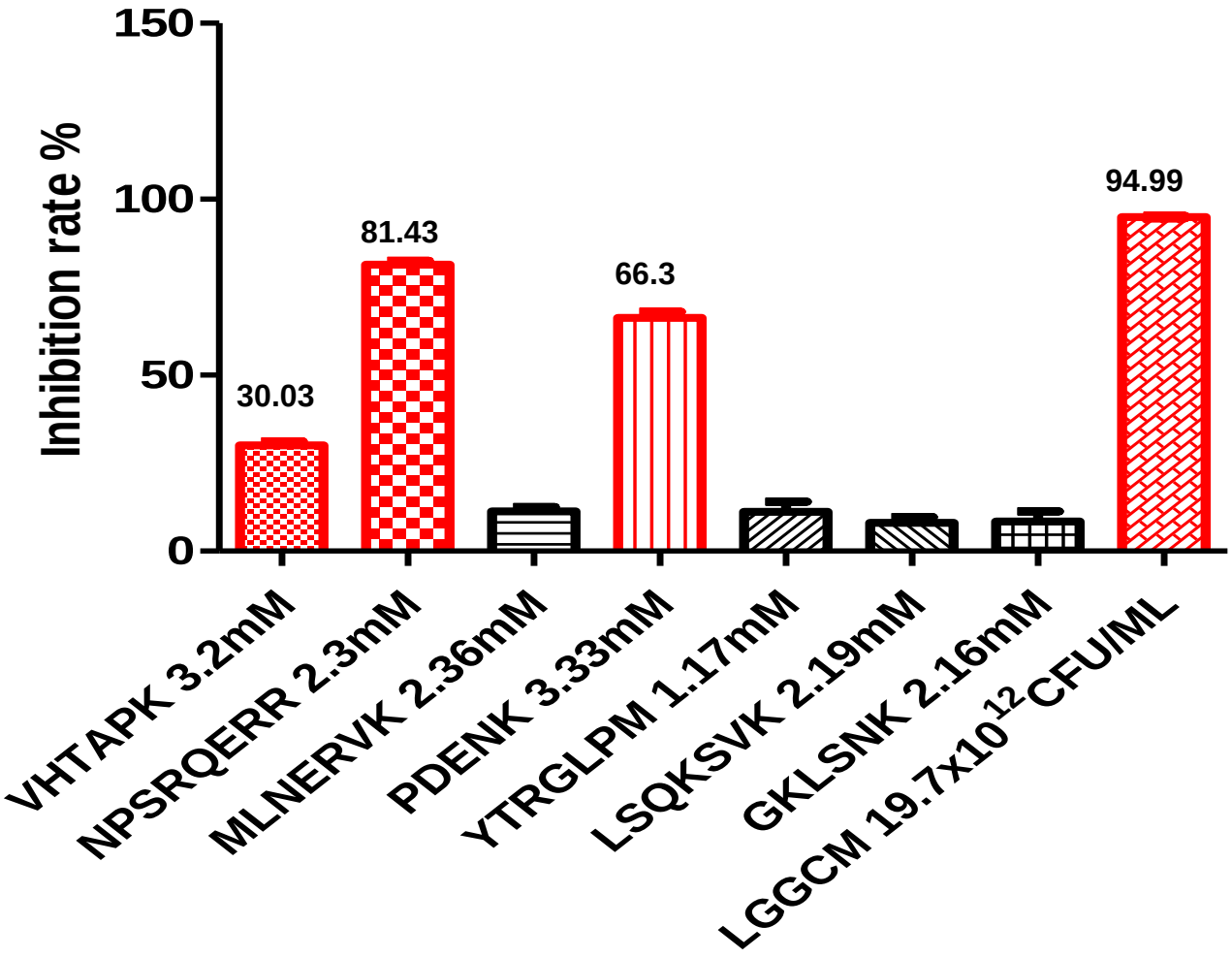
Peptides synthesized

Results

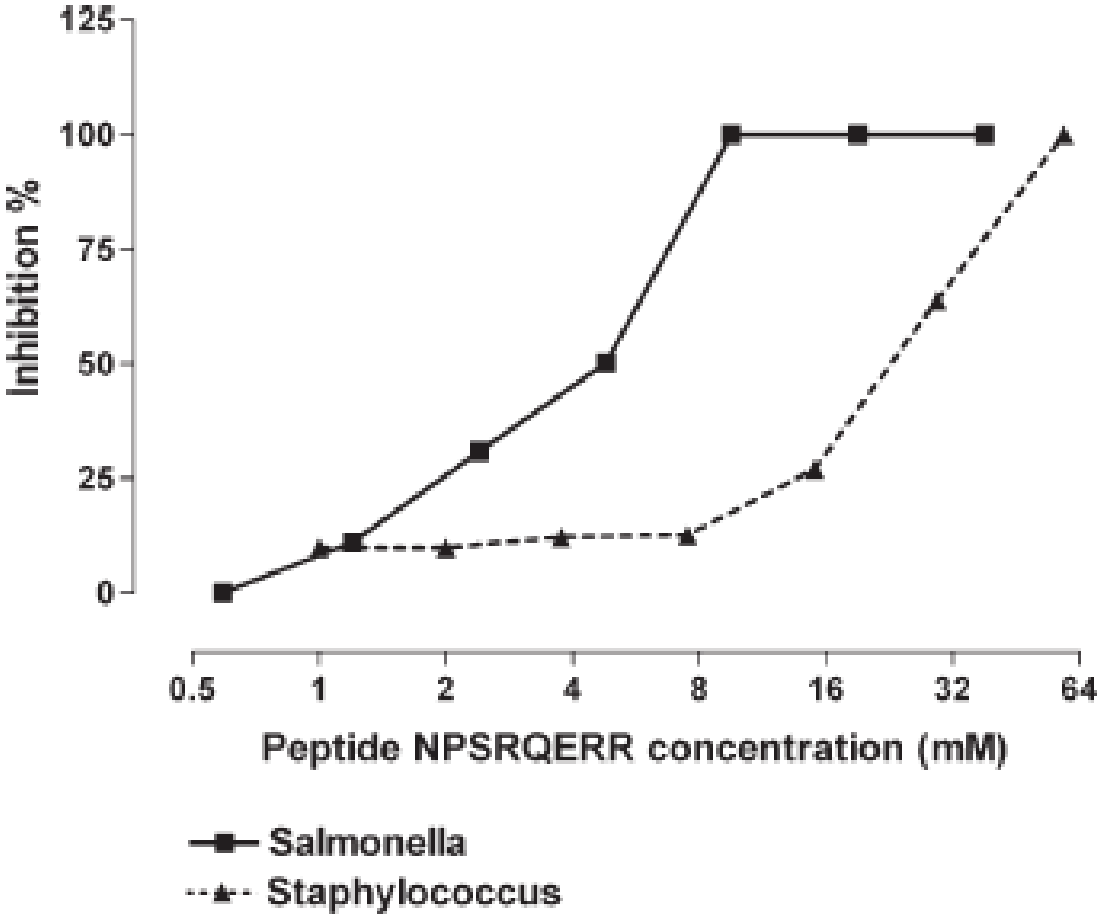
LGGCM and 7 synthetic peptides relative inhibition activities on *E. coli* growth.

Growth inhibition assay: Mean *E.coli* record at A_{600} in the presence of different peptides (n=3) or LGGC (positive; n=7). Readings are normalized with media alone



RESULTS

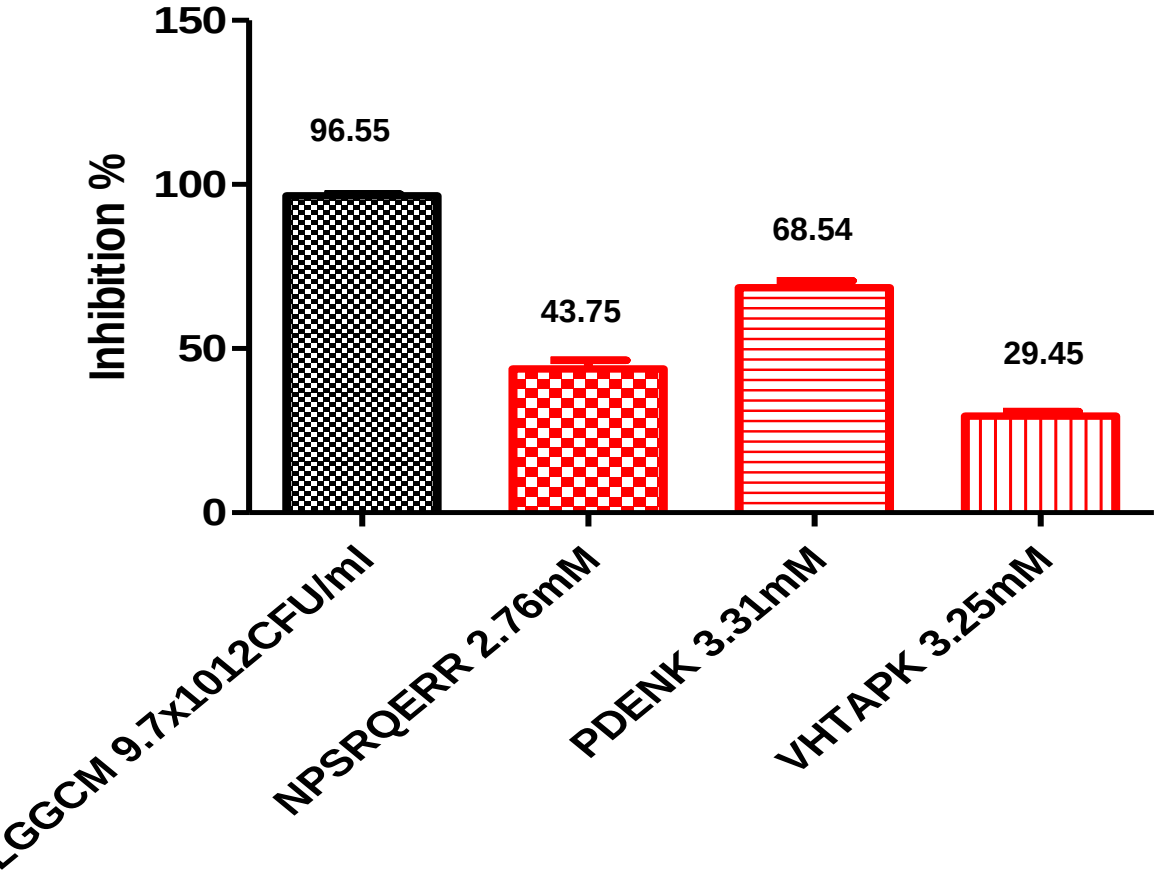
Peptide NPSRQERR shows does-dependent antibacterial activities on both *Salmonella typhi* and *Staphylococcus aureus*.



Peptide NPSRQERR showed a dose-dependent antibacterial effect that was more potent on *S. typhi* (in which 100% inhibition was reached at the peptide concentration of 8 mmol/L) than on *S. aureus* (in which 100% was reached at a peptide concentration of 64 mmol/L).

Results

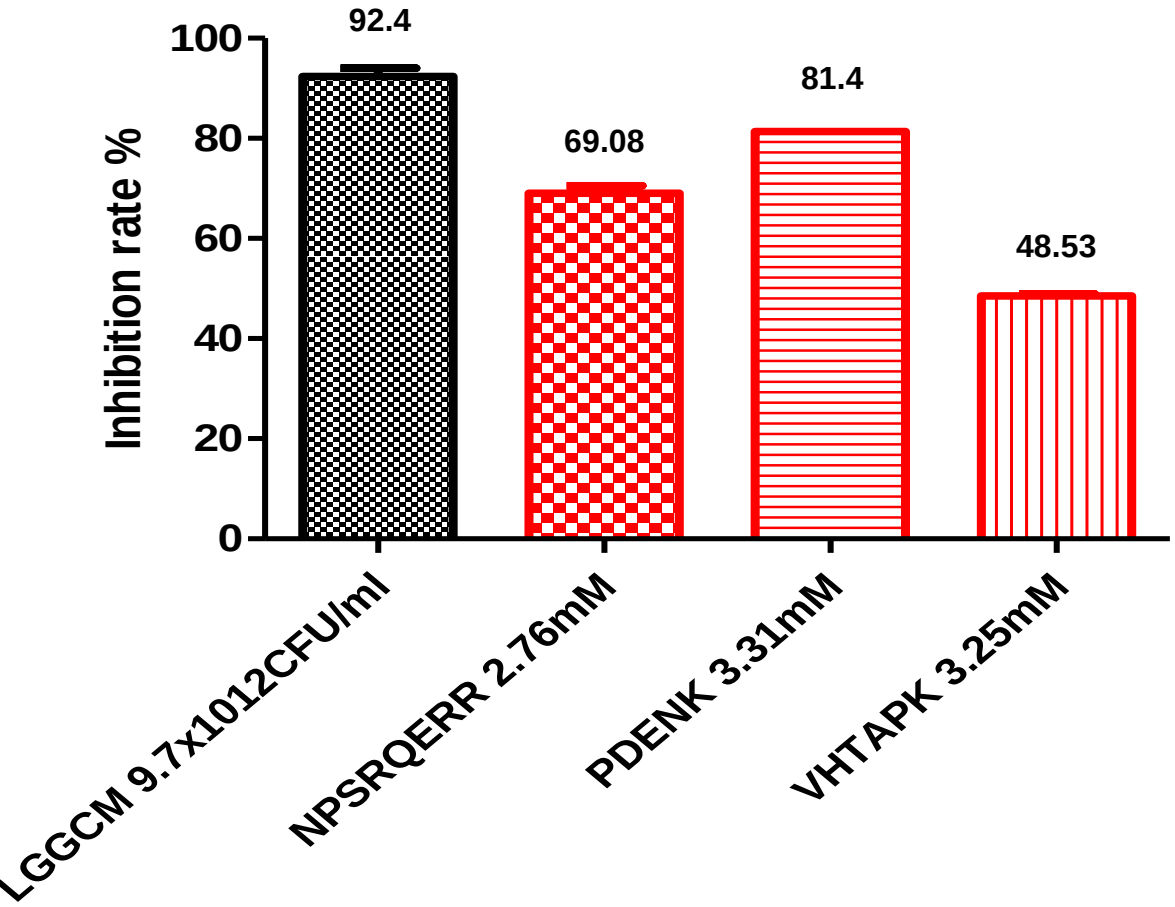
Peptides NPSRQERR, PDENK and VHTAPK inhibition on *Kanamycine-resistant E. coli SM10 λpir* growth.



Readings are normalized with media alone
Data show Average (n≥3) ± SE .
LGGCM($9.7X10^{12}$ CFU/ML) as positive control.

Results

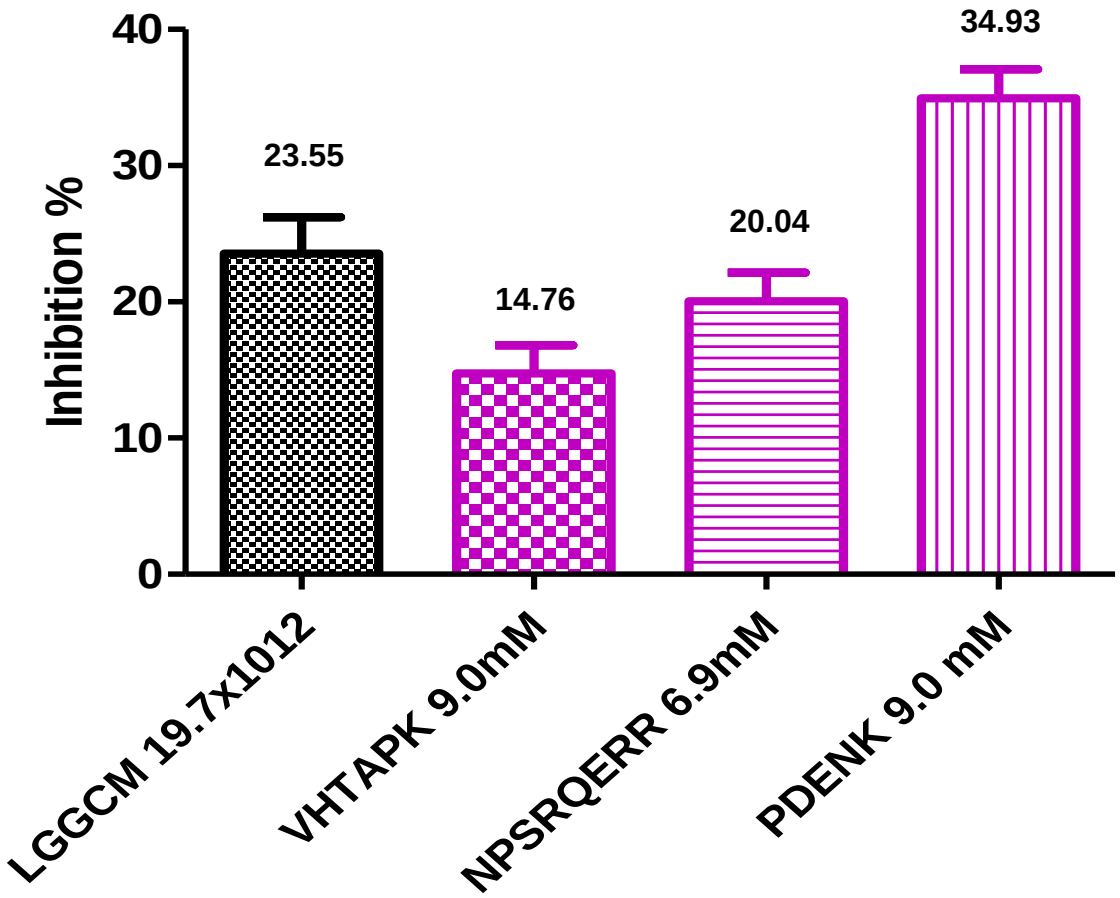
Peptides NPSRQERR, PDENK and VHTAPK inhibition on *Tetracycline-resistant E. coli TOPO10* growth.



Readings are normalized with media alone
Data show Averages (n≥3)±SE.
LGGCM(9.7X10^{12} CFU/ML) as positive control.

Results

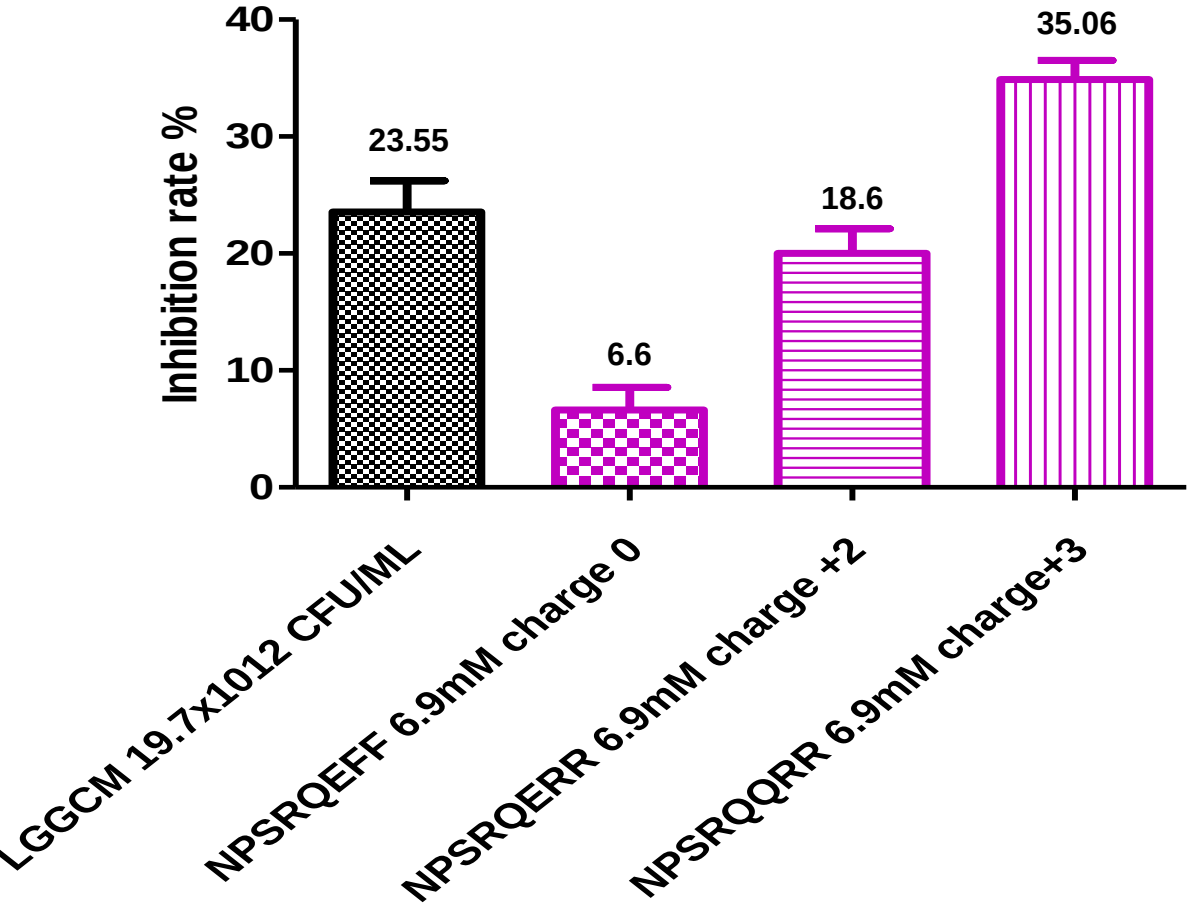
Peptide NPSRQERR, PDENK and VHTAPK inhibition on *MRSA* growth



Readings are normalized with media alone
Data show Average (n≥3) ±SE.
LGGCM(19.7X10^{12} CFU/mL) as positive control.

Results

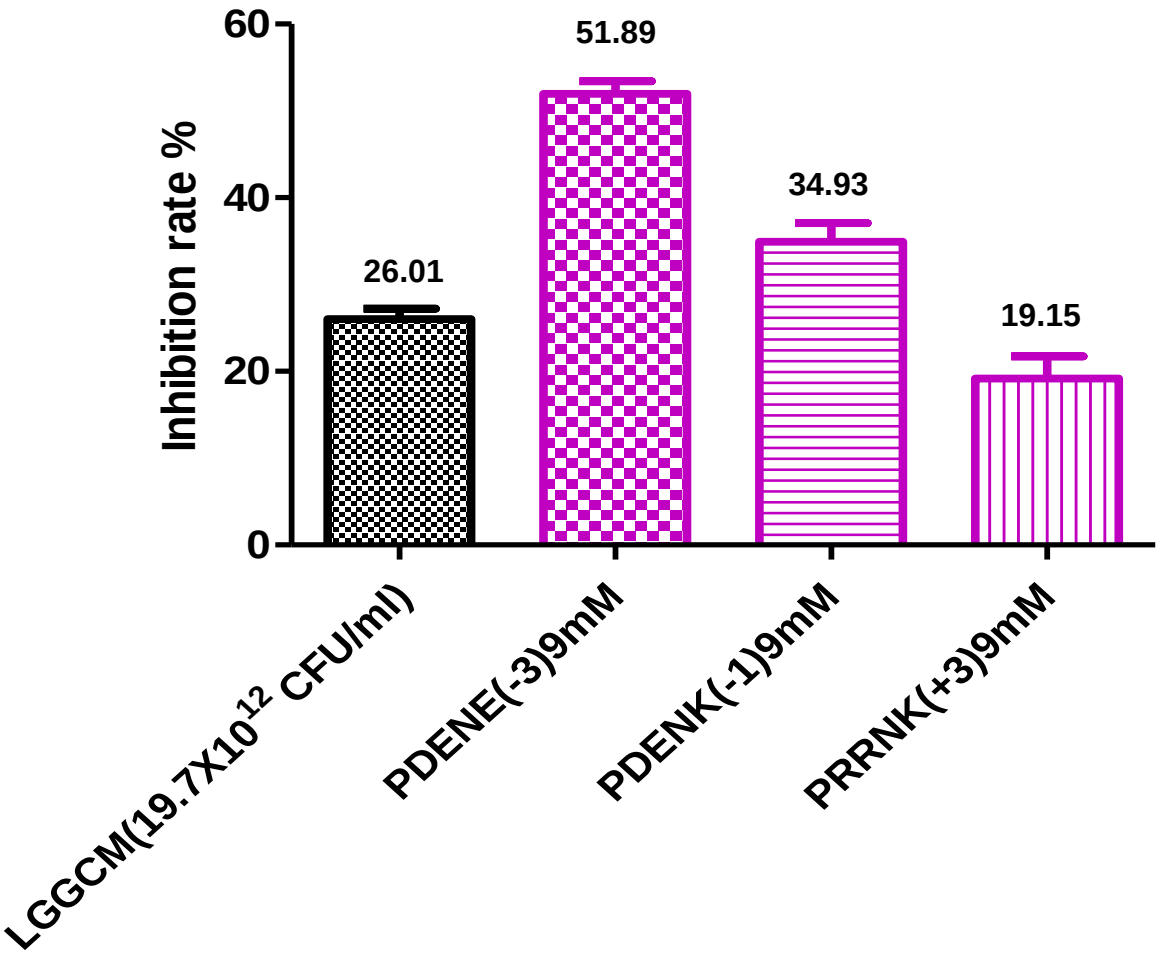
Structure – activity relationship of peptide NPSRQERR and its analogs on *MRSA* growth.



Picture shows Average (n≥3)±SE. LGGCM($19.7X10^{12}$ CFU/ML) as positive control.

Results

Structure - activity relationship of peptide PDENK and its analogs on *MRSA* growth



Data show average(n≥3)± SE. LGGCM(19.7X10^{12} CFU/mL) was used as positive control.

CONCLUSIONS

1. **Seven small peptides identified from Lactobacillus GG conditional media showed different inhibition activities on *E.Coli* growth.**
2. **Peptide NPSRQERR has inhibitory activities on both *Staphylcoccus aureus* and *Salmonella* Growth.**
3. **Peptides NPSRQERR, PDENK and VHTAPK showed inhibitory activities on several *antibiotics-resistant bacteria*.**
4. **Peptides NPSRQERR, PDENK and VHTAPK with potential to be customized**