Designing selective peptide binders for Methicillin resistance *Staphylococcus aureus*

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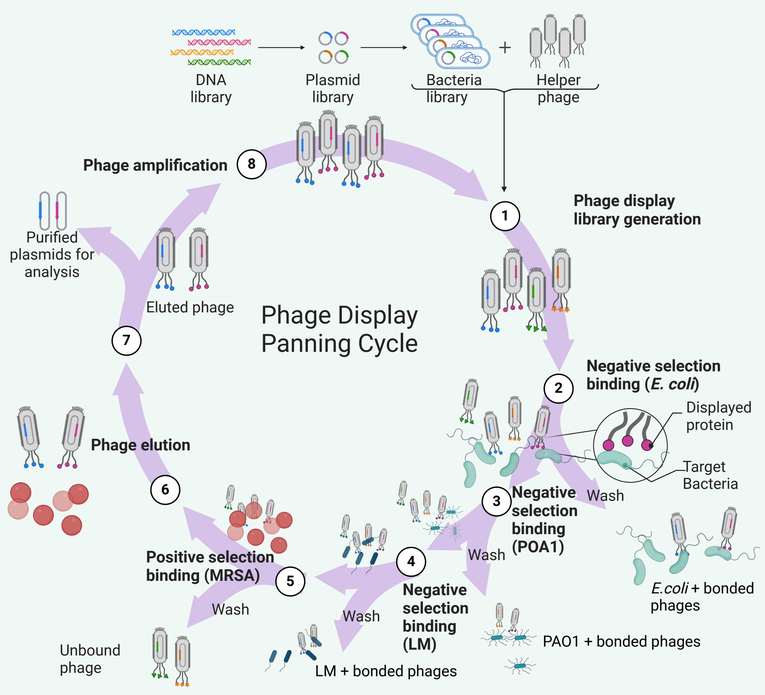
Abstract

The growing global crisis of antibiotic resistance highlights the need for novel approaches in diagnosing and treating bacterial infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) presents a particularly severe threat, especially in healthcare environments. This study aims to advance the development of rapid and selective peptide binders for MRSA as a model bacterium. Peptides, with their potential for modification and cost-effectiveness, offer significant promise for creating effective bacterial binders. We employed phage display technology to identify unique peptide binders with high affinity for MRSA cells. This method involved using whole bacterial cells in suspension during the assay, which increased the diversity of the selection process. During the phage display screening, the peptide library was exposed to various types of bacteria (negative selection) to enhance selectivity towards MRSA (the target bacteria). Subsequent Next Generation Sequencing and bioinformatics analysis allowed us to select sequences that were present in high abundance in the MRSA treatment compared to the negative selection bacterial cells.

The identified lead peptides were synthesized, purified, and characterized using two methods: Flow Cytometry for suspension-based interactions and gold surface immobilization for interactions on surfaces. The lead peptides demonstrated strong binding affinities for MRSA in both immobilized and suspension conditions, with promising specificity. Chemical modifications further enhanced the binding affinities, resulting in peptides that showed even greater affinity for MRSA than the original sequences. Notably, the two leading peptide binders, WPG and TST, along with the modified peptide WPG11A, exhibited high selectivity for MRSA, even in mixed bacterial populations. Importantly, these peptides showed no toxicity towards either bacterial or mammalian cells, highlighting their potential for safe application in diagnostic and therapeutic settings.

Our study successfully identifies selective peptide binders for MRSA using innovative phage display technology. The characterized peptides demonstrate promising selectivity and high binding affinity for MRSA, laying the basis for the development of rapid and specific bacteria detection technologies.

1. Peptide screening and isolation

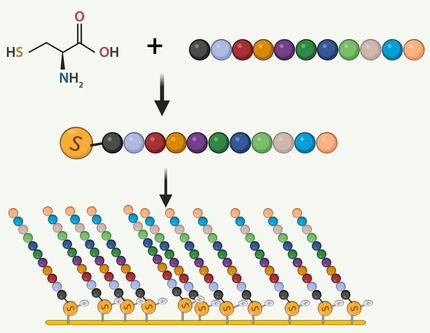


Phage display methodology that was Used at the current study:

1. **Initial Phage Library Preparation:** A diverse library of phage-displayed peptides was created, featuring a broad spectrum of peptide sequences.
2. **First Negative Selection:** The phage library was incubated with *Escherichia coli* (*E. coli*) cells. Phages that bind to *E. coli* are removed, leaving only those that did not interact with *E. coli*.
3. **Second Negative Selection:** The remaining phages were then incubated with *Pseudomonas aeruginosa* (PAO1) bacterial cells. Phages that bind to PAO1 were removed, isolating those that did not interact with PAO1.
4. **Third Negative Selection:** The process was repeated with *Listeria monocytogenes* (LM) bacterial cells. Phages that bind to LM were discarded, retaining only those that did not bind to LM.
5. **Positive Selection:** The remaining phages were incubated with Methicillin-resistant *Staphylococcus aureus* (MRSA) cells. Phages that specifically bind to MRSA were kept, while unbound phages were removed.
6. **Elution:** Bound phages were eluted from MRSA cells to isolate those as a candidate as the peptide binders.
7. **Phage Amplification:** The eluted phages were amplified in *E. coli* DH5α bacteria to increase the quantity of the selected phages.
8. **Iteration:** Steps 2 through 7 were repeated three times independently to enrich for phages with the highest specificity and affinity for MRSA.

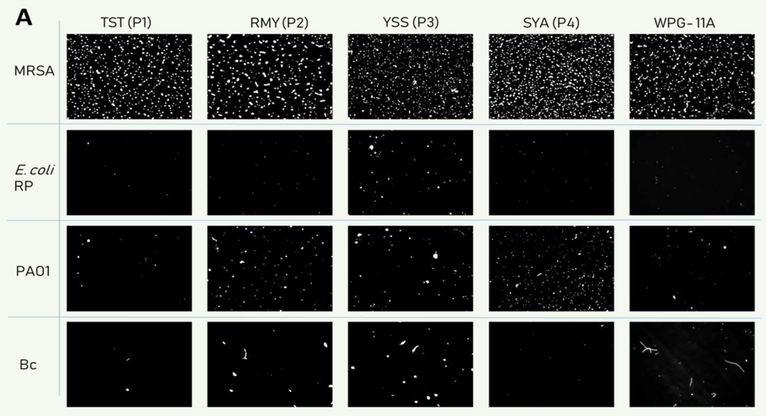
This refined process enhanced the selection and enrichment of peptide binders that specifically target MRSA, while minimizing non-specific interactions with other bacterial species.

2. Characterizing the binding affinity of the lead peptides



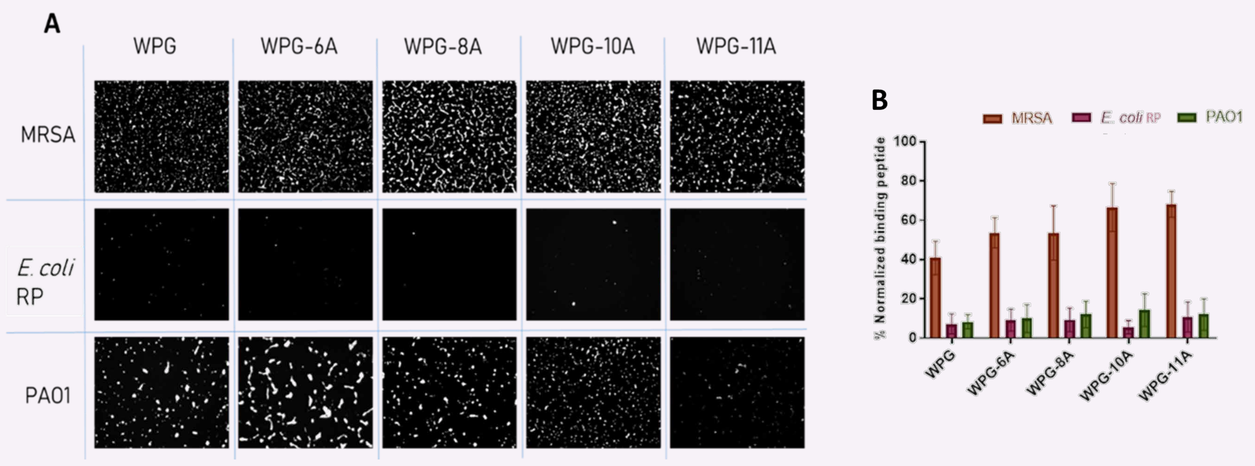
Schematic Illustration for the Immobilization of Peptides on a Gold Surface:

All peptides were synthesized with a linker at the N-terminus, consisting of three glycine residues followed by a cysteine residue (Gly-Gly-Gly-Cys) to covalently immobilize the peptides on the gold surfaces through the thiol group (-SH) in the cysteine side chain. This thiol-gold interaction covalently attaches the peptide to the gold surface, resulting in a peptide-coated gold surface.



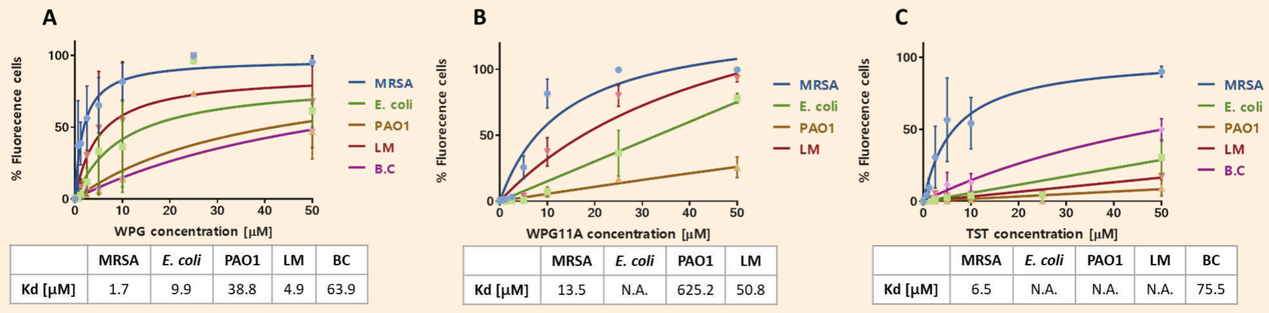
Peptide-Bacteria Binding Affinity Detection:
Peptide sequences identified from phage display as having high copy numbers for Methicillin-resistant *Staphylococcus aureus* (MRSA) and low copy numbers in negative control bacteria were assessed for bacterial affinity against MRSA, *Pseudomonas aeruginosa* (PAO1), *Escherichia coli* (E. coli RP), and *Bacillus cereus* (Bc). **(A). Immobilized peptide binding analysis**: peptides were covalently attached to a gold surface at a concentration of 10 µM using a thiol-gold interaction. Bacterial cells were then stained with 4',6-diamidino-2-phenylindole (DAPI) and observed using Evos fluorescence microscopy to visualize binding. **(B). flow cytometry analysis:** bacterial cells at a density of approximately 10^8 CFU/mL were incubated with 10 µM fluorescein-tagged peptide. After removing unbound peptides, flow cytometry was used to measure the proportion of fluorescein-labeled bacteria relative to the total bacterial population, providing quantitative data on peptide binding (mean of three replicates ± standard error).

3. Chemical modification of the lead peptide



WPG lead peptide chemical modifications:
The WPG peptide and four derivatives were evaluated for bacterial affinity against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (PAO1), and *Escherichia coli* (E. coli RP). **(A). Immobilized peptide binding analysis:** peptides were covalently attached to a gold surface at a concentration of 10µM via a thiol-gold interaction. Bacterial cells were stained with 4',6-diamidino-2-phenylindole (DAPI) and visualized using Evos fluorescence microscopy to assess bacterial cells binding. **(B). Flow cytometry analysis**: bacterial cells at an approximate density of 10^8 CFU/mL were incubated with 10µM fluorescein-tagged peptide. Following the removal of unbound peptides, flow cytometry measured the proportion of fluorescein-labeled bacteria relative to the total bacterial population, providing quantitative analysis on peptide binding, expressed as the mean of three replicates ± standard error.

4. Quantifying the binding affinities of the lead peptides



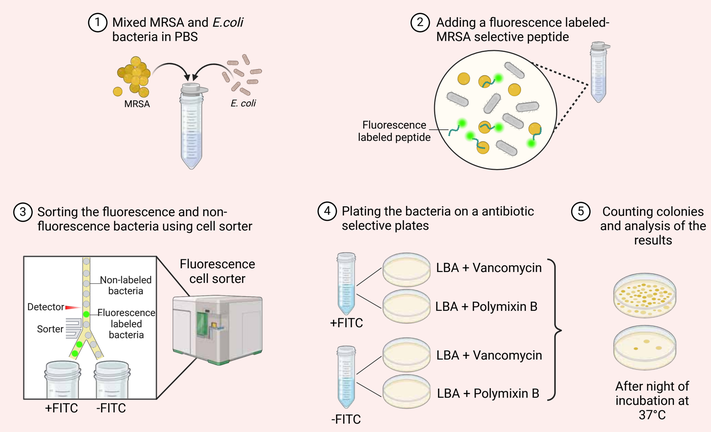
	MRSA	*E. coli*	PAO1	LM	BC
Kd [µM]	1.7	9.9	38.8	4.9	63.9

	MRSA	*E. coli*	PAO1	LM
Kd [µM]	13.5	N.A.	625.2	50.8

	MRSA	*E. coli*	PAO1	LM	BC
Kd [µM]	6.5	N.A.	N.A.	N.A.	75.5

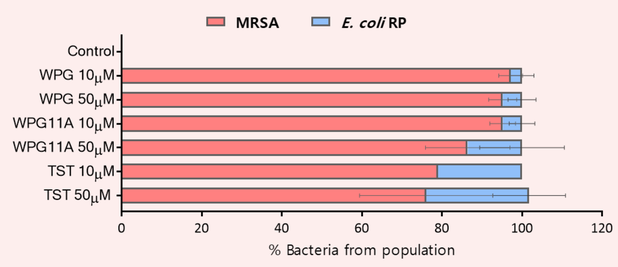
Binding curves for lead peptide bacterial binders:
The binding curves for peptides WPG (A), WPG11A (B), and TST (C) are shown. Fluorescently labeled peptides were used at varying concentrations: 0.65, 1.25, 2.5, 5, 10, 25, and 50 µM. Each peptide concentration was incubated with bacteria at a density of 10^8 CFU/ml in PBS for 30 minutes. After incubation, the bacteria were washed twice with PBS and fixed with 4% glutaraldehyde. Flow cytometry was then employed to measure the proportion of FITC-labeled bacteria at each peptide concentration. The dissociation constant (K*d*) for each peptide-bacteria interaction was calculated using PRISM software. Data are presented as the mean of three replicates ± standard error (S.E.).

5. Competition binding assay



Competition assay of the lead peptide bacterial binders:

1. **Bacterial Preparation:** Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E. coli*) were cultured separately in LB medium to a density of 10^8 CFU/ml, then washed three times with PBS. The bacteria were mixed in equal ratios.
2. **Peptide Incubation:** Fluorescence-labeled peptides were added to the bacterial mixture at concentrations of 10 and 50 µM and incubated for 30 minutes. After incubation, the bacteria were washed with PBS.
3. **Separation by FACS:** Fluorescence-activated cell sorting (FACS) was used to distinguish between FITC-labeled bacteria and non-labeled bacteria.
4. **Plating and Incubation:** Both labeled and non-labeled bacteria were plated on LB agar plates separately containing selective antibiotics and incubated overnight at 37°C.
5. **Colony Counting:** Colonies were counted the following day to determine the proportion of each bacterial type.



Quantification of MRSA and *E. coli* cells that the lead peptides bind:

The distribution of bacteria within the FITC-positive population was assessed as follows. MRSA and *E. coli* were cultured and processed as described. After separation by fluorescence-activated cell sorting (FACS) and plating on LB agar plates with selective antibiotics, colonies were counted the following day. The bacterial load for each type was normalized to the total number of FITC-positive cells. Data are presented as the mean of three replicates ± standard error (S.E.).

Conclusions

Our study successfully identified selective peptide binders for Methicillin-resistant *Staphylococcus aureus* (MRSA) using a phage display technique that utilized whole bacterial cells in suspension to enhance peptide diversity. This method led to the discovery of peptides with good specificity and high binding affinity for MRSA cells. The chemically modified peptides, including WPG and its derivatives, demonstrated enhanced binding affinities compared to the original sequence. Importantly, these peptides exhibited no toxicity towards either bacterial or mammalian cells, highlighting their safety for potential diagnostic and therapeutic applications.
In conclusion, our findings highlight the potential of our approach as a basis for developing rapid and specific any pathogenic bacterial detection abilities for various applications.