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Investigations on novel aedesin-derived peptides against ESKAPE pathogens

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Antimicrobial peptides (AMPs) are promising alternatives to conventional antibiotics, yet strategies to optimize novel peptide templates remain limited. In this study, *aedesin*, a cecropin-like peptide from *Aedes aegypti*, was investigated and found to adopt a helix-bend-helix structure. Systematic modifications were introduced on the N- or C-terminal helices to enhance antimicrobial efficiency and selectivity. N-terminal derivatives showed greater potency compared to C-terminal modifications. Notably, peptides 2D11 and 2D12 exhibited minimum inhibitory concentrations (MICs) of 8-16 µg/mL against *Acinetobacter baumannii*, while peptide 3E1-E3 displayed broad-spectrum activity against ESKAPE pathogens with MICs of 16-64 µg/mL. Similarly, peptides 4B8-B11 demonstrated MICs of 8-32 µg/mL against most bacteria, excluding *Enterococcus faecium*. Among them, peptide 4B11 showed the strongest activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strain MW2, with an MIC of 8 µg/mL. Cytotoxicity assays confirmed that the peptides were non-toxic to HEPG2 cells at their MICs and to human red blood cells up to 128 µg/mL. Mechanistic studies revealed a membrane-disruptive mode of action, supported by propidium iodide uptake and membrane depolarization assays. Furthermore, peptides effectively eliminated antibiotic-induced persister cells and protected *Galleria mellonella* from MRSA infection. These findings highlight optimized aedesin-derived peptides as potential candidates against ESKAPE pathogens.

Keywords: Antimicrobial peptides, *Galleria mellonella*, HEPG2 cell lines, Minimal inhibitory concentration

According to the Infectious Diseases Society of the United States declared in recent years, a group of pathogens has been capable of escaping from the action of currently used antimicrobials. The panel is responsible for causing the most nosocomial infections in humans with increased pathogenesis and virulence that lead to life-threatening infections. Bacteria including, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species* are included in the ESKAPE pathogen panel. The Centers for Disease Control and Prevention (CDC) estimates that at least 2 million Americans contract an antibiotic-resistant infection each year, which results in 23, 000 deaths. The mortality rate associated with this condition has already eclipsed the number from HIV infections. Furthermore, the British groups' estimations are far more concerning. As a result, if nothing is done, the toll will increase to 10 million by 2050 at the very least. Furthermore, the system is further confounded by the rate at which these ESKAPE microorganisms acquire resistance to the widely used antibiotics. Due to the limited rate at which new antibiotic classes are identified, it would be nearly impossible to counteract[1].

Several studies have consensus to develop antimicrobial peptides (AMPs) as alternatives to antibiotics[2]. These are short peptides less than 50 amino acids and have a broadactivity spectrum. They are produced by nearly all the life kingdoms including; plants, animals, fungi, bacteria, and even archaea as the first line of defense. AMPs are mostly cationic, α-helical with specific hydrophobic characters, while β, αβ peptides rich in specific amino acids are less numerically reported[3]. A balance in the change and the hydrophobicity renders an amphipathic structure required for the AMPs to bind the negatively charged bacterial membranes[4]. This property edges the selectivity over its mammalian cell to its zwitterionic properties imparted by the presence of cholesterol moieties. Although non-membrane targets of the AMPs are also well known as proline-rich AMPs work by inhibiting protein synthesis in *E. coli* and defensins like Human β-Defensin 3 inhibit cell wall biosynthesis in *S. aureus*[5]. However, most AMPs have short killing times because of their membrane-interacting nature. The property doesn't give sufficient selection pressure for the development

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of drug resistance[6]. In addition, AMPs are known to elevate the host immune response. Expression of selective cytokines like MCP-1 and CXCL-10 is known to clear up the infection by macrophage-assisted phagocytosis or by direct killing[3]. The mentioned properties illustrate their suitability for future drug developments.

Aedesin is a 36-residue-long cecropin-like peptide discovered from the salivary glands of dengue virus-infected mosquitoes (*Aedes aegypti*)[7]. The immature peptide containing the signal sequence was found to have anti-Leishmanial activity and helical propensity. And, when the mature peptide with a sequence of GGLKKLGKKLEGAGKRVFKASEKALPVVVGIK AIGK was analyzed structurally with NMR studies carried out in the presence of 50% Trifluoroethanol (TFE) revealed a helix-band-helix motif. Helix 1 starts from residues Lys30 to Lys48, and helix 2 from residues Val52 to Ile59, with the band in between (Fig. 1)[8]. The structure is well-characterized as cecropin-like. And since cecropin-derived peptides are found to be highly effective[9]. This investigation was systematically performed to obtain short Aedesin-derived peptides. Though the activity of the full molecule is known to inhibit Gram-negatives, a detailed activity spectrum analysis is also justified.

In this study, the efficacy of short peptides (16-18 residues) designed from either the N or C terminus corresponding to the two major helical segments of the molecule, was evaluated. Knowledge from both established and novel peptide design principles was incorporated to address infections caused by a panel of ESKAPE pathogens. Hemolytic activity and cell cytotoxicity of these peptides were also examined against human red blood cells and liver-derived HEPG2 cell lines in order to have a better understanding of toxicity.

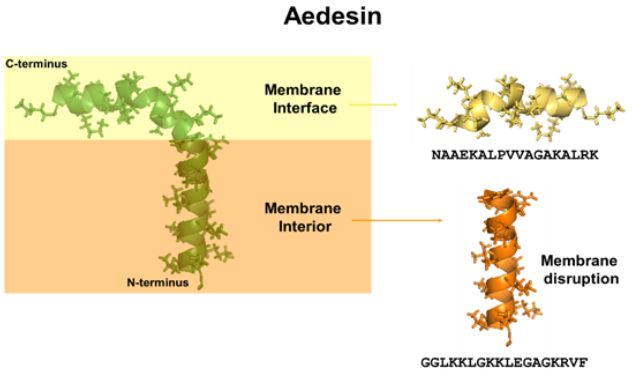

Fig. 1 — Schematic structural representation of the peptide Aedecin representing the two helical fragments with membrane disruption and interface interacting regions. The figure is adapted from (PMID: 25162372)

Materials and Methods

Microbial strains and growth parameters

A. baumannii ATCC 17978, *P. aeruginosa* PA14, *E. aerogenes* ATCC 13048, *S. aureus MW2, K. pneumonia* WGLW2, and *E. faecium* E007 are the bacterial strains employed in this investigation. Brain-heart infusion (BHI) broth was used to cultivate *E. faecium* (BD, Franklin Lakes, NJ, USA). The four Gram-negative bacteria, namely *E. aerogenes*, *P. aeruginosa, A. baumannii,* and *S. aureus*, were cultured in tryptic soy broth (TSB) purchased from BD Biosciences, Franklin Lakes, NJ, USA. All bacteria were cultivated in an incubator shaker at 200 rpm and 37°C.

Determination of Minimum inhibitory concentrations (MICs)

By performing broth micro dilution assay, the Minimal Inhibitory Concentration (MIC) of peptides was ascertained by the protocol outlined by the Clinical and Laboratory Standards Institute (CLSI). To sum up, serial dilutions (50 µL) in duplicates of each peptide was prepared in Mueller-Hinton broth (MHB; BD Biosciences) using a 96-well plate (broadly diluted). 50 µL of bacterial inoculum prepared the ratio at 1:1000 fold of overnight culture in fresh media was laid them. For every peptide, the final concentration falls between 64 and 1 µg/mL. After adding the bacteria, the plates were incubated at 35°C for 18 h. The minimum concentration of peptide required to inhibit bacterial growth was known as the minimum inhibitory concentration, or MIC, and it was determined by monitoring the OD_{600}. MIC assays were performed in duplicate with each experiment repeated twice.

Checkerboard assay

The checkerboard assay was used to understand how the peptide interacted with reference antibiotics that are currently in use. In short, one component was stacked vertically, and the other horizontally, after two compounds were serially diluted at a 2X concentration. The bacteria that were used on the plates were exactly the same as those that were used in the previously described susceptibility test. The fractional inhibitory concentration (HFIC), which illustrates the synergistic effects of the peptides and antibiotics, is calculated using the formula FIC = FIC peptide + FIC antibiotic. The minimum inhibitory

concentration (MIC) of a peptide in combination or of the peptide alone is known as FIC peptide, while the MIC of an antibiotic in combination or alone is known as FIC antibiotic.

Red blood cells (RBCs) Lysis activity

The effectiveness of the peptide to cause hemoglobin leakage was evaluated as described in a previous study[10]. Human erythrocytes were obtained from Rockland Immunochemicals (Limerick, PA, USA). Briefly, from a stock of 4% Human Red Blood Cells (hRBCs (in PBS), 100 µL of was added to 100 µL of two-fold serial dilutions of peptides in PBS ranging from 128-4 µg/mL in a 96-well microtiter plate. 1% Triton-X 100 and PBS served as the positive and negative controls respectively. Upon addition, the samples were incubated at 37°C for 1 h and then centrifuged at 500×g for 5 min. 100 µL of the supernatant was transferred to a fresh 96-well plate, and absorbance was read at 540 nm. Percent hemolysis was calculated taking 100% hemolysis caused by 1% Triton X-100, and 0% in PBS. Values were represented as mean duplicates.

Mammalian cell cytotoxicity assays

As previously mentioned, HepG2 cells were employed to evaluate the cytotoxicity of AMPs on mammalian cells[11]. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin/streptomycin (Gibco) and maintained at 37°C in 5% CO_2. Cells were harvested and resuspended in DMEM., 100 µL of cells were added to a 96-well plate at 5×10^4 cells/well. AMPs were serially diluted in serum and antibiotic-free DMEM was added to the monolayer and the plates were incubated at 37°C in 5% CO_2 for 24 h. At 4 h before the end of the incubation period, 10 µL of 2-(4-iodophenyl) -3- (4-nitrophenyl) -5- (2, 4-disulfophenyl) -2H-tetrazolium (WST-1) solution (Roche, Mannheim, Germany) was added to each well. WST-1 reduction was monitored at 450 nm using a V_{max} microplate reader. Experiments were carried out in three independent replicates and the percentage survival was calculated by DMSO-treated vehicle controls.

Membrane depolarization

Minor adjustments were made to an existing methodology to perform measurements of bacterial membrane potential. After being cultured in fresh MHB media, exponential phase *S. aureus* MW2 bacteria were three times rinsed in PBS and then resuspended in the same buffer at double the starting volume. The cells were energized by adding 25 mM of glucose for 15 min. After that, 50 µL was divided across 96-well black, clear-bottom plates (Corning no. 3904), and 5.0 µM of DISC3 (5) (3, 3′ - Dipropylthiadicarbocyanine iodide) (Molecular Probes/Thermo Fisher Scientific, ON, Canada) was added to the bacterial growth. At 610 nm for excitation and 660 nm for emission, the plate's fluorescence was monitored for 20 min, or until the baseline stabilized.

Propidium iodide-based membrane permeability

The purpose of the fluorescence-based bacterial permeation experiment was to monitor the membrane-oriented interactions between the bacteria and the proposed peptides. The propidium iodide probe is typically utilized to determine how AMPs create pores. To put it briefly, *S. aureus* MW2 was cultured in MHB medium for the entire night at 37°C and 220 rpm of shaking. To reach the exponential phase of growth, a new inoculation was carried out in the same media the next day. After that, the culture was resuspended, cleaned three times in PBS, and adjusted to an OD_{600} of around 0.3 in PBS. In a 96-well black, clear-bottom plate (Corning no. 3904), 48 µL of this culture was added to 50 µL of serially diluted 2x peptide concentrations.

Laurdan-based membrane fluidity assay

The fluidity of the bacterial membrane induced by the peptide was assessed by slightly altering an established methodology. To put it briefly, *S. aureus* MW2 bacteria were regrown from a culture prepared by overnight incubation in new MHB media during their exponential phase. Following a 3x PBS wash, the cells were resuspended in half the initial culture volume that was removed for washing. The bacteria were exposed to a final concentration of 10 µM of Laurdan dye (Sigma-Aldrich, Cat. # 40227) at room temperature and in the absence of light. Black plates were loaded with serially diluted peptides, clear-bottomed, 96-well plates (Corning no. 3904) with 100 µL of this dye/bacteria mixture added.

Persisters cell generation and membrane permeability

An established approach was followed for the production of antibiotic-induced persister cells. After reaching the stationaryphase, a 25 mL culture of *S. aureus* MW2 was exposed to 20 µg/mL of gentamicin for 4 h in order to generate MRSA persisters. PBS

(phosphate-buffered saline) was used to wash the bacteria three times in the same volume. PBS was used to dilute the cleaned cells to an OD600 = 0.4 (~2×10^8 CFU/mL). 30 min were spent at room temperature in the dark with SYTOX Green (Molecular Probes) added to 10 mL of the diluted persister suspension, resulting in a final concentration of 5 μM. Ninety-six black, clear-bottom 96-well plates (Corning no. 3904) had 50 μL of the persister/SYTOX Green mixture poured into each well.

Killing kinetics of persister cells

The *S. aureus* MW2 persister cells were generated as described above. After the PBS washing, cells were adjusted to approximately 1 $\times10^8$ cells/mL (OD_{600}= 0.3) in PBS and exposed to the peptide at 10 times the MIC for 120 min. Aliquots of bacteria at 0, 30, 60, 90, and 120 min were diluted accordingly and plated in MH agar plates. Plates were then incubated at 37°C, 18 h for colony enumeration. Vancomycin at 10x MIC (MIC; 1 μg/mL) was served as a control.

In vivo* protection assay on *Galleria mellonella

The well-established wax moth model system was established to investigate the protective effects of the peptide 4B11 in an *in vivo* setting. A separate batch of 16 randomly chosen worms (*G. mellonella* larvae; Vanderhorst Wholesale, St. Mary's, OH, USA) was distributed. After an overnight culture, *S. aureus* MW2 bacteria were developed to the exponential phase. Following three PBS washes, the cells were eventually suspended in PBS with an OD_{600}~0.3. The four experimental groups were therapy (group), PBS (vehicle), bacterial infection (group), and untouched (no injection). The positive control in this experiment was vancomycin. The corresponding group received an injection of 10 μL (2×10^6 cells/mL) of the produced bacterium, which was followed by a one-hour peptide treatment.

Results and Discussion

Evaluation of antimicrobial properties of the designed peptides

Set1: Antimicrobial activity of N-terminal derived peptides from Cecropin A2

For the first set of peptides, the design was based on the cecropin A2 template[12]. The reason behind the selection of this template lies in the sequence similarity with aedesin. Peptide design parameters were subsequently incorporated into aedesin-derived peptides. This set consists of 16 amino acids with 16 designed peptides based on different principles. The template started from the first α-helical Lysine corresponding to Lys29 (Lys1 here w.r.t the short template) of the cecropin A2 and up to Phe43 (Phe11), which is the last residue in the first helix conserved in both cecropin A2 and aedesin corresponding to a sequence of KKLEGAGKRVFNAAEK (Table 1).

Table 1 — Antimicrobial activity of the 1st set of peptides. MIC is denoted in μg/mL. Peptides were derived from the N-terminus of cecropin A, AB; *A. baumannii*, KP; *K. pneumoniae*, PA; *P. aeruginosa*, and *S. aureus.*

Sequence	Label	AB	KP	PA	SA
GGLKKLGKKLEGAGKRVFNAAEKALPVVAGAKALRK	1A1	8	32	> 64	>64
KKLEGAGKRVFNAAEK	1A2	> 64	> 64	> 64	> 64
KKLEGAGKRVFNAAEK	1A3	> 64	> 64	> 64	> 64
KKIEGAGKRVFNAAEK	1A4	> 64	> 64	> 64	> 64
KKAEGAGKRVFNAAEK	1A5	> 64	> 64	> 64	> 64
KKVEGAGKRVFNAAEK	1A6	> 64	> 64	> 64	> 64
KKSEGAGKRVFNAAEK	1A7	> 64	> 64	> 64	> 64
KKFEGAGKRVFNAAEK	1A8	> 64	> 64	> 64	> 64
KKKEGAGKRVFNAAEK	1A9	> 64	> 64	> 64	> 64
KKLEGAGKRVFRAAEK	1A10	> 64	> 64	> 64	> 64
KKLEGAGKRVFKAAEK	1A11	> 64	> 64	> 64	> 64
KKLEGAGKRVFQAAEK	1A12	> 64	> 64	> 64	> 64
KKLEGAGKKVFNAAEK	1B1	> 64	> 64	> 64	> 64
KKLEGAGKLVFNAAEK	1B2	> 64	> 64	> 64	> 64
FYGKKLEGAGKRVFNAAEK	1B3	> 64	> 64	> 64	> 64
GKKLEGAGKRVFNAAEK	1B4	> 64	> 64	> 64	> 64
Gentamycin	GEN	2	0.5	2	0, 5
Ciprofloxacin	CIPRO	0.25	0.06	0.25	0, 25

Notably, all peptides in this set were amidated to maintain structural stabilization of the terminal amide to inner backbones by hydrogen bonding[13].

Also, the symmetric-to-asymmetric conversion of the first hydrophobic amino acid the Leu3 to Ile, Ala, Val, Ser, Phe, and Lys was evaluated to see any structural influence when the amidated Asn12 was substituted with charged both positive (Arg or Lys) or negative Gln at the position. Lys to Arg substitutions were also incorporated at the residue. In addition, a few residues were also added on the N-terminus to mask the effects of the exopeptidase that imparts resistance to degradation. This set of peptides was tested against *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* and none of the peptides was active against any of the tested strains up to 64 µg/mL. Also, any of the peptides designed for the C-terminal interfacial regions are not effective. Hence, it's concluded that the template selection from cecropin A2 was not appropriate, and further work proceeded with the helix portions of aedesin.

Aedesin comprises two well-defined amphipathic α-helices located in the Lys30-Lys48 (N-terminal) and Val52-Ile59 (C-terminal) regions. The N-terminal helix is rich in positively charged residues, including six lysines, whereas the C-terminal helix is predominantly hydrophobic, containing two isoleucines and three valines, interrupted by a single lysine residue (Fig. 2).

Set 2: Antimicrobial activity of N-terminal derived peptides from Aedesin

The sequence starting from the N-terminus up to the last homologous residue of both peptides with the sequence GGLKKLGKKLEGAGKRVF. This group of peptides was incorporated with parameters of flexible motif deletion, an increase in the positive charge in both hydrophilic and hydrophobic interface, increased hydrophobic moments, etc. The N-terminal sequence was not active by itself against any of the tested bacteria panels including *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. Interestingly, an increment in the hydrophobicity by interchange of the smaller hydrophobic units12thand 13th from the N-terminus by two Leu units showed promising activity against *A. baumannii* and *S. aureus*. The peptides 2D11 and 2D12 have MICs of 8-16 µg/mL against *A. baumannii* and 32 µg/mL against *S. aureus,* respectively (Table 2). Peptides from Sepacin B, from the flesh fly *Sarcophaga peregrine* has increased antimicrobial activity by the incorporation of a string of Leu[14]. The peptides tested in this group pointed us in the right direction for the template selection.

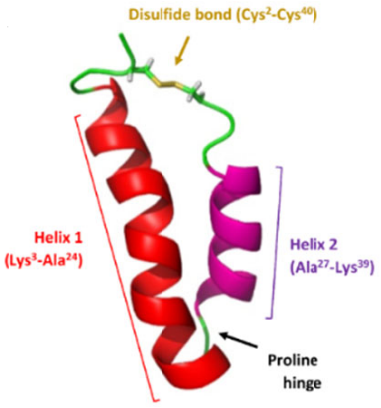

Fig. 2 — Structural representation of Aedesin showing C and H terminal with the L residue highlighted in red and pink

Synergy with antibiotics

The peptide 4B11 was tested for synergy in a checkerboard assay with four traditionally used antibiotics (Table 2). It included daptomycin, gentamycin, vancomycin, and oxacillin. Interestingly, the peptide was found to be synergistic with gentamycin with a ΣFIC value of 0.5. The rest of the antibiotics were found to have a ΣFIC value of ≥ 1 suggesting additive or partial synergistic.

Evaluation of the hemolytic activity

The hemolysis of the peptides was done in the presence of 2% hRBCs (Fig. 3). The peptides were hemolytic in the sequence of 4C1>4B12>4B9>4B8 with an HL_{50} of ~30, 45, 52, 60 µg/mL. Interestingly, the best peptides like 4B10 and 4B11 were found to be least hemolytic with no HL_{50} values up to 128 µg/mL. The hemolysis experiment was only conducted for the 4th set of the peptides, because of their best microbial activity. The peptides 4B10 and 4B11 have the least MIC of 16 µg/mL, which is much less than the tested concentration here. This imparts the flexibility of using the peptide even a few times more of its MIC values.

Evaluation of the cellular cytotoxicity on HEPG2 cell lines

AMPs administration via intravenous that reaches the liver and the process of detoxification occurs.

Table 2 — Synergistic activity of the peptide 4B11 with various antibiotics against *S. aureus* MW2

SL. No.	Antibiotic	ΣFIC values
1	Vancomycin	0.575
2	Daptomycin*	0.5375
3	Gentamycin	0.5
4	Oxacillin	0.75

Hence, liver toxicity needs to be tested with any novel anti-infectives. HepG2 cells were used (Hepatic cancer cell lines) to assess the hepatic toxicity of proposed AMPs. The IC_{50} of AMPs such as 4B8, 4B10-12, 4C1-3 seems to be 64 µg/mL respectively (Fig. 4). However, the AMP-B9 shows the IC_{50} of 32 µg/mL.

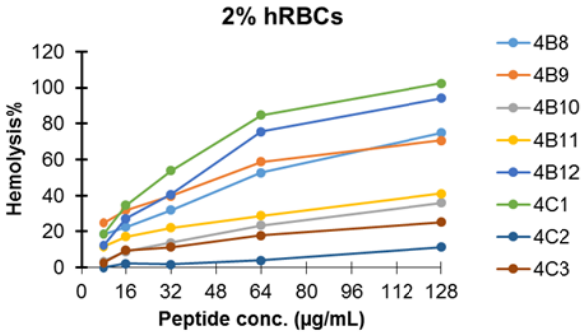

Fig. 3 — The effect of the model peptides (4th set) on hemolysis of hRBCs

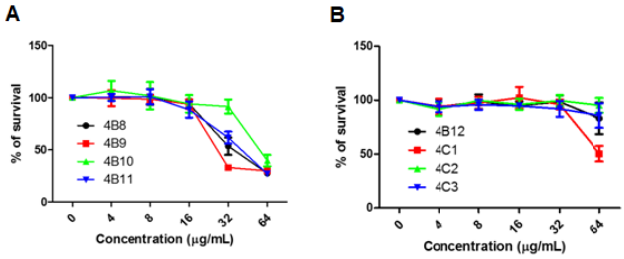

Fig. 4 — The peptide's cellular toxicity against the HEPG2 cell line generated from the liver

Membrane depolarization

The alteration of the membrane potential by the membrane-active agents is established[15]. Compared to the 1% Triton-100, the peptide showed considerably similar *S. aureus* MW2 membrane depolarization, even at 8 µg/mL (Fig. 5A) At 60 min, both the peptides 4B10 and 4B11 were seen having similar depolarizing capabilities although the antimicrobial activity of 4B11 is 4 × 4B11 against *S. aureus* MW2. It is worth mentioning that both peptides exhibit comparable sequences except for the carboxyl-terminal, one with an open acid and amide end.

Propidium iodide-based membrane permeability

Propidium iodide builds up in the cytoplasm and is an impermeable dye. However, the peptide-membrane contact causes pore development, which permits the dye to enter cells, combine with DNA, and intensify fluorescence. The 4B11 peptide, at 32 µg/mL, demonstrated comparable outcomes when it came to *S. aureus* MW2 (Fig. 5B). About 50 min into the incubation period, there is a 100% bacterial permeabilization, indicating a pore-like or even carpet-like mode of action.

Kinetic killing of the persistant cells against *S. aureus*

Activity of the most effective peptide, 4B11, on *S. aureus* MW2 persister cells was explored through membrane permeabilization and killing kinetics of the

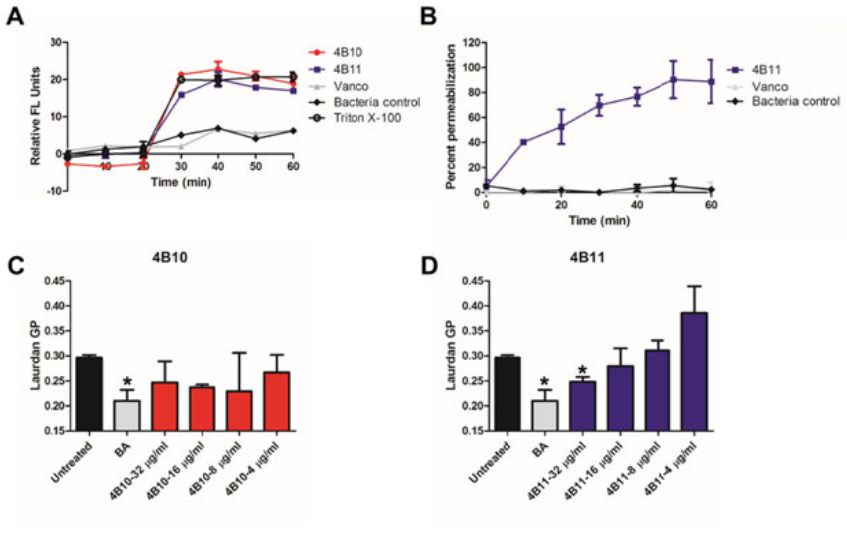

Fig. 5 — The proposed peptides' interaction with the membrane of *S. aureus* MW2. (A) The bacterial surface membrane depolarizes when treated with a peptide and an antibiotic at a concentration of 8 µg/mL; (B) Propidium iodide membrane permeability serves as a marker for the pore formation mechanism; (C and D) The membrane fluidity change caused by the interactions of peptides 4B10 and 4B11, respectively, with bacterial cells is measured using Laurdan GP plots. A control membrane fluidizer with a 50 mM concentration is benzoyl alcohol. (*) A student t-test was used to calculate significance ($P > 0.05$)

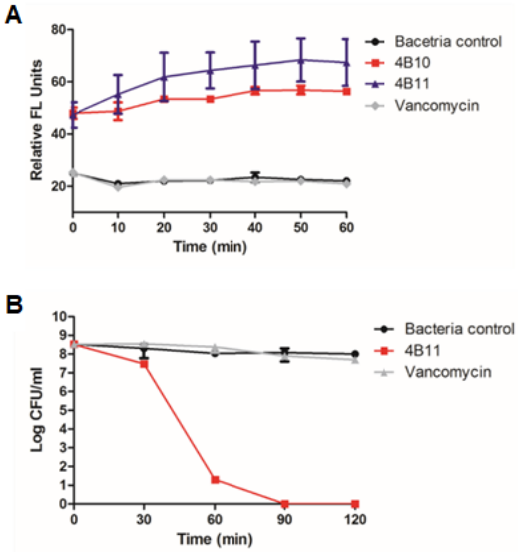

Fig. 6 — Action of the best peptide 4B11 on *S. aureus* MW2 persister cells. (A) A time-dependent plot of SYTOX-based membrane permeabilization of the persister cells upon peptide treatment and vancomycin at 32 µg/mL; and (B) Kinetic killing of the persister cells treated with 10× MIC of peptide and vancomycin

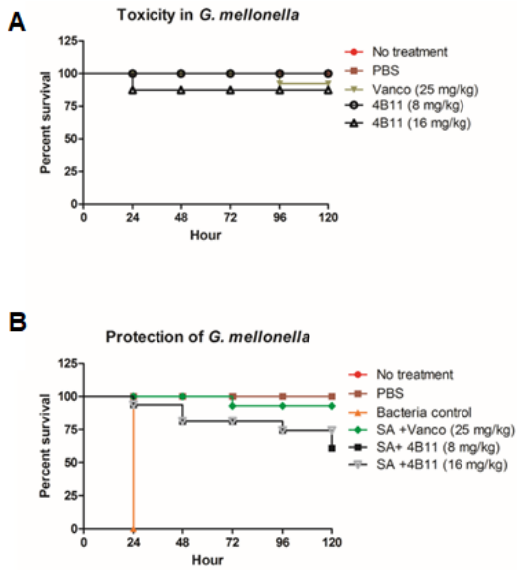

Fig. 7 — The proposed peptides' *in vivo* activity in a *G. mellonella* model. (A) The peptide's toxicity throughout the entire animal model. Peptides were employed in two doses: 8 mg/kg and 16 mg/kg. Vancomycin was used as a control at a dosage of 25 mg/kg; and (B) The wax moth larvae were safeguarded against MRSA infection by the antibiotic and peptide. The toxicity and the real protective effects of the medicines were compared using similar concentrations

bacteria. Figure 6A indicates the SYTOX-based membrane permeabilization assay. Peptide 4B11 treatment (32 µg/mL) produced a fast and time-dependent increase in fluorescence, reflecting extensive membrane disruption. Vancomycin treatment at the same level had little effect on membrane integrity, similar to the nontreated bacterial control. These results indicate that 4B11 is bactericidal by causing direct disruption of bacterial membranes, a mechanism of action different from vancomycin.

The bactericidal kinetics versus persister cells are presented in (Fig 6B). 10× MIC of 4B11 treatment resulted in a significant decrease in the number of viable cells, with close to complete elimination of persister cells within 90 min. Vancomycin did not decrease CFU counts during the same time frame, with levels close to the untreated control. This establishes that 4B11 is extremely potent in killing persister cells, which are generally resistant to conventional antibiotics. Cumulatively, these findings emphasize the significant anti-persister activity of 4B11 through the action of rapid membrane permeabilization and bactericidal activity. This activity is especially needed to fight recurrent and chronic infections due to multidrug-resistant *S. aureus*, in which traditional antibiotics fail.

In vivo* protection assay on *Galleria mellonella

In a wax moth *in vivo* model, the peptide 4B11 was found to be very effective (Fig. 7). The bacterial group receiving only infections was found to be 100% dead by the next day of observation (after 24 h). Interestingly, upon treatment with the peptide at 8 mg/kg and 16 mg/kg, protection of 60% and 75% respectively was observed for MRSA-treated larvae even after 5 days. At similar concentrations of the peptide injected alone, it did not show any toxicity at 8 mg/kg and a slight toxicity of 12.5% at 16 mg/kg. The positive control vancomycin was found to protect 90% of the larvae from death without showing any toxicity. Also, the PBS (vehicle) treated and no mortality was observed in the untreated controls that suggesting the model worked.

Conclusion

In conclusion, the investigation into the antibacterial activity of adhesion-derived peptides has provided valuable insights into their potential as promising agents for combating bacterial infections. The ability of these peptides to inhibit bacterial growth and disturb biofilm formation highlights their multifaceted mechanism of action. Moreover, the diverse sources from which adhesion-derived peptides can be derived, including natural proteins and synthetic mimetics, offer a wide range of possibilities for the design and optimization of novel antibacterial agents. The ongoing research in this

field holds promise for the development of next-generation antibiotics that can address the challenges posed by antibiotic resistance. These shorter fragments have an extended activity spectrum and low toxicities. Though, further experiments establishing stability, and *in vivo* efficacy in advanced animal models are required to establish these preliminary candidates for future drug development.

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Conflict of interest

Both the authors declare no conflict of interest.

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