

## Original Article

# The Antibacterial Effects of Antimicrobial Peptides OP-145 against Clinically Isolated Multi-Resistant Strains

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**SUMMARY:** OP-145 is a synthetic antimicrobial peptide developed from the human cathelicidin LL-37. The purpose of this investigation was to evaluate the effect of the antimicrobial peptide OP-145 against clinically isolated drug-resistant strains. Ten methicillin-resistant *Staphylococcus aureus* (MRSA) strains were obtained from our hospital's clinical inspection center, and the activity of OP-145 on growth and biofilm formation of these strains was evaluated by colony counts and scanning electron microscopy. The antimicrobial peptide OP-145 showed significant antibacterial activity against 9 MRSA strains. For the biofilm experiments, MRSA counts in the biofilms decreased significantly after 24 h ( $P < 0.05$ ). OP-145 strongly reduced growth and biofilm formation of clinically isolated drug-resistant strains in vitro, and the use of this class of antimicrobial agents may be an important new approach in controlling bacterial infections.

## INTRODUCTION

With the development of modern medicine and the use of antibiotics, an increasing trend of nosocomial infections has been observed (1). Moreover, with increasing bacterial resistance, there is also an increased risk of treatment failure, recurrent infections, and death (2).

The misuse of antimicrobials is a major cause of increased bacterial resistance in hospitals (3), which has led to an increased prevalence in drug-resistant strains (4). Thus, the proper choice of antimicrobial agents is of great importance in combating drug-resistant bacteria (5). Antimicrobial peptides (AMPs) are one of the types of antimicrobial agents used to treat infection diseases (6). Natural antibacterial peptides have a wide antimicrobial spectrum (7), particularly with the ability to eradicate multidrug-resistant bacteria (8), and antibacterial peptides exhibit good thermal stability and solubility in water. One of the best-studied AMP is human LL-37, which is the only cathelicidin peptide found in humans. Studies have shown that LL-37 inhibits growth and biofilm formation of pathogenic bacteria (9, 10). As a synthetic antimicrobial peptide developed from the human cathelicidin LL-37, OP-145 exhibits strong antibacterial activities against pathogens, suggesting a novel method for treating infectious diseases (11). Although the relative efficacy of LL-37 in inhibiting the in vitro growth of various pathogenic bacteria has been determined (12), there is a lack of information on the activity of OP-145 against pathogenic bacteria, particularly drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA).

In this study, we investigated the antibacterial effects of the antimicrobial peptide OP-145 on MRSA. We measured the effects of OP-145 on biofilm formation of MRSA and aimed to verify the effects of OP-145 in the treatment of infectious diseases in vivo.

## MATERIALS AND METHODS

**Peptide:** OP-145 (acetyl-IGKEFKRIVERIKRFLRELVRPLR-amide) peptide was synthesized by Shanghai Apeptide Co. Ltd (Shanghai, China). OP-145 was purified by high-performance liquid chromatography, the identity of the peptide was confirmed by SDS-PAGE, and its purity ( $> 95\%$ ) and mass were confirmed by electrospray ionization mass spectrometry (13).

**Strains and culture conditions:** Ten MRSA strains were obtained from our hospital's clinical inspection center. All isolates were sent to the CDC for identification, testing, and storage by the methods of Klevens et al (14). The strain *Staphylococcus aureus* (*S. aureus*) 99,308 was used as the reference strain in this study. The *S. aureus* strains used to screen for antibacterial activity were cultured overnight in brain heart infusion broth (BHI, Difco Laboratories Inc. Detroit, MI, USA) at 37 °C with gentle agitation and maintained on blood agar plates (bioMérieux, Marcy-l'Étoile, France).

**Interference test:** OP-145 was diluted to 1 mg/mL in phosphate buffered saline (PBS). *S. aureus* clinical isolates were cultured on Columbia sheep blood agar under microaerophilic conditions at 37 °C for 24 h. After incubation, the *S. aureus* culture concentration was adjusted to 0.1 McFarland standard ( $3 \times 10^7$  CFU/mL) with PBS using a Densicheck analyzer (bioMérieux). OP-145 (500 µL) was mixed with 10 mL of BHI agar in Petri dishes. Finally, 100 µL of *S. aureus* cultures was evenly spread on BHI plates and incubated under microaerophilic conditions at 37 °C for 24 h. PBS (100 µL) was used as the blank control.

After the 24-h incubation period, the colonies were counted using image analysis software Image Pro (Media

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Cybernetics, Inc. Rockville, MD, USA).

**Biofilm formation:** For this assay, 5 of the 10 clinically isolated *S. aureus* strains were randomly selected as test strains. The selected strains were cultured on Columbia sheep blood agar under microaerophilic conditions at 37 °C for 24 h and were then diluted in PBS at a concentration of 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/mL). The bacterial solutions were transferred to sterile 9-cm Petri dishes with 10 mL BHI broth, and 0.1 mL OP-145 suspension (0.1 mg/mL) was added to the Petri dishes with a cover glass. The Petri dishes were incubated under microaerophilic conditions at 37 °C for 24 h. Biofilm formation was examined after 24 h of incubation (15).

**Scanning electron microscopy (SEM) of biofilms:** After 24 h of incubation, the cover glasses were fixed with 2.5% glutaraldehyde at 4 °C for 1 h and were observed under a SEM (Hitachi SU-70, Hitachi High-Technology Corp. Tokyo, Japan) in high-vacuum mode at 3 kV.

**Statistical analysis :** All experiments were performed 3 times and expressed as mean  $\pm$  SD. SPSS 14.0 software for Windows (IBM, New York, NY, USA) was used for data analysis. Data were analyzed for statistical significance using Student's test.  $P < 0.05$  was considered statistically significant.

## RESULTS

**Interference test:** Among the 10 clinically isolated *S. aureus* strains, OP-145 showed significant antibacterial activity against 9 strains ( $P < 0.05$ ; Fig. 1). OP-145 showed no significant antibacterial effect against strain 9 ( $P > 0.05$ , Fig. 1). The response of the *S. aureus* strains to OP-145 and PBS loaded onto disks is shown in Fig. 2.

**Inhibition of biofilm formation:** After 24 h of biofilm formation, the number of bacteria in the biofilm formed by strains 1, 3, 4, and 5 decreased significantly compared to those in the control group ( $P < 0.05$ ; Fig. 3), whereas there was no obvious difference in the bacterial count in the biofilm of strain 2 ( $P > 0.05$ , Fig. 3). The images of SEM are shown in Fig. 4.

## DISCUSSION

Antimicrobial peptides (AMP) are small peptides that exist widely in nature (16) ,and form an important part

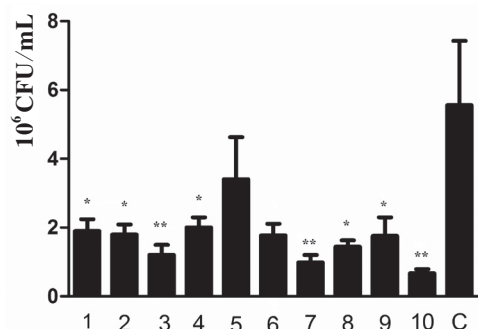


Fig.1 Viable *S. aureus* cells in the presence of OP-145 or PBS (1-10, isolated *S. aureus* strain No.1-10; C, control.), \*  $P < 0.05$ , \*\*  $P < 0.01$  compared with PBS controls (paired-t test).

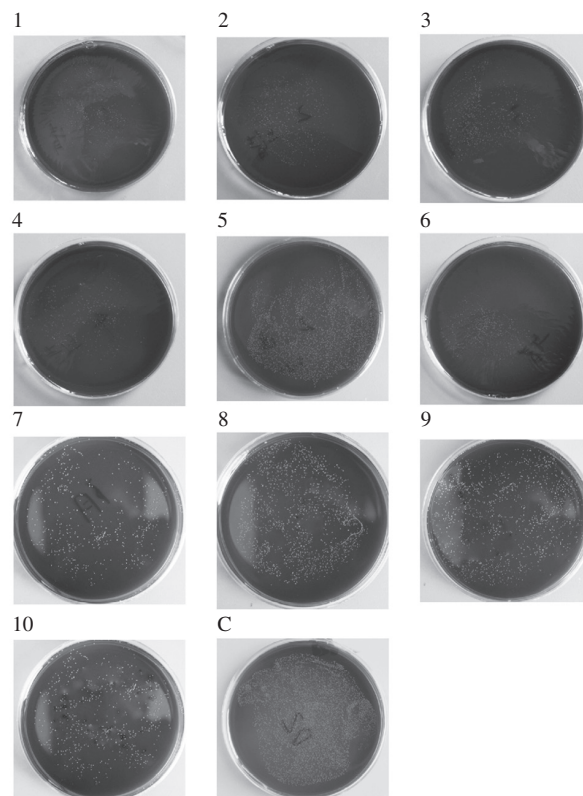


Fig. 2. Response of OP-145 and PBS with disks containing 1ml of OP-145 solution and PBS (1-5, isolated *S. aureus* strain No.1-5; C, control.).

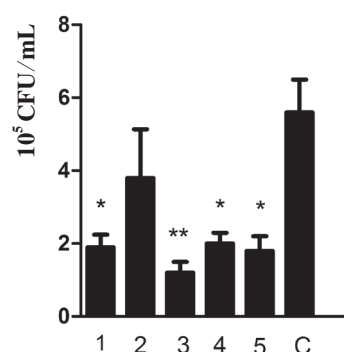


Fig. 3. Viable *S. aureus* cells in the biofilms in the presence of OP-145 or PBS (1-5, isolated *S. aureus* strain No.1-5; C, control.), \*  $P < 0.05$ , \*\*  $P < 0.01$  compared with PBS controls (paired-t test).

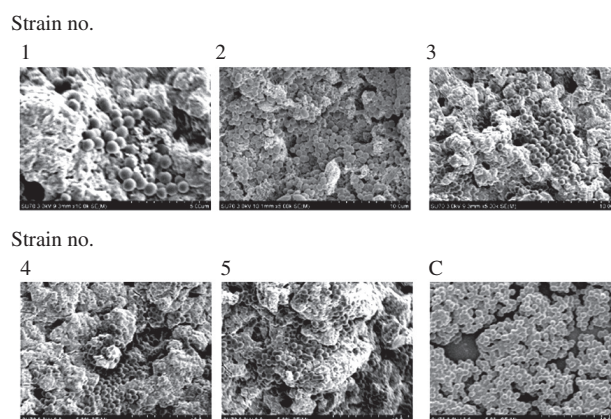


Fig. 4. SEM images of the biofilm of MRSA (1-10, isolated *S. aureus* strain No.1-10; C, control.).

of the innate immune system of an organism. Antimicrobial peptides have a wide range of activities against bacteria, fungi, parasites, viruses, and tumors (17). AMPs usually possess high efficiency, broad-spectrum, and potential antitumor activity, and can accelerate the body's immune defenses and wound healing ability of a body, making them an alternative candidate to traditional antibiotics to treat infectious diseases. Our study demonstrated that the AMP OP-145 can inhibit growth and biofilm formation of *S. aureus* in vitro, which is consistent with previous reports (11, 17).

*S. aureus* is the most important infectious disease pathogen (18), and invasive *S. aureus* infections are often lethal (19). In the 1940s, penicillin was used to treat *S. aureus* infections; however, it was not long before penicillin-resistant strains such as MRSA evolved (20). MRSA presence was then observed in many hospitals all over the world. It is reported that the MRSA acquisition rate was increased significantly when an outbreak of severe acute respiratory syndrome (SARS) occurred in China from 12 March to 31 May 2003(21).

This increase in the rate of MRSA cases suggests that the last line of defense for the treatment of MRSA seems be on the verge of collapse. MRSA has been well-studied in recent years owing to its heterogeneity and multiple drug resistance.

Moreover, any additional potential mechanisms of action of AMPs have not yet been thoroughly studied. Therefore, further studies on the antibacterial effects of AMPs using in vivo animal models are still necessary to fully understand the role AMPs may play in the prevention of implant-associated infections. In the meantime, our study serves to confirm the short-term effects of AMPs on clinically relevant strains; however, further studies are required to determine their long-term effects.

**Conclusion:** The antimicrobial peptide OP-145 coating holds promise for further clinical development as an alternative to coatings that release conventional antibiotics associated with the development of resistance. In addition, the present coating has the potential for rapid translation in humans, particularly because all compounds present in the coating, including OP-145, have already been approved for human use.

**Conflict of interest** None to declare.

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