Effects of the antimicrobial peptide L12 against multidrug-resistant *Staphylococcus aureus*

FU XIONG[1], XIAOTIAN DAI[1], YU-XUE LI[2], RUI WEI[3], LI AN[4], YAJUAN WANG[4] and ZHENHONG CHEN[4]

[1]Department of Pulmonology, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038; [2]Department of Respiratory Medicine, The First Hospital of Shijiazhuang, Shijiazhuang, Hebei 050011; [3]Department of Respiratory Medicine, North China University of Science and Technology of Clinical Medicine, Tangshan, Hebei 063000; [4]Department of Nanlou Pulmonology and National Clinical Research Center of Geriatrics Disease, Chinese People's Liberation Army General Hospital, Beijing 100853, P.R. China

Received April 19, 2018; Accepted December 3, 2018

DOI: 10.3892/mmr.2019.9988

Abstract. Methicillin-resistant *Staphylococcus aureus* (*S. aureus*; MRSA) is one of the most common bacterial pathogens and MRSA infections are characterized by high mortality rates. Antimicrobial peptides are considered one of the most promising drugs for the treatment of resistant strains of *S. aureus*. The present study aimed to examine the antimicrobial activity of L12 against numerous bacterial species using the broth microdilution method. Furthermore, the synergistic effect of L12 combined with various antibacterial drugs was tested, and its antibacterial mechanism was investigated by a checkerboard assay. The alterations in bacterial morphology were detected by electron microscopy, and biofilm formation and removal were tested by crystal violet staining. The present results suggested that L12 affected the growth of gram-positive strains, particularly *S. aureus*. Electron microscopy analysis suggested that L12 may target the cell membrane, and L12 increased the antibacterial activity of vancomycin and levofloxacin, exerting a synergistic effect. However, the minimal inhibitory concentrations (MICs) of L12 were not correlated with antibiotic resistance, the strains resistant to more antibiotics were not more resistant to L12. A sub-MIC of L12 was able to inhibit biofilm formation in a dose-dependent manner; however, concentrations of L12 ≤10 times the MIC were not sufficient to degrade previously formed biofilm. Collectively, the present study suggested that L12 may represent a novel potential therapeutic molecule for the treatment of *S. aureus* infections.

Correspondence to: Professor Zhenhong Chen, Department of Nanlou Pulmonology and National Clinical Research Center of Geriatrics Disease, Chinese People's Liberation Army General Hospital, 28 Fuxing Road, Haidian, Beijing 100853, P.R. China
E-mail: zhenhong_chen2013@163.com

Key words: methicillin-resistant *Staphylococcus aureus*, antimicrobial peptide, L12, resistance, synergistic effect

Introduction

Staphylococcus aureus (*S. aureus*) is the most common bacterial pathogen, and due to the increase of drug-resistant *S. aureus* strains, *S. aureus* infection remains a public health threat worldwide. Methicillin-resistant *S. aureus* (MRSA) strains exhibit a multidrug-resistant phenotype and the available antimicrobial treatments are ineffective (1). MRSA infections account for ~50% of nosocomial and community-associated staphylococcal infections (2). MRSA infections are characterized by high mortality rates and prolonged hospitalization, thus increasing the costs of health care. At present, to the best of the authors' knowledge, there is no effective treatment for MRSA infection, thus it is necessary to identify novel molecules targeting MRSA strains. Antimicrobial peptides (AMPs) are considered among the most promising drugs against resistant strains (3).

AMPs are synthesized by multicellular organisms as a defense mechanism against pathogenic microbes (4,5). Previous studies demonstrated that AMPs exhibit antimicrobial and anticancer activities, and serve as regulators of the innate immune system (6-8). The majority of natural cationic peptides are characterized by low biological activity or by high toxicity, and these peptides require modification in order to achieve high and broad-spectrum activity without toxicity (9). In addition, the potency of synthetic peptides is identical to that of natural peptides, and it is possible to produce large quantities of highly pure AMPs ready to be used in clinical applications (10). A 66-amino acid peptide was designed in the laboratory at the Department of Nanlou Pulmonology & National Clinical Research Center of Geriatrics Disease, Chinese People's Liberation Army General Hospital and derived from LCT-EF128 enterocin (11). Based on the 66-amino-acid peptide, three AMPs composed of 9-12 amino acids were designed and their antimicrobial activities against gram-positive and gram-negative strains were examined *in vitro*. The present results suggested that L12 is able to target gram-positive strains, particularly *S. aureus*. In addition, the synergistic action of L12 combined with various antibacterial drugs was tested, and its antibacterial mechanism was investigated.

Materials and methods

Bacterial strains and antibiotics. The bacteria selected in the present study were gram-positive and gram-negative isolates, and common clinical and standard strains (Table I). The gram positive clinical isolates (*S. aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Enterococcus faecium*) and gram negative clinical isolates (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*) were collected from The Southwest Hospital (Chongqing, China) and are not commercially available. The standard strains (Vichita, N315, FDA Strain PCI 1200, RP62A, Boston 41501 and FDA strain Seattle 1946) were purchased from China Center for Type Culture Collection (Wuhan, China) and stored in the laboratory of the Pulmonology Department of Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China. Oxacillin and linezolid were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The following antibiotics were purchased from The National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China): erythromycin, tetracycline, ceftazidime, levofloxacin, gentamycin and vancomycin. The antibiotics were diluted in water or the recommended solvent to obtain a working concentration. L12, whose structure is presented in Fig. 1, was purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

Analysis of antibacterial activity. The minimal inhibitory concentrations (MICs) of L12 and various antibiotics were calculated using the broth microdilution method (12) for each type of strain, according to the guidelines of The Clinical and Laboratory Standards Institute (2015) (13). The MIC for each drug was set as the lowest concentration required to inhibit bacterial growth. *S. aureus* (American Type Culture Collection, Manassas, VA, USA; cat. no. 29213) strain Wichita was used as a control strain.

Checkerboard assays. The checkerboard assay method was used to measure the combinatorial effects of L12 with multiple antibiotics (14). Solutions containing two drugs were prepared in 96-well plates. Serial two-fold dilutions were prepared for each column, relative to drug 'A', and for each row, relative to drug 'B'. The starting concentration was two times the MIC for each drug. Bacterial suspensions at the mid-log phase of growth (1-5x10^5 Cfu/ml) were added to the plates, and the plates were subsequently incubated at 37˚C for 24 h. The effects of the combinations were analyzed by calculating the fractional inhibitory concentration index (FICI) of each combination as follows: ($MIC_{drug'A'}$ in combination)/($MIC_{drug'A'}$ alone)+($MIC_{drug'B'}$ in combination)/($MIC_{drug'B'}$ alone). The effect was considered synergistic if the FICI was ≤0.5, additive if the FICI was >0.5 and ≤4.0, and antagonistic if the FICI was >4.0 (15).

Time-kill curves of various antibiotics alone and in combination with L12. Freshly prepared colonies of *S. aureus* were suspended in tryptic soy broth (TSB) medium (Solarbio Science and Technology Co., Ltd., Beijing, China) and incubated at 37˚C in a shaker at 180 rpm for 18 h. The cultures were diluted to 5x10^5 Cfu/ml with TSB to obtain a final volume of 10 ml. Vancomycin and L12 alone or in combination were added to the prepared bacterial suspensions to meet the MIC. The negative control was not treated with any drugs. The treated samples were incubated at 37˚C in a shaker at 180 rpm, and bacterial counts were measured at 0, 15, 30, 60, 120, 240, 360, 480, 720 and 1,440 min. The results were expressed in Cfu/ml on a logarithmic scale. The limit of detection was defined as 100 Cfu/ml and lower bacterial numbers were considered not detectable.

Transmission electron microscope (TEM) examination. The standard control *S. aureus* strain Wichita was treated with or without L12 at the MIC in TSB medium and subsequently incubated at 37˚C in a shaker at 180 rpm for 30 min. The cultures were centrifuged at 7,620 x g for 15 min prior to harvesting. The bacteria were subsequently fixed in 2.5% glutaraldehyde overnight at 4˚C. The samples were washed three times with PBS and incubated in 1% osmium tetraoxide for 2 h at 4˚C. Following washing with water, the samples were dehydrated with an acetone series (50, 70, 90 and 100%) for 15 min at each concentration. The samples were subsequently embedded in epoxy resin at 70˚C for ~9 h, cut into ultrathin (60 nm) sections, and stained with uranyl acetate and lead citrate for 15 min respectively at room temperature prior to examination. Each specimen was examined using a TEM (H-600; Hitachi, Ltd., Tokyo, Japan; magnification, x50,000).

Scanning electron microscope (SEM) examination. The standard control *S. aureus* strain Wichita was treated with or without L12 at the MIC in TSB medium and incubated at 37˚C in a shaker at 180 rpm for 30 min. The solutions were centrifuged at 7,620 x g for 2 min and washed twice with PBS. The bacteria were resuspended in PBS and subsequently added to polylysine-treated 8-mm cover glasses. The samples were dried at room temperature and fixed with 2.5% glutaraldehyde overnight at 4˚C. Following fixation, the samples were washed with 0.9% NaCl for 5 min. The samples were subsequently dehydrated at room temperature with increasing concentrations of ethanol (30, 50, 70, 95 and 100%), and the solvent was replaced with a tert-butanol series for 6 min following incubation with each concentration. The samples were point dried with CO_2, mounted on aluminum stubs and sputter-coated with gold. The samples were examined using an SEM (S-3400N II; Hitachi, Ltd.; magnification, x10,000).

Biofilm assay. Biofilm formation and removal were tested using the 96-well crystal violet staining method. An overnight culture of *S. aureus* was diluted 1:1,000 in TSB. The effect of L12 on the biofilm formation of *S. aureus* was tested as follows: 100 µl bacterial suspensions were mixed with 100 µl L12 at concentrations ranging between the MIC and MIC/32, resulting in final concentrations ranging between MIC/2 and MIC/64, or with 100 µl TSB. The resulting solutions were added to the 96-well plate. The effect of L12 on biofilm removal was evaluated as follows: 200 µl bacterial suspension was added to the 96-well plate, and the plate was incubated at 37˚C for 24 h. The medium was removed, and the wells were gently rinsed three times with sterile distilled water. Subsequently, 200 µl TSB was mixed with L12 at a concentration ranging between 2x MIC and 10x MIC, or without L12, and the resulting

Table I. MICs of L12 against gram-positive and gram-negative bacteria.

A, Gram-positive bacteria

Species	Strain	MIC of L12, μg/ml
Staphylococcus aureus		
	Wichita	8
	N315	8
	S26	8
	S28	16
	S29	4
Staphylococcus epidermidis		
	FDA strain PCI 1200	16
	RP62A	16
	48	16
	49	16
	50	32
Enterococcus faecalis		
	167	32
	169	16
	170	32
	171	32
	172	16
Enterococcus faecium		
	175	32
	176	4
	177	4
	178	16
	179	32

B, Gram-negative bacteria

Species	Strain	MIC of L12, μg/ml
Pseudomonas aeruginosa		
	Boston 41501	>256
	P496	>256
	P501	>256
	P544	>256
	P630	>256
Escherichia coli		
	FDA strain Seattle 1946	64
	E259	>256
	E260	>256
	E270	>256
	E282	>256
Klebsiella pneumoniae		
	1206	>256
	1220	>256
	1240	>256
	1248	>256
	1314	>256

Table I. Continued.

B, Gram-negative bacteria

Species	Strain	MIC of L12, μg/ml
Acinetobacter baumannii		
	aba658	>256
	aba659	>256
	aba660	>256
	aba661	>256
	aba662	>256

MIC, minimal inhibitory concentration.

solution was added to the 96-well plate. Following incubation at 37˚C for 24 h, the media from the two experiments were removed, and the wells were gently rinsed three times with sterile distilled water. The plates were air-dried, stained with 1% crystal violet for 10 min at room temperature, rinsed three times with distilled water, and air-dried. Following drying, 100 μl acetic acid at 30% were added to each well. The biofilms were examined at 590 nm using a MicroELISA reader (Dynatech Laboratories, Inc., Alexandria, VA, USA). Each assay was performed in triplicate, and wells without biofilm were used as blank controls.

Statistical analysis. All the experiments were repeated independently three times. The bacterial biofilm data are presented as the mean ± standard deviation and were analyzed by one-way analysis of variance with the Least Significant Difference post hoc test, using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). The correlation between the MICs of L12 and antibiotic resistance was calculated by Spearman's correlation analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Antibacterial activity analysis. To investigate the antimicrobial effect of L12, five strains of each species of bacteria were selected. The MICs of L12 against gram-positive and gram-negative bacteria are presented in Table I. The MICs of L12 against gram-positive bacteria ranged between 4 and 32 μg/ml. However, the majority of MICs of L12 against gram-negative bacteria were >256 μg/ml. In the present study, L12 exhibited an increased effect on *S. aureus* compared with the other three types of gram-positive bacteria. Therefore, only *S. aureus* strains were analyzed in the following experiments. Since the present study aimed to investigate the potential of L12 in treating infections caused by resistant strains, 30 MRSA isolates (which are not commercially available) were selected. The MICs of L12 against MRSA strains ranged between 4 and 32 μg/ml. Specifically, the MICs relative to MRSA strains 11, 10, 5 and 4 were 4, 8, 16 and 32 μg/ml, respectively (data not shown). The antibiotic resistance of MRSA strains and the correlation between antibiotic resistance and

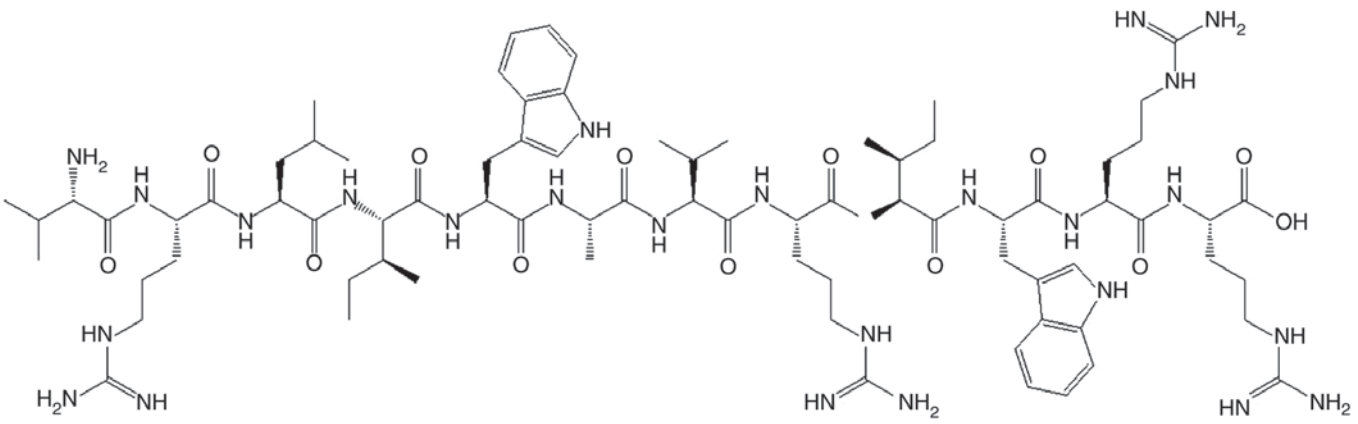

Figure 1. Structure of the antimicrobial peptide L12.

the MICs of L12 for all the 30 MRSA strains are presented in Table II. The MRSA strains were highly resistant to the majority of the antibiotics tested, the resistance rates of oxacillin, erythromycin, tetracycline, levofloxacin and gentamycin were 100.0% (30 strains), 83.3% (25 strains), 76.7% (23 strains), 76.7% (23 strains), 73.3% (22 strains) and 66.7% (20 strains). However, they were wholly sensitive to vancomycin and linezolid. A correlation analysis suggested that no correlation was present between the MICs of L12 and antibiotic resistance, the strains resistant to more antibiotics were not more resistant to L12.

Checkerboard assays. The synergistic effect of L12 with various antibiotics was examined by calculating the FICI. The MICs of the antibiotics against the majority of the MRSA strains were not obtained since the tested concentrations were not sufficient to inhibit the growth of the strains, thus five MRSA strains were selected for this experiment. The MICs of L12 against five MRSA strains ranged between 4 and 32 μg/ml, the MICs of vancomycin, levofloxacin, tetracycline, gentamycin and ceftazidime were 1-2, 16-64, 16-64, 64-128 and 32-128 μg/ml, respectively. As presented in Table III, L12 manifested a synergistic effect when used in combination with vancomycin and levofloxacin. However, L12 exhibited an additive effect when used in combination with gentamicin, tetracycline and ceftazidime.

Time-kill curves. The time-killing curves suggested that L12 induced bacterial death of *S. aureus* in 60 min, and the number of bacteria decreased continuously when treated with vancomycin. Furthermore, samples cotreated with L12 and vancomycin induced bacterial death in 30 min (Fig. 2).

TEM observations. TEM images of *S. aureus* cells treated with L12 are presented in Fig. 3. Untreated *S. aureus* cells exhibited an intact cellular architecture with a uniform cytoplasmic density, whereas, the cells treated with L12 presented severe damage, disrupted cell walls, leakage of the cytoplasmic contents and misshapen or fragmented cells.

SEM observations. SEM images of *S. aureus* cells that were treated with L12 are presented in Fig. 4. The untreated cells were round and plump, whereas, the majority of the cells treated with L12 were shriveled, and exhibited a disrupted cell wall and cell membrane.

Biofilm assay. The effect of L12 on biofilms of *S. aureus* is presented in Fig. 5. The MRSA isolate S37, which exhibits the strongest biofilm formation, and the standard strain N315, which is the biofilm-positive strain were selected. Compared with the control group, concentrations ranging between MIC/2 and MIC/16 of L12 significantly inhibited the biofilm formation of *S. aureus* strains N315 and S37 (Fig. 5A and C). However, concentrations ranging between 2x MIC and 10x MIC L12 did not degrade the previously formed biofilms of *S. aureus* strains N315 and S37 (Fig. 5B and D).

Discussion

To resolve the problem of antimicrobial resistance, multiple AMPs have been developed in recent years as alternative antibiotics (16-19), and a number of AMPs exhibited high antimicrobial efficacy and broad-spectrum activity (17,20). The present study suggested that L12 was effective in susceptibility tests against gram-positive bacteria, particularly *S. aureus*. The decreased effectiveness of L12 against other gram-positive bacteria may be due to differences in the cell wall composition. In particular, Enterococcus has a thick cell wall and grows at a slow rate (21), and these two features may be responsible for the observed poor antibacterial activity of L12. However, the molecular mechanism underlying Enterococcus resistance to L12 requires further investigation in future studies. The MIC of L12 against *S. aureus* was similar to traditional antibacterial drugs, and the antibacterial activity of L12 was comparable with other AMPs (22-25). Furthermore, the MICs of L12 exhibited no correlation with resistance to the tested antibiotics, the strains resistant to more antibiotics were not more resistant to L12, which suggested that L12 may be used to treat infections caused by MRSA strains. However, L12 exhibited little effect on gram-negative bacteria. The variable susceptibility between gram-negative and gram-positive bacteria was likely due to structural variations in the cell wall and the cell membrane. The cell membrane is the target of the majority of AMPs (26,27). Notably, according to SEM and TEM analysis, L12 may additionally target the cell membrane.

Table II. Resistance of methicillin-resistant *Staphylococcus aureus* strains to antibiotics, and the correlations between antibiotic resistance and L12 minimal inhibitory concentration.

Antibiotic	Resistance rate, %	Resistant strains, n	Spearman's correlation coefficient r	P-value
Oxacillin	100.0	30	0.060	0.751
Erythromycin	83.3	25	0.110	0.563
Tetracycline	76.7	23	-0.045	0.813
Ceftazidime	76.7	23	0.238	0.205
Levofloxacin	73.3	22	0.006	0.976
Gentamycin	66.7	20	-0.147	0.437
Vancomycin	0.0	0	0.342	0.064
Linezolid	0.0	0	0.173	0.362

Table III. Fractional inhibitory concentration indexes of L12 in combination with multiple antibiotics.

	Treatment				
Strain	Vancomycin+L12	Gentamycin+L12	Levofloxacin+L12	Tetracycline+L12	Ceftazidime+L12
S26	0.375	0.750	0.313	0.750	1.000
S29	0.531	0.531	0.531	0.750	2.000
S37	0.531	0.531	0.531	0.531	0.625
S47	0.281	0.750	0.500	1.000	0.531
S49	0.188	0.625	0.125	0.750	2.000

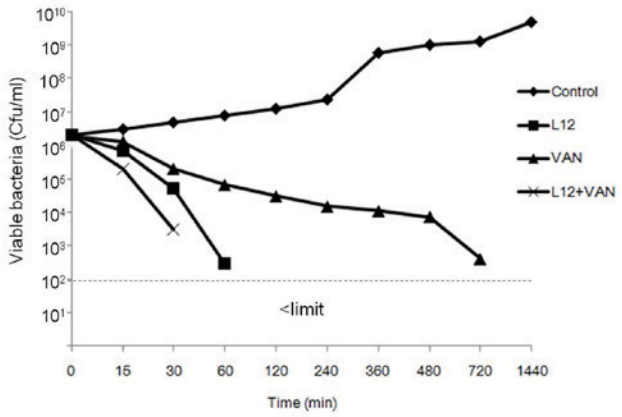

Figure 2. Synergistic effects of L12 and VAN. VAN, vancomycin.

In the present study, L12 exerted a synergistic effect with vancomycin and levofloxacin. The majority of AMPs exhibit synergistic effects with traditional antibacterial drugs (24,28), which may broaden the scope of their use. The additional antibacterial molecules tested presented additive effects. A previous study demonstrated that antibacterial drugs with similar molecular mechanisms or that target the same molecular components, exert improved synergistic effects compared with combinations of drugs with dissimilar function (17). Additionally, vancomycin and ceftazidime inhibit the cell wall synthesis of gram-positive bacteria by multiple mechanisms (29), which is hypothesized to be responsible for their synergistic effects with L12. According to the present study, ceftazidime was not among the most effective drugs against *S. aureus* and exhibited a decreased antibacterial effect compared with vancomycin. Since synergistic effects are due to similar antibacterial mechanism, further studies are necessary to investigate the antibacterial mechanisms of the tested compounds, thus improving the clinical use of these antimicrobial drugs.

S. aureus is considered one of the most important pathogens involved in biomaterial-associated infections. *S. aureus* adheres to the surfaces of medical devices to form biofilms resistant to the action of the immune system (30). The results of the present study suggested that sub-MIC of L12 may inhibit biofilm formation in a dose-dependent manner; however, concentrations ≤10x MIC were not sufficient to degrade previously formed biofilms. Biofilms are colonies of bacteria embedded by a self-secreted extracellular polymeric substance, and this extracellular polymeric substance serves as a selective barrier that allows the exchange of nutrients with the surroundings, preventing xenobiotics from entering the biofilm. L12 is a large molecule that is not able to penetrate the biofilm (31). The present results suggested that L12 may only serve as a preventive drug and not as a therapeutic drug for the treatment of biofilm infection.

A number of peptides have been developed as novel pharmaceuticals and tested in clinical trials (5,32) and these antimicrobial peptides possess promising potential. Further technological developments may allow researchers to precisely modify AMP sequences in order to modulate their antibacterial potency and cytotoxicity. Furthermore, it is crucial to identify novel strategies to shorten the peptide sequences to

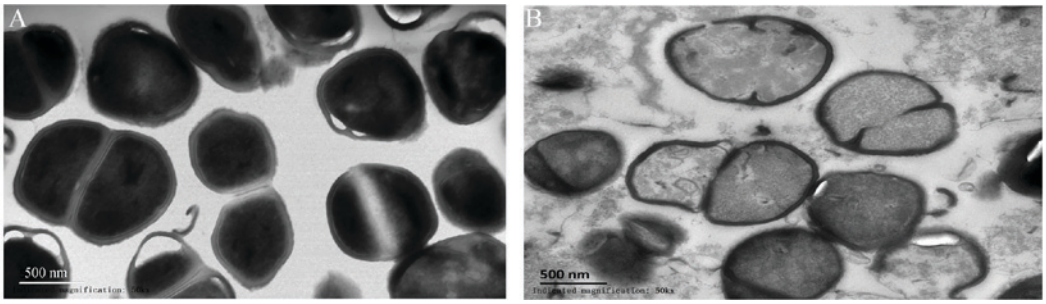

Figure 3. Effect of L12 on *S. aureus* by transmission electron microscopy analysis. (A) Untreated control. (B) *S. aureus* treated with L12 at the minimal inhibitory concentration. *S. aureus*, *Staphylococcus aureus*.

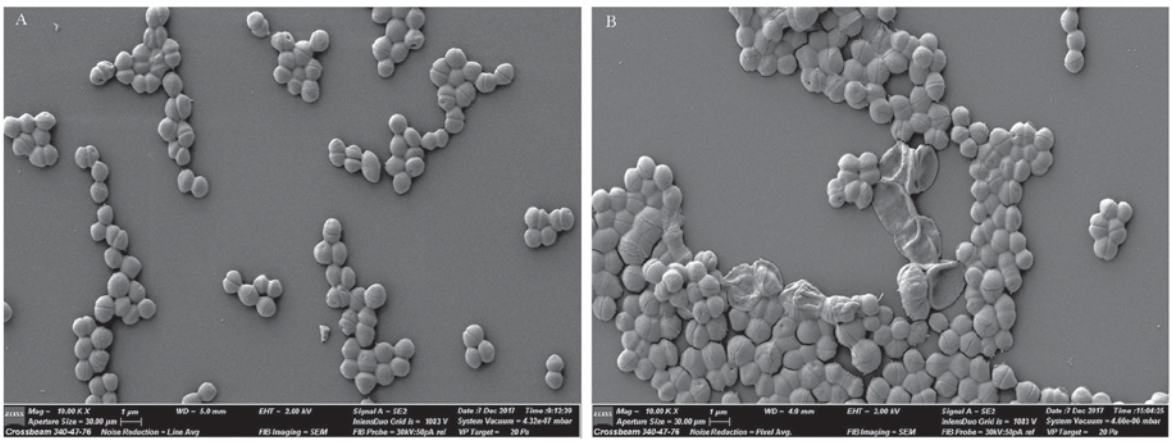

Figure 4. Effect of L12 on *S. aureus* by scanning electron microscopy analysis. (A) Untreated control. (B) *S. aureus* treated with L12 at the minimal inhibitory concentration. *S. aureus*, *Staphylococcus aureus*.

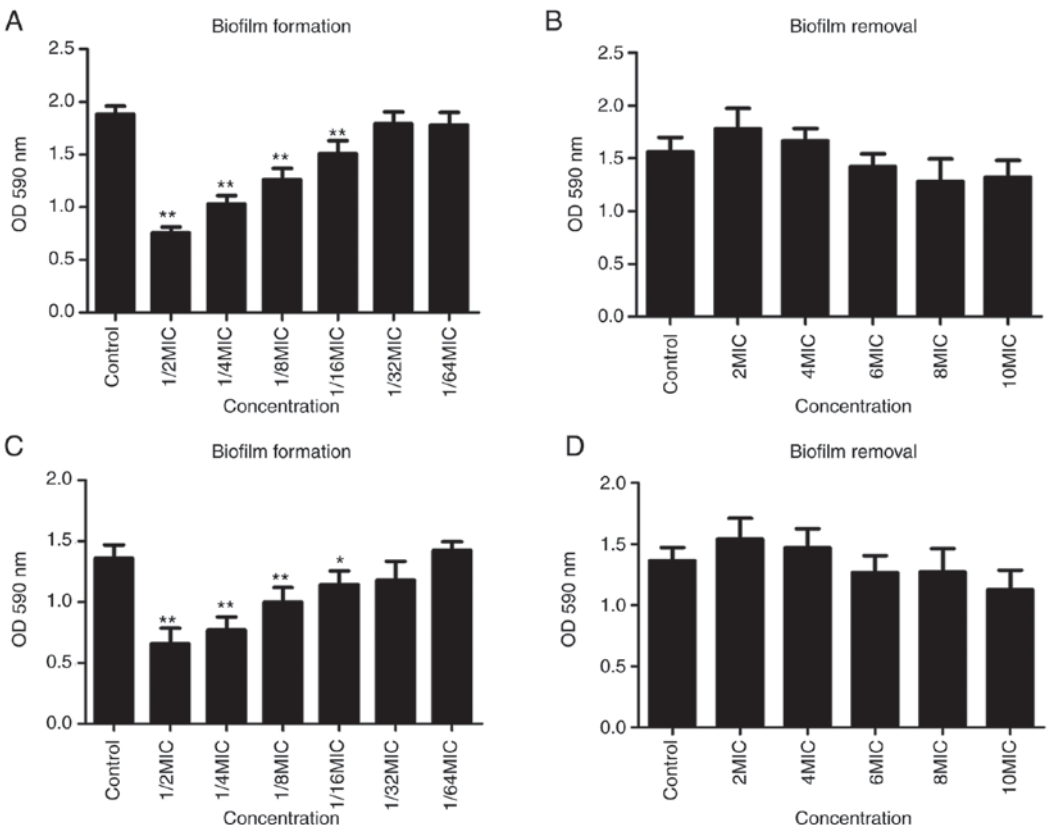

Figure 5. Effect of L12 on *Staphylococcus aureus* biofilm. (A) Biofilm formation of strain N315. (B) Biofilm removal of strain N315. (C) Biofilm formation of isolate S37. (D) Biofilm removal of isolate S37. *P<0.05, **P<0.01 vs. control. MIC, minimal inhibitory concentration; OD, optical density.

improve their clinical application (33). In the present study, it was demonstrated that L12, derived from enterocin, a molecule isolated from *E. faecium* (14), exerts antibacterial effects against *S. aureus* and biofilm production. The present study suggested that L12 may represent a potential therapeutic drug for the treatment of *S. aureus* infection.

Acknowledgements

Not applicable.

Funding

The present study was supported by The Nature Science Foundation of Beijing, China (grant no. 7152132).

Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Authors' contributions

FX designed the present study and wrote the manuscript. XD and YL performed the experiments. RW, LA and YW analyzed the data. ZC was involved in the design of the present study and provided financial support for the present study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Palavecino EL: Clinical, epidemiologic, and laboratory aspects of methicillin-resistant *Staphylococcus aureus* infections. Methods Mol Biol 1085: 1-24, 2014.
2. Bassetti M and Righi E: Multidrug-resistant bacteria: What is the threat? Hematology Am Soc Hematol Educ Program 2013: 428-432, 2013.
3. Kang HK, Kim C, Seo CH and Park Y: The therapeutic applications of antimicrobial peptides (AMPs): A patent review. J Microbiol 55: 1-12, 2017.
4. Zasloff M: Antimicrobial peptides of multicellular organisms. Nature 415: 389-395, 2002.
5. Fjell CD, Hiss JA, Hancock RE and Schneider G: Designing antimicrobial peptides: Form follows function. Nat Rev Drug Discov 11: 37-51, 2011.
6. Zasloff M: Antibiotic peptides as mediators of innate immunity. Curr Opin Immunol 4: 3-7, 1992.
7. Tripathi AK, Kumari T, Tandon A, Sayeed M, Afshan T, Kathuria M, Shukla PK, Mitra K and Ghosh JK: Selective phenylalanine to proline substitution for improved antimicrobial and anticancer activities of peptides designed on phenylalanine heptad repeat. Acta Biomater 57: 170-186, 2017.
8. Kazmirchuk T, Dick K, Burnside DJ, Barnes B, Moteshareie H, Hajikarimlou M, Omidi K, Ahmed D, Low A, Lettl C, *et al*: Designing anti-Zika virus peptides derived from predicted human-Zika virus protein-protein interactions. Comput Biol Chem 71: 180-187, 2017.
9. Xia X, Cheng L, Zhang S, Wang L and Hu J: The role of natural antimicrobial peptides during infection and chronic inflammation. Antonie Van Leeuwenhoek 111: 5-26, 2018.
10. Ciociola T, Giovati L, Conti S, Magliani W, Santinoli C and Polonelli L: Natural and synthetic peptides with antifungal activity. Future Med Chem 8: 1413-1433, 2016.
11. Chen Z, Chang D, Zou Y, Su L, Zhu Y, Fang X, Wang J, Guo Y, Zhao J, Li D, *et al*: Genome sequence of enterococcus faecium clinical isolate LCT-EF128. J Bacteriol 194: 4765, 2012.
12. Ouyang J, Sun F, Feng W, Sun Y, Qiu X, Xiong L, Liu Y and Chen Y: Quercetin is an effective inhibitor of quorum sensing, biofilm formation and virulence factors in *Pseudomonas aeruginosa*. J Appl Microbiol 120: 966-974, 2016.
13. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing; 25th Informational Supplement. CLSI document M100-S24. Clinical Laboratory Standards Institute, Wayne, PA, 2015.
14. Pankey GA and Ashcraft DS: In vitro synergy of ciprofloxacin and gatifloxacin against ciprofloxacin-resistant *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 49: 2959-2964, 2005.
15. Menegucci TC, Albiero J, Migliorini LB, Alves JL, Viana GF, Mazucheli J, Carrara-Marroni FE, Cardoso CL and Tognim MC: Strategies for the treatment of polymyxin B-resistant acinetobacter baumannii infections. Int J Antimicrob Agents 47: 380-385, 2016.
16. Wuerth KC, Falsafi R and Hancock REW: Synthetic host defense peptide IDR-1002 reduces inflammation in *Pseudomonas aeruginosa* lung infection. PLoS One 12: e0187565, 2017.
17. Wu X, Li Z, Li X, Tian Y, Fan Y, Yu C, Zhou B, Liu Y, Xiang R and Yang L: Synergistic effects of antimicrobial peptide DP7 combined with antibiotics against multidrug-resistant bacteria. Drug Des Devel Ther 11: 939-946, 2017.
18. Bechinger B and Gorr SU: Antimicrobial peptides: Mechanisms of action and resistance. J Dent Res 96: 254-260, 2017.
19. Mangoni ML, McDermott AM and Zasloff M: Antimicrobial peptides and wound healing: Biological and therapeutic considerations. Exp Dermatol 25: 167-173, 2016.
20. Ge Y, MacDonald DL, Holroyd KJ, Thornsberry C, Wexler H and Zasloff M: In vitro antibacterial properties of pexiganan, an analog of magainin. Antimicrob Agents Chemother 43: 782-788, 1999.
21. Wellinghausen N, Chatterjee I, Berger A, Niederfuehr A, Proctor RA and Kahl BC: Characterization of clinical Enterococcus faecalis small-colony variants. J Clin Microbiol 47: 2802-2811, 2009.
22. Schillaci D, Cusimano MG, Spinello A, Barone G, Russo D, Vitale M, Parrinello D and Arizza V: Paracentrin 1, a synthetic antimicrobial peptide from the sea-urchin paracentrotus lividus, interferes with *Staphylococcal* and *Pseudomonas aeruginosa* biofilm formation. AMB Express 4: 78, 2014.
23. Gottschalk S, Gottlieb CT, Vestergaard M, Hansen PR, Gram L, Ingmer H and Thomsen LE: Amphibian antimicrobial peptide fallaxin analogue FL9 affects virulence gene expression and DNA replication in *Staphylococcus aureus*. J Med Microbiol 64: 1504-1513, 2015.
24. Hu Y, Liu A, Vaudrey J, Vaiciunaite B, Moigboi C, McTavish SM, Kearns A and Coates A: Combinations of beta-lactam or aminoglycoside antibiotics with plectasin are synergistic against methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. PLoS One 10: e0117664, 2015.
25. Wang Z, Zhang L, Wang J, Wei D, Shi B and Shan A: Synergistic interaction of PMAP-36 and PRW4 with aminoglycoside antibiotics and their antibacterial mechanism. World J Microbiol Biotechnol 30: 3121-3128, 2014.
26. Sani MA and Separovic F: Antimicrobial peptide structures: From model membranes to live cells. Chemistry 24: 286-291, 2018.
27. Marin-Medina N, Ramirez DA, Trier S and Leidy C: Mechanical properties that influence antimicrobial peptide activity in lipid membranes. Appl Microbiol Biotechnol 100: 10251-10263, 2016.
28. Amani J, Barjini KA, Moghaddam MM and Asadi A: In vitro synergistic effect of the CM11 antimicrobial peptide in combination with common antibiotics against clinical isolates of six species of multidrug-resistant pathogenic bacteria. Protein Pept Lett 22: 940-951, 2015.

29. Karampatakis V, Papanikolaou T, Giannousis M, Goulas A, Mandraveli K, Kilmpasani M, Alexiou-Daniel S and Mirtsou-Fidani V: Stability and antibacterial potency of ceftazidime and vancomycin eyedrops reconstituted in BSS against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Acta Ophthalmol 87: 555-558, 2009.
30. Montanaro L, Speziale P, Campoccia D, Ravaioli S, Cangini I, Pietrocola G, Giannini S and Arciola CR: Scenery of staphylococcus implant infections in orthopedics. Future Microbiol 6: 1329-1349, 2011.
31. Stewart EJ, Ganesan M, Younger JG and Solomon MJ: Artificial biofilms establish the role of matrix interactions in staphylococcal biofilm assembly and disassembly. Sci Rep 5: 13081, 2015.
32. Domingues MM, Inacio RG, Raimundo JM, Martins M, Castanho MA and Santos NC: Biophysical characterization of polymyxin B interaction with LPS aggregates and membrane model systems. Biopolymers 98: 338-344, 2012.
33. Haney EF, Mansour SC and Hancock RE: Antimicrobial peptides: An introduction. Methods Mol Biol 1548: 3-22, 2017.