SUPPLEMENTARY APPENDIX

Efficacy and Safety of PL-5 (Peceleganan) Spray for Wound Infections:
A Phase IIb Randomized Clinical Trial

Yating Wei, PhD1, Jun Wu, MD1, 2*, Yuxin Chen, PhD3*, Kunwu Fan, MD1, Xuming Yu, MD1, Xiaojian Li, MD2, Yaohua Zhao, MD3, Yi Li, MD4, Guozhong Lv, MD5, Guodong Song, MD5, Xinzhou Rong, MD5, Cai Lin, MD5, Haitao Wang, MD5, Xiaodong Chen, MD5, Pihong Zhang, MD5, Chunmao Han, MD4, Hongxu Zu, MD1, Wenjun Liu, MD1, Yi Zhang, MD1, Chang Liu, MD1, Yongtao Su, MD1, Baolin Zhang, MD2, Bingwei Sun, MD2, Lei Wang, MD2, Wen Lai, MD2, Jinhui Liu, MD2, Chengde Xia, MD2, Geng Ji, MD2, Feng Zhu, MD2, Jia’ao Yu, MD2, Akebaer.Ahemaiti MD2, Hu Dong, Msc3, Mingxia Chen, Msc3, on behalf of the PL-5 Investigators

TABLE OF CONTENTS

List of Investigators and Study Sites.................................................................1

In vitro tests of antibacterial activity................................................................3

Overview of Preclinical Pharmacokinetic Study..................................................15

Toxicity tests........................................................................................................16

Allergy test............................................................................................................19

Irritation test.......................................................................................................20

Phase I clinical trial............................................................................................21

Phase IIa clinical trial..........................................................................................23

Table S1. The Skin Infection Rating Scales (SIRS)..............................................25

Table S2. Inclusion and exclusion criteria of the phase IIb clinical trial.............26

Table S3. Demographic and clinical characteristics of the patients at baseline ..................................................27

Table S4. The SIRS evaluations before and after interventions........................28

Table S5. The results of bacteria cultures at baseline ........................................31

Table S6. The results of adverse event ..............................................................32
List of Investigators and Study Sites

Dr. Yating Wei, Shenzhen Second People’s Hospital, No. 3002, Sungang West Road, Futian District, Shenzhen, Guangdong Province, China 518035
Dr. Dr. Jun Wu, Shenzhen Second People’s Hospital, No. 3002, Sungang West Road, Futian District, Shenzhen, Guangdong Province, China 518035
Dr. Kunwu Fan, Shenzhen Second People’s Hospital, No. 3002, Sungang West Road, Futian District, Shenzhen, Guangdong Province, China 518035
Dr. Xuming Yu, Shenzhen Second People’s Hospital, No. 3002, Sungang West Road, Futian District, Shenzhen, Guangdong Province, China 518035
Dr. Xiaojian Li, Guangzhou Red Cross Hospital, No.396 ,Tongfu Middle Road, Guangzhou, Guangdong Province, China 510240
Dr. Yaohua Zhao, Jiangyin People’s Hospital, No.163 Shoushan Road, Jiangyin, Wuxi, Jiangsu Province, China 214499
Dr. Vi Li, Qinghai University Affiliated Hospital, No. 29 Tongren Road, Xining, Qinghai Province, China 810012
Dr. Guozhong Lv, Affiliated Hospital of Jiangnan University, No. 1000 He Feng Road, Wuxi, Jiangsu Province, China 214043
Dr. Guodong Song, Jinan Central Hospital, NO.105, Jiefang Road, Lixia District, Jinan, Shandong Province, China 250013
Dr. Xinzhou Rong, Guangzhou First people's Hospital, No.1, Panfu Road, Yuxiu District, Guangzhou, Guangdong Province, China 510180
Dr. Cai Lin, The First Affiliated Hospital of WMU, New District of the First Hospital of Wenyi Hospital, Nanbaixiang, Ouhai District, Wenzhou, Zhejiang Province, China 325015
Dr. Haitao Wang, Weihai Municipal Hospital, NO.70, Heping Road, Huancui District, Weihai, Shandong Province, China 264299
Dr. Xiaodong Chen, The First People's Hospital of Foshan, No. 81, North Lingnan Avenue, Chancheng District, Foshan, Guangdong Province, China 528010
Dr. Phong Zhang, Xiangya Hospital of Central South University, No.87, Xiangya Road, Changsha, Hunan Province, China 410008
Dr. Chunmao Han, The Second Affiliated Hospital Zhejiang University School of Medicine, NO.88, Jiefang Road, Shangcheng District, Hangzhou, China 310003
Dr. Hongxu Zu, The First Affiliated Hospital of Henan University of Science and Technology, NO.24, Jinghua Road, Jianxi District, Luoyang, Henan Province, China 450052
Dr. Wenjun Liu, The Second Affiliated Hospital of Kunming Medical University, No. 374, Dian Mian Road, Wuhua District, Kunming, Yunnan Province, China 650106
Dr. Yi Zhang, Affiliated Hospital of Nantong University, NO.20, Xisi Road, Nantong, Jiangsu Province, China 226006
Dr. Chang Liu, Affiliated Hospital of Jiangsu University, No.438 Jiefang Road, Zhenjiang, Jiangsu Province, China 212001
Dr. Yongtao Su, PKU care Luzhong Hospital, NO.65,Taigong Road, Linzi District, Zibo, Shandong Province, China 045099
Dr. Baolin Zhang, First Hospital of Shanxi Medical University, NO.85, Jiefang South Road, Yingzhe District, Taiyuan, Shanxi Province, China 030001
Dr. Bingwei Sun, Suzhou Municipal Hospital, NO.242, Guangji Road, Gusu District, Suzhou, Jiangsu Province, China 215008
Dr. Lei Wang, Zhongda Hospital Southeast University, No.87 Dingjiaqiao, Gulou District, Nanjing, Jiangsu Province, China 210009
Dr. Wen Lai, Guangdong Provincial People's Hospital, No. 106, Zhongshan 2nd Road, Yuxiu District, Guangzhou, Guangdong Province, China 510080
Dr. Jinhui Liu, Jilin Province People's Hospital, No. 1183 Gongnong Road, Chaoyang District, Changchun, Jilin Province, China 130021
Dr. Chengde Xia, Zhengzhou First People’s Hospital, NO.56, East Street, Huizu District, Guancheng, Zhengzhou, Henan Province, China 451475
Dr. Geng Ji, Taizhou People's Hospital, No.366 Taihu Road, Taizhou Medical High-tech Zone, Taizhou, Jiangsu Province, China 225399
Dr. Feng Zhu, Sir Run Run Hospital Nanjing Medical University, No. 109 Longmian Dadao, Jiangning District, Nanjing, Jiangsu Province, China 211112
Dr. Jia’ao Yu, The First Hospital of Jilin University, No.1 Xinmin Dajie, Chaoyang District, Changchun City, Jilin Province, China 130031
Dr. Akebaer Ahemaiti, The First Affiliated Hospital of Xinjiang Medical University, No. 137 Li Yu Shan Nan Lu, Urumqi, Xinjiang Uyghur Autonomous Region, China 830011
In vitro tests of antibacterial activity

Antibacterial and bactericidal effects of PL-5 against 13 strains in vitro (MIC and MBC determination)

1. Antibacterial and bactericidal effects of antimicrobial peptide PL-5 on Staphylococcus in vitro

The antibacterial and bactericidal effects of antimicrobial peptide PL-5 on 130 strains of methicillin resistant Staphylococcus aureus (MRSA), 67 strains of methicillin sensitive Staphylococcus aureus (MSSA), 121 strains of methicillin resistant coagulase negative Staphylococcus (MRSCNS) and 61 strains of methicillin sensitive coagulase negative Staphylococcus (MSSCNS) were determined using microdilution method and nutrient agar plate culture method, and vancomycin was used as the control drug.

The results showed that the MIC range of PL-5 to four kinds of Staphylococcus were 2–16 μg/ml, 2–16 μg/ml and 2–16 μg/ml; the geometric mean of MIC was 5.54 μg/ml, 2.43 μg/ml, 4.49 μg/ml and 4.48 μg/ml respectively; MIC50 were 4 μg/ml, 4 μg/ml, 4 μg/ml and 4 μg/ml respectively; MIC90 were 8 μg/ml, 8 μg/ml, 8 μg/ml and 8 μg/ml respectively. The cumulative antibacterial percentage showed that at the concentration of 16 μg/ml, PL-5 could inhibit the growth of four kinds of Staphylococcus. The MIC ranges of four staphylococci were 4 ~ > 64 μg/ml, 2 ~ > 64 μg/ml, 2 ~ > 64 μg/ml and 2 ~ > 64 μg/ml respectively; MBC50 were 8 μg/ml, 4 μg/ml, 8 μg/ml, and 8 μg/ml respectively; MBC90 is 32 μg/ml, 16 μg/ml, 64 μg/ml and 64 μg/ml. The cumulative bactericidal percentage of MRSA and MSSA at the concentration of 32 μg/ml exceeded 90%; while at the concentration of 64 μg/ml, the cumulative bactericidal percentage was more than 90%.

The above results showed that antimicrobial peptide PL-5 had strong antibacterial and bactericidal effects on the growth of four kinds of Staphylococcus. Considering the content of pure peptide to be 84.34%, the MIC range and MBC range of MRSA were 1.6868 ~ 13.4944 μg/ml and 3.3736 ~ > 53.9776 μg/ml respectively; MIC50 and MBC50 were 6.7472 μg/ml and 26.9888 μg/ml respectively; The MIC range and MBC range of MSSA were 1.6868 ~ 13.4944 μg/ml and 1.6868 ~ > 53.9776 μg/ml respectively, and MIC90 and MBC90 were 6.7472 μg/ml and 13.4944 μg/ml respectively; The MIC range and MBC range of MRSCNS were 1.6868 ~ 13.4944 μg/ml and 1.6868 ~ > 53.9776 μg/ml respectively; and MIC50 and MBC50 were 6.7472, respectively μ G / ml and 53.9776 μ g/ml ; The MIC range and MBC range of MSSCNS were 1.6868 ~ 13.4944 μg/ml and 1.6868 ~ > 53.9776 μg/ml respectively, and MIC90 and MBC90 were 6.7472 μg/ml and 53.9776 μg/ml respectively.

Antibacterial effect of antimicrobial peptide PL-5 and vancomycin on Staphylococcus aureus and coagulase negative Staphylococcus in vitro

| Sample | Bacteria Strains | Number of Strains | Strains of bacteria not growing under different drug concentrations (μg/ml) | MIC range (μg/ml) | MBC50 (μg/ml) | MBC90 (μg/ml) | MBC Geometric mean (μg/ml) |
|--------|-----------------|------------------|-------------------------------------------------|-----------------|---------------|---------------|-------------------------|
|        |                 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 1/2 | 1/4 | solvent | Culture medium |
| PL-5   | Staphylococcus aureus (ATCC 29213) | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 8 | 8 | 8 | 8 |
|        | Staphylococcus aureus (MRSA) | 130 | 130 | 130 | 130 | 124 | 70 | 5 | 0 | 0 | 0 | - | 0 | 130 | 2–16 | 4 | 8 | 5.54 |
|        | Staphylococcus aureus (MSSA) | 67 | 67 | 67 | 67 | 65 | 47 | 3 | 0 | 0 | 0 | - | 0 | 67 | 2–16 | 4 | 8 | 4.87 |
|        | Coagulase negative Staphylococcus (MRSCNS) | 121 | 121 | 121 | 121 | 117 | 90 | 15 | 0 | 0 | 0 | - | 0 | 121 | 2–16 | 4 | 8 | 4.49 |
|        | Coagulase negative Staphylococcus (MSSCNS) | 61 | 61 | 61 | 61 | 58 | 45 | 9 | 0 | 0 | 0 | - | 0 | 61 | 2–16 | 4 | 8 | 4.48 |
|        | Staphylococcus aureus (ATCC 29213) | 1 | - | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
|        | Staphylococcus aureus (MRSA) | 130 | - | 130 | 130 | 130 | 130 | 127 | 118 | 43 | 0 | 0 | 130 | 0.5–4 | 1 | 1 | 0.86 |
|        | Staphylococcus aureus (MSSA) | 67 | - | 67 | 67 | 67 | 67 | 63 | 59 | 26 | - | 0 | 67 | 0.5–4 | 1 | 2 | 0.87 |
|        | Coagulase negative Staphylococcus (MRSCNS) | 121 | - | 121 | 121 | 121 | 121 | 120 | 105 | 25 | 0 | 0 | 121 | 0.5–4 | 1 | 2 | 0.96 |
|        | Coagulase negative Staphylococcus (MSSCNS) | 61 | - | 61 | 61 | 61 | 61 | 58 | 45 | 15 | 0 | 0 | 61 | 0.5–4 | 1 | 2 | 1.05 |
**Bactericidal effect of antimicrobial peptide PL-5 and vancomycin on *Staphylococcus aureus* and coagulase negative *Staphylococcus in vitro***

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MBC<sub>cp</sub> (μg/ml) | MBC<sub>cp</sub> (μg/ml) |
|--------|-----------------|-------------------|---------------------------------|-----------------|-------------------|
| PL-5   | *Staphylococcus aureus* (ATCC 29213) | 1 | 100 | 100 | 95 | 54 | 4 | 0 | 0 | 0 | 0 | 2 | 4 |
|        | *Staphylococcus aureus* (MSSA) | 67 | 100 | 100 | 100 | 97 | 70 | 4 | 0 | 0 | 0 | 1 | 2 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 121 | 120 | 108 | 95 | 67 | 33 | 5 | 0 | 0 | 0 | 2 | 8 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 61 | 100 | 100 | 100 | 95 | 74 | 15 | 0 | 0 | 0 | - | 2 |
| Vanco- | *Staphylococcus aureus* (ATCC 29213) | 130 | 100 | 100 | 100 | 95 | 54 | 4 | 0 | 0 | 0 | 2 | 4 |
| mycin  | *Staphylococcus aureus* (MSSA) | 67 | 100 | 100 | 100 | 97 | 70 | 4 | 0 | 0 | 0 | 1 | 2 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 121 | 120 | 108 | 95 | 67 | 33 | 5 | 0 | 0 | 0 | 2 | 8 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 61 | 100 | 100 | 100 | 95 | 74 | 15 | 0 | 0 | 0 | - | 2 |

**Cumulative antibacterial percentage of antimicrobial peptide PL-5 and vancomycin on *Staphylococcus aureus* and coagulase negative *Staphylococcus in vitro***

| Sample | Bacteria Strains | Number of Strains | Cumulative antibacterial percentage of different concentrations (μg/ml) of tested drugs (%) | MBC<sub>cp</sub>/MIC<sub>cp</sub> | MBC<sub>cp</sub>/MIC<sub>cp</sub> |
|--------|-----------------|-------------------|---------------------------------|-----------------|-------------------|
| PL-5   | *Staphylococcus aureus* (ATCC 29213) | 130 | 100 | 100 | 100 | 95 | 54 | 4 | 0 | 0 | 0 | 2 | 4 |
|        | *Staphylococcus aureus* (MSSA) | 67 | 100 | 100 | 100 | 97 | 70 | 4 | 0 | 0 | 0 | 1 | 2 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 121 | 120 | 108 | 95 | 67 | 33 | 5 | 0 | 0 | 0 | 2 | 8 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 61 | 100 | 100 | 100 | 95 | 74 | 15 | 0 | 0 | 0 | - | 2 |
| Vanco- | *Staphylococcus aureus* (ATCC 29213) | 130 | 100 | 100 | 100 | 95 | 54 | 4 | 0 | 0 | 0 | 2 | 4 |
| mycin  | *Staphylococcus aureus* (MSSA) | 67 | 100 | 100 | 100 | 97 | 70 | 4 | 0 | 0 | 0 | 1 | 2 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 121 | 120 | 108 | 95 | 67 | 33 | 5 | 0 | 0 | 0 | 2 | 8 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 61 | 100 | 100 | 100 | 95 | 74 | 15 | 0 | 0 | 0 | - | 2 |

**Cumulative bactericidal percentage of antimicrobial peptide PL-5 and vancomycin on *Staphylococcus aureus* and coagulase negative *Staphylococcus in vitro***

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MBC<sub>cp</sub>/MIC<sub>cp</sub> | MBC<sub>cp</sub>/MIC<sub>cp</sub> |
|--------|-----------------|-------------------|---------------------------------|-----------------|-------------------|
| PL-5   | *Staphylococcus aureus* (ATCC 29213) | 130 | 100 | 100 | 100 | 95 | 54 | 4 | 0 | 0 | 0 | 2 | 4 |
|        | *Staphylococcus aureus* (MSSA) | 67 | 100 | 100 | 100 | 97 | 70 | 4 | 0 | 0 | 0 | 1 | 2 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 121 | 120 | 108 | 95 | 67 | 33 | 5 | 0 | 0 | 0 | 2 | 8 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 61 | 100 | 100 | 100 | 95 | 74 | 15 | 0 | 0 | 0 | - | 2 |
| Vanco- | *Staphylococcus aureus* (ATCC 29213) | 130 | 100 | 100 | 100 | 95 | 54 | 4 | 0 | 0 | 0 | 2 | 4 |
| mycin  | *Staphylococcus aureus* (MSSA) | 67 | 100 | 100 | 100 | 97 | 70 | 4 | 0 | 0 | 0 | 1 | 2 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 121 | 120 | 108 | 95 | 67 | 33 | 5 | 0 | 0 | 0 | 2 | 8 |
|        | Coagulase negative *Staphylococcus aureus* (MSSCNS) | 61 | 100 | 100 | 100 | 95 | 74 | 15 | 0 | 0 | 0 | - | 2 |
2. Antibacterial and bactericidal effects of antimicrobial peptide PL-5 on *Streptococcus pyogenes* in vitro

The antibacterial effect and bactericidal efficacy of antimicrobial peptide PL-5 on 19 erythromycin resistant strains and 21 erythromycin sensitive strains of *Streptococcus pyogenes* were tested in vitro using microdilution method and nutrient agar plate culture method, and vancomycin was used as the control drug.

The results showed that the MIC range of PL-5 to erythromycin resistant and erythromycin sensitive strains of *Streptococcus pyogenes* were 0.5–64 μg/ml and <0.03125–64 μg/ml; the geometric mean of MIC were 23.0448 μg/ml and 15.48 μg/ml, respectively; MIC<sub>50</sub> and MIC<sub>90</sub> were both 32 μg/ml. At the concentration of 64 μg/ml, PL-5 could 100% inhibit the growth of two kinds of *Streptococcus pyogenes*. The MBC ranges of two kinds of *Streptococcus pyogenes* were 1–64 and <0.03125–64 μg/ml respectively; MBC<sub>50</sub> were 32 μg/ml, and MBC<sub>90</sub> is 64 μg/ml. At the concentration of 64 μg/ml, the cumulative bactericidal percentage of PL-5 on erythromycin resistant strains reached 95%; while that was 100% on erythromycin sensitive strains. MBC<sub>50</sub>/MIC<sub>90</sub> was 1 and MBC<sub>90</sub>/MIC<sub>90</sub> was 2.

The results showed that antimicrobial peptide PL-5 had certain antibacterial and bactericidal effects on erythromycin resistant and erythromycin sensitive strains of *Streptococcus pyogenes*. Considering the content of pure peptide to be 84.34%, the MIC range and MBC range of erythromycin resistant strains of *Streptococcus pyogenes* were 0.4217–53.9776 μg/ml and 0.8434–53.9776 μg/ml respectively; MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively; The MIC range and MBC range of erythromycin sensitive strains of *Streptococcus pyogenes* were <0.0264–53.9776 μg/ml and <0.0264–53.9776 μg/ml respectively, and MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively.

### Antibacterial effect of antimicrobial peptide PL-5 and vancomycin on *Streptococcus pyogenes* in vitro

| Sample | Bacteria Strain | Number of Strains | MIC<sub>90</sub> 50 and MBC<sub>90</sub>/MIC<sub>90</sub> respectively, and MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |
|---------|----------------|------------------|--------------------------------------------------|
|          |                |                  | Geometric mean of MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |
|          |                |                  | Geometric mean of MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |

### Bactericidal effect of antimicrobial peptide PL-5 and vancomycin on *Streptococcus pyogenes* in vitro

| Sample | Bacteria Strain | Number of Strains | MIC<sub>90</sub> 50 and MBC<sub>90</sub>/MIC<sub>90</sub> respectively, and MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |
|---------|----------------|------------------|--------------------------------------------------|
|          |                |                  | Geometric mean of MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |
|          |                |                  | Geometric mean of MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |

### Cumulative antibacterial percentage of antimicrobial peptide PL-5 and vancomycin on *Streptococcus pyogenes* in vitro

| Sample | Bacteria Strain | Number of Strains | Cumulative antibacterial percentage of different concentrations (μg/ml) of tested drugs (%) |
|---------|----------------|------------------|--------------------------------------------------|
|          |                |                  | Geometric mean of MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |
|          |                |                  | Geometric mean of MIC<sub>90</sub> and MBC<sub>90</sub> were 26.9888 μg/ml and 53.9776 μg/ml respectively. |

---

5
Cumulative bactericidal percentage of antimicrobial peptide PL-5 and vancomycin on *Streptococcus pyogenes in vitro*

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) |
|--------|------------------|-------------------|------------------------------------------------------------------------------------------------|
| PL-5   | *Streptococcus pyogenes* resistant strain | 19 | 95 89 26 5 5 5 5 0 0 0 0 - |
|        | *Erythromycin* resistant strain | 21 | 100 81 33 5 5 5 5 5 5 5 5 - |
| Vanco- mycin | *Streptococcus pyogenes* resistant strain | 19 | - 84 68 58 37 21 21 16 11 0 0 0 |
|        | *Erythromycin* resistant strain | 21 | - 71 52 52 43 38 38 33 24 5 5 0 0 |

3. Antibacterial and bactericidal effects of antimicrobial peptide PL-5 on *Enterococcus in vitro*

The antibacterial effect and bactericidal efficacy of antimicrobial peptide PL-5 on 40 strains of *Enterococcus faecalis* and 40 clinical isolates of *Enterococcus faecium* were tested in vitro using microdilution method and nutrient agar plate culture method, and vancomycin was used as the control drug.

The results showed that the MIC range of PL-5 to *Enterococcus faecalis* and *Enterococcus faecium* were both 2->64 μg/ml; the geometric mean of MIC was 14.17 and 11.31 μg/ml, respectively; MIC50 were 16 and 4 μg/ml respectively, and MIC90 were >64 and 64 μg/ml respectively. At the concentration of 64 μg/ml, PL-5 could inhibit the growth of *Enterococcus faecalis* and *Enterococcus faecium* by 80% and 93% respectively. The MBC ranges of two kinds of bacteria were both 2->64 μg/ml; MBC50 were 16 μg/ml, and MBC90 was >64 μg/ml. At the concentration of 64 μg/ml, the cumulative bactericidal percentage of PL-5 on both bacteria reached 78% and 85% respectively.

The above results showed that antimicrobial peptide PL-5 had certain antibacterial and bactericidal effects on *Enterococcus faecalis* and *Enterococcus faecium* only at high concentrations. Considering the content of pure peptide to be 84.34%, the MIC range and MBC range of *Enterococcus faecalis* and *Enterococcus faecium* were 1.6868->53.9776 μg/ml and 1.6868->53.9776 μg/ml respectively; MIC50 and MBC90 were >53.9776 μg/ml and >53.9776 μg/ml respectively; The MIC range and MBC range of *Enterococcus faecalis* and *Enterococcus faecium* were 1.6868->53.9776 μg/ml and 1.6868->53.9776 μg/ml respectively; the MIC50 and MBC90 were 53.9776 μg/ml and >53.9776 μg/ml respectively.

**Antibacterial effect of antimicrobial peptide PL-5 and vancomycin on *Enterococcus faecalis* and *Enterococcus faecium in vitro***

| Sample | Bacteria Strains | Number of Strains | Strains of bacteria not growing under different drug concentrations (μg/ml) | MIC range (μg/ml) | MBC range (μg/ml) | MIC90 (μg/ml) | MBC90 (μg/ml) | Minimum Geometric mean (μg/ml) |
|--------|------------------|-------------------|--------------------------------------------------------------------------|------------------|------------------|--------------|--------------|-----------------------------|
| PL-5   | *Enterococcus faecalis* (ATCC 29212) | 1 | 1 1 1 0 0 0 0 0 0 0 0 | 0 | 1 1 | 32 32 32 32 | 32 32 32 32 | 32 32 32 32 |
|        | *Enterococcus faecalis* | 40 | 32 26 22 19 17 3 0 0 0 0 0 | 0 | 40 | 2->64 | 16 | >64 | 14.17 |
|        | *Enterococcus faecium* | 40 | 37 29 25 22 21 3 0 0 0 0 0 | 0 | 40 | 2->64 | 4 | 64 | 11.31 |
| Vanco- mycin | *Enterococcus faecalis* (ATCC 29212) | 1 | 1 1 1 1 1 1 1 0 0 0 0 | 1 1 1 1 1 1 | 0.5 | 2 | 0.5 | 1 | 0.68 |
|        | *Enterococcus faecalis* | 40 | 40 40 40 40 40 39 23 0 0 0 0 | 0 | 40 | 0.5 | 2 | 1 | >32 | 1.71 |
Bactericidal effect of antimicrobial peptide PL-5 and vancomycin on *Enterococcus faecalis* and *Enterococcus faecium* in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MBC range (μg/ml) | MBC<sub>50</sub> (μg/ml) | MBC<sub>90</sub> (μg/ml) |
|--------|------------------|-------------------|-------------------------------------------------|-------------------|-----------------|-----------------|
| PL-5   | *Enterococcus faecalis* (ATCC 29212) | 1 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 1/2 | 1/4 | 0 | 1 | 32 | 32 | 32 | 32 |
|        | *Enterococcus faecalis* | 40 | 31 | 25 | 22 | 16 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 2->64 | 16 | >64 |
|        | *Enterococcus faecium* | 40 | 34 | 27 | 22 | 19 | 10 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 2->64 | 16 | >64 |
| Vanco- mycin | *Enterococcus faecalis* (ATCC 29212) | 1 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | >32 | >32 | >32 |
|        | *Enterococcus faecalis* | 40 | - | 33 | 10 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 8->32 | 32 | >32 |
|        | *Enterococcus faecium* | 40 | - | 20 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | >32 | 32 | >32 |

Cumulative antibacterial percentage of antimicrobial peptide PL-5 and vancomycin on *Enterococcus faecalis* and *Enterococcus faecium* in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative antibacterial percentage of different concentrations (μg/ml) of tested drugs (%) | MBC<sub>50</sub>/MIC<sub>90</sub> | MBC<sub>90</sub>/MIC<sub>90</sub> |
|--------|------------------|-------------------|-------------------------------------------------|-----------------|-----------------|
| PL-5   | *Enterococcus faecalis* | 40 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 1 | 3 | 4 | 64 | >3 |
|        | *Enterococcus faecium* | 40 | 93 | 73 | 63 | 35 | 24 | 8 | 0 | 0 | - | - | - | - | - | 4 | >3 |
| Vanco- mycin | *Enterococcus faecalis* | 40 | - | 100 | 100 | 100 | 100 | 98 | 98 | 98 | 98 | 98 | 0 | 64 | 64 | >3 |
|        | *Enterococcus faecium* | 40 | - | 80 | 80 | 80 | 80 | 80 | 65 | 38 | 0 | 0 | 32 | 32 | - | - |

Cumulative bactericidal percentage of antimicrobial peptide PL-5 and vancomycin on *Enterococcus faecalis* and *Enterococcus faecium* in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MBC<sub>50</sub>/MIC<sub>90</sub> | MBC<sub>90</sub>/MIC<sub>90</sub> |
|--------|------------------|-------------------|-------------------------------------------------|-----------------|-----------------|
| PL-5   | *Enterococcus faecalis* | 40 | 78 | 63 | 55 | 40 | 14 | 10 | 3 | 0 | - | - | - | - | - | - |
|        | *Enterococcus faecium* | 40 | 85 | 68 | 55 | 48 | 25 | 25 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vanco- mycin | *Enterococcus faecalis* | 40 | - | 83 | 25 | 8 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | *Enterococcus faecium* | 40 | - | 50 | 10 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

4. Antibacterial and bactericidal effects of antimicrobial peptide PL-5 on *Escherichia coli* and *Klebsiella pneumoniae* in vitro

The antibacterial effect and bactericidal efficacy of antimicrobial peptide PL-5 on 119 clinical isolates of *Escherichia coli* (79 strains of producing extended spectrum beta lactamases ESBLs and 40 non-producing ESBLs) and 120 *Klebsiella pneumoniae* (80 strains of producing ESBLs and 40 non-producing ESBLs) were tested in vitro using microdilution method and nutrient agar plate culture method, and Imipenem Cilastatin sodium was used as the control drug. The results showed that the MIC range of PL-5 to ESBLs producing *Escherichia coli* and ESBLs non-producing *Escherichia coli* were both 2->64μg/ml; the geometric mean of MIC was 4.68 and 8.14μg/ml, respectively; MIC<sub>50</sub> were 4 and 8μg/ml respectively, and MIC<sub>90</sub> were 32 and 64μg/ml respectively. According to the cumulative antibacterial percentage, at the concentration of 64 μg/ml, PL-5 could inhibit the growth of ESBLs producing *Escherichia coli* and ESBLs non-producing *Escherichia coli* by 94% and 93% respectively. The MBC ranges of two kinds of bacteria were both 2->64μg/ml; MBC<sub>50</sub> were 4μg/ml and 8μg/ml, and MBC<sub>90</sub> were 64 and >64 μg/ml. At the concentration of 64 μg/ml, the cumulative bactericidal percentage of PL-5 on *Escherichia coli* reached 94% and 88% respectively; MIC range of PL-5 to ESBLs producing *Klebsiella pneumoniae* and ESBLs non-producing *Klebsiella pneumoniae* were 1->64μg/ml and 1->64μg/ml respectively; the geometric mean of MIC were 6.22 and 5.96μg/ml, respectively; MIC<sub>50</sub> were 4 and 8μg/ml respectively, and MIC<sub>90</sub> were both 8μg/ml. According to the cumulative antibacterial percentage, at the concentration of 64 μg/ml, PL-5 could inhibit both the growth of ESBLs producing *Klebsiella pneumoniae* and ESBLs non-producing *Klebsiella pneumoniae* by 100%. The MBC ranges of two kinds of bacteria were 2->64μg/ml and 2->64μg/ml respectively; MBC<sub>50</sub> were both 8μg/ml, and MBC<sub>90</sub> were 64 and >64 μg/ml. At the concentration of 64 μg/ml, the cumulative bactericidal percentage of PL-5 on ESBLs producing *Klebsiella pneumoniae* and ESBLs non-producing *Klebsiella pneumoniae* were 96% and 100% respectively. The above results showed that antimicrobial peptide PL-5 had antibacterial and bactericidal effects on clinical isolates of ESBLs producing *Escherichia coli*, ESBLs non-producing *Escherichia coli*, ESBLs producing *Klebsiella pneumoniae* and ESBLs non-producing *Klebsiella pneumoniae*. Considering the content of pure peptide to be 84.34%, the MIC range and MBC range of producing *Escherichia coli* were 1.6865->53.9776μg/ml and 1.6865->53.9776μg/ml respectively; MIC<sub>50</sub> and MBC<sub>90</sub> were >26.9884μg/ml and >53.9776μg/ml respectively; The MIC range and MBC range of ESBLs non-
producing *Escherichia coli* were 1.6868→53.9776μg/ml and 1.6868→53.9776μg/ml respectively; the MIC<sub>90</sub> and MBC<sub>90</sub> were 53.9776μg/ml and >53.9776μg/ml respectively. The MIC range and MBC range of ESBLs producing *Klebsiella pneumoniae* were 0.8434→53.9776μg/ml and 1.6868→53.9776μg/ml respectively; the MIC<sub>90</sub> and MBC<sub>90</sub> were 6.7472μg/ml and >53.9776μg/ml respectively. The MIC range and MBC range of ESBLs non-producing *Klebsiella pneumoniae* were 1.6868→53.9776μg/ml and 1.6868→53.9776μg/ml respectively; the MIC<sub>90</sub> and MBC<sub>90</sub> were 6.7472μg/ml and 26.9888μg/ml respectively.

**Antibacterial effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Escherichia coli* in vitro**

| Sample | Bacteria Strains | Number of Strains | MIC range (μg/ml) | MBC<sub>90</sub> (μg/ml) | MBC<sub>90</sub> (μg/ml) | MIC geometric mean (μg/ml) |
|--------|-----------------|-------------------|-------------------|-----------------|-----------------|----------------------|
| PL-5   | ESBLE E. Coli (ATCC 35218) | 1 | 4 | 4 | 4 | 4 |
| Non-ESBLE E. Coli (ATCC 25922) | 1 | 4 | 4 | 4 | 4 |
| ESBLE E. Coli (ATCC 35218) | 79 | 2→64 | 4 | >64 | >64 |
| ESBLE E. Coli (ATCC 25922) | 79 | 2→64 | 4 | >64 | >64 |
| ESBLE E. Coli (ATCC 35218) | 40 | 0.25→16 | 4 | >16 | >16 |
| Non-ESBLE E. Coli (ATCC 25922) | 40 | 0.25→16 | 4 | >16 | >16 |

**Bactericidal effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Escherichia coli* in vitro**

| Sample | Bacteria Strains | Number of Strains | No. of Strains | MIC range (μg/ml) | MBC<sub>90</sub> (μg/ml) | MBC<sub>90</sub> (μg/ml) |
|--------|-----------------|-------------------|---------------|-------------------|-----------------|-----------------|
| PL-5   | ESBLE E. Coli (ATCC 35218) | 1 | 4 | 4 | 4 | 4 |
| Non-ESBLE E. Coli (ATCC 25922) | 1 | 4 | 4 | 4 | 4 |
| ESBLE E. Coli (ATCC 35218) | 79 | 2→64 | 4 | >64 | >64 |
| ESBLE E. Coli (ATCC 25922) | 79 | 2→64 | 4 | >64 | >64 |
| ESBLE E. Coli (ATCC 35218) | 40 | 0.25→16 | 4 | >16 | >16 |
| Non-ESBLE E. Coli (ATCC 25922) | 40 | 0.25→16 | 4 | >16 | >16 |

**Cumulative antibacterial percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Escherichia coli* in vitro**

| Sample | Bacteria Strains | Number of Strains | Cumulative antibacterial percentage of different concentrations (μg/ml) of tested drugs (%) | MIC<sub>90</sub>/MIC<sub>90</sub> | MBC<sub>90</sub>/MBC<sub>90</sub> |
|--------|-----------------|-------------------|-----------------------------------------------|-----------------|-----------------|
| PL-5   | ESBLE E. Coli (ATCC 35218) | 79 | 0.125 | 1 |
| Non-ESBLE E. Coli (ATCC 25922) | 40 | 0.125 | 1 |
| ESBLE E. Coli (ATCC 35218) | 79 | 0.125 | 1 |
| Non-ESBLE E. Coli (ATCC 25922) | 40 | 0.125 | 1 |
| Enterococcus faecalis | 79 | 0.125 | 1 |
| Enterococcus faecium | 40 | 0.125 | 1 |

**Cumulative bactericidal percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Escherichia coli* in vitro**

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MIC<sub>90</sub>/MIC<sub>90</sub> |
|--------|-----------------|-------------------|-----------------------------------------------|-----------------|
| PL-5   | Enterococcus faecalis | 79 | 0.125 | 1 |
| Enterococcus faecium | 40 | 0.125 | 1 |

**Cumulative bactericidal percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Escherichia coli* in vitro**

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MIC<sub>90</sub>/MIC<sub>90</sub> |
|--------|-----------------|-------------------|-----------------------------------------------|-----------------|
| PL-5   | Enterococcus faecalis | 79 | 0.125 | 1 |
| Enterococcus faecium | 40 | 0.125 | 1 |
### Antibacterial effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Klebsiella pneumoniae in vitro*

| Sample | Bacteria Strains | Number of Strains | Strains of bacteria not growing under different drug concentrations (μg/ml) | MIC range (μg/ml) | MBC\text{C}_{50} | MBC\text{C}_{90} | MIC\text{C}_{90} | MBC\text{C}_{90}/MIC\text{C}_{90} |
|--------|-----------------|------------------|----------------------------------------------------------------|------------------|------------------|------------------|------------------|------------------------|
|        | Klebsiella pneumoniae (ATCC 1706) | 1 | 1 1 1 0 0 0 0 0 | - - 0 1 16 16 16 16 |
|        | Klebsiella pneumoniae (ATCC 1706) | 1 | 1 1 1 0 0 0 0 0 | - - 0 1 16 16 16 16 |
|        | ESBL Klebsiella pneumoniae (ATCC 700605) | 1 | 1 1 1 1 1 1 0 0 | - - 0 1 16 16 16 16 |
|        | ESBL Klebsiella pneumoniae (ATCC 700605) | 1 | 1 1 1 1 1 1 0 0 | - - 0 1 16 16 16 16 |

### Bactericidal effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Klebsiella pneumoniae in vitro*

| Sample | Bacteria Strains | Number of Strains | Strains of bacteria not growing under different drug concentrations (μg/ml) | MBC (μg/ml) | MBC\text{C}_{50} | MBC\text{C}_{90} |
|--------|-----------------|------------------|----------------------------------------------------------------|-------------|------------------|-----------------|
|        | Klebsiella pneumoniae (ATCC 1706) | 1 | 1 1 1 0 0 0 0 0 | - - 0 1 16 16 16 16 |
|        | Klebsiella pneumoniae (ATCC 1706) | 1 | 1 1 1 0 0 0 0 0 | - - 0 1 16 16 16 16 |
|        | ESBL Klebsiella pneumoniae (ATCC 700605) | 1 | 1 1 1 1 1 1 0 0 | - - 0 1 16 16 16 16 |
|        | ESBL Klebsiella pneumoniae (ATCC 700605) | 1 | 1 1 1 1 1 1 0 0 | - - 0 1 16 16 16 16 |

### Cumulative antibacterial percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Klebsiella pneumoniae in vitro*

| Sample | Bacteria Strains | Number of Strains | Cumulative antibiotic percentage of different concentrations (μg/ml) of tested drugs (%) | MBC\text{C}_{50}/MIC\text{C}_{90} |
|--------|-----------------|------------------|-----------------------------------------------------------------------------------------|------------------------|
|        | ESBL Klebsiella pneumoniae | 80 | 100 98 95 90 51 4 1 0 | - - 2 8 |
|        | Non-ESBLs Klebsiella pneumoniae | 40 | 100 98 98 98 40 10 0 0 | - - 1 4 |
|        | ESBL Klebsiella pneumoniae | 80 | 90 90 90 90 89 78 66 51 3 8 | >4 |
|        | Non-ESBLs Klebsiella pneumoniae | 40 | 100 98 98 95 90 90 80 60 0 64 | >16 |

### Cumulative bactericidal percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Klebsiella pneumoniae in vitro*

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MBC\text{C}_{50}/MIC\text{C}_{90} |
|--------|-----------------|------------------|-----------------------------------------------------------------------------------------|------------------------|
|        | ESBLs Klebsiella pneumoniae | 80 | 96 80 73 63 35 3 0 0 | - - |
|        | Non-ESBLs Klebsiella pneumoniae | 40 | 100 90 88 78 33 5 0 0 | - - |
|        | ESBLs Klebsiella pneumoniae | 80 | - - 90 90 90 90 89 78 66 51 3 8 | >4 |
|        | Non-ESBLs Klebsiella pneumoniae | 40 | - - 100 98 98 95 90 90 80 60 0 64 | >16 |
5. **Antibacterial and bactericidal effects of antimicrobial peptide PL-5 on Pseudomonas aeruginosa and Acinetobacter baumannii in vitro**

The antibacterial effect and bactericidal efficacy of antimicrobial peptide PL-5 on clinical isolates of *Pseudomonas aeruginosa* (81 strains of IPM-R and 43 IPM-S) and *Acinetobacter baumannii* (81 strains of IPM-R and 33 IPM-S) were tested in vitro using microdilution method and nutrient agar plate culture method, and Imipenem Cilastatin sodium was used as the control drug.

The results showed that the MIC range of PL-5 to IPM-R and IPM-S *Pseudomonas aeruginosa* were 2→128 and 4→16μg/ml respectively; the geometric mean of MIC was 7.16 and 7.03μg/ml respectively; MIC50 were 8μg/ml and MIC90 were 16μg/ml respectively. According to the cumulative antibacterial percentage, at the concentration of 128μg/ml, PL-5 could inhibit the growth of IPM-R *Pseudomonas aeruginosa* by 100%; at the concentration of 16μg/ml, PL-5 could inhibit the growth of IPM-S *Pseudomonas aeruginosa* by 100%. The MBC ranges of two kinds of bacteria were 4→128 and 4→32μg/ml respectively; MBC50 were 16μg/ml and 8μg/ml, and MBC90 were 64 and 32μg/ml. At the concentration of 128μg/ml, the cumulative bactericidal percentage of PL-5 on IPM-R strains reached 96%, while at the concentration of 32μg/ml, the cumulative bactericidal percentage of PL-5 on IPM-S strains reached 100%. MIC range of PL-5 to IPM-R *Acinetobacter baumannii* and IPM-S *Acinetobacter baumannii* were both 2→64μg/ml; the geometric mean of MIC was 3.47 and 3.17μg/ml respectively; MIC50 were 4 and 2μg/ml respectively, and MIC90 were both 8μg/ml. According to the cumulative antibacterial percentage, at the concentration of 32μg/ml, PL-5 could inhibit the growth of IPM-R *Acinetobacter baumannii* by 99%; at the concentration of 64μg/ml, PL-5 could inhibit the growth of IPM-S *Acinetobacter baumannii* by 97%. The MBC ranges of two kinds of bacteria were both 2→64μg/ml; MBC50 were 4 and 2μg/ml respectively, and MBC90 were 16 and 8μg/ml respectively. At the concentration of 64μg/ml, the cumulative bactericidal percentage of PL-5 on IPM-R *Acinetobacter baumannii* and IPM-S *Acinetobacter baumannii* were 95% and 97% respectively.

The above results showed that antimicrobial peptide PL-5 had certain antibacterial and bactericidal effects on clinical isolates of IPM-R *Pseudomonas aeruginosa*, IPM-S *Pseudomonas aeruginosa*, IPM-R *Acinetobacter baumannii* and IPM-S *Acinetobacter baumannii*. Considering the content of pure peptide to be 84.34%, the MIC range and MBC range of IPM-R *Pseudomonas aeruginosa* were 1.6868→107.9552μg/ml and 3.3736→107.9552μg/ml respectively; MIC50 and MBC50 were 13.4944μg/ml and 53.9776μg/ml respectively; The MIC range and MBC range of IPM-S *Pseudomonas aeruginosa* were 3.3736→13.4944μg/ml and 3.3736→26.9888μg/ml respectively; The MIC50 and MBC50 were 13.4944μg/ml and 26.9888μg/ml respectively. The MIC range and MBC range of IPM-R *Acinetobacter baumannii* were 1.6868→53.9776μg/ml and 1.6868→53.9776μg/ml respectively; The MIC50 and MBC50 were 6.7472μg/ml and 13.4944μg/ml respectively. The MIC range and MBC range of IPM-S *Acinetobacter baumannii* were 1.6868→53.9776μg/ml and 1.6868→53.9776μg/ml respectively; The MIC50 and MBC50 were 6.7472μg/ml and 6.7472μg/ml respectively.

### Antibacterial effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Pseudomonas aeruginosa* in vitro

| Strain          | Bacteria          | MICs (μg/ml) | MIC range (μg/ml) | MBC50 (μg/ml) | MBC90 (μg/ml) | MBC geometric mean (μg/ml) |
|-----------------|------------------|--------------|------------------|--------------|--------------|--------------------------|
| Pseudomonas      | PL-5             | 1            | 1                | 1             | 4            | 1                        | 4944μg/ml |
| aeruginosa      |                  | 81           | 81               | 81            | 81           | 81                       | 7376μg/ml |
| (ATCC 27853)    |                  | 63           | 43               | 43            | 43           | 43                       | 7804μg/ml |
| Pseudomonas      |                  | 1            | 1                | 1             | 1            | 1                        | 26μg/ml  |
| aeruginosa      |                  | 43           | 43               | 43            | 43           | 43                       | 84μg/ml  |
| (IPM-R)         |                  |              |                  |              |              |                          |          |
| Acinetobacter    | PL-5             | 1            | 1                | 1             | 1            | 1                        | 2       |
| baumannii       |                  | 81           | 81               | 81            | 81           | 81                       | 2→128 μg/ml |
| (ATCC 19606)    |                  | 63           | 43               | 43            | 43           | 43                       | 128μg/ml |
| (IPM-R)         |                  |              |                  |              |              |                          |          |
| Pseudomonas      | PL-5             | 1            | 1                | 1             | 1            | 1                        | 2μg/ml  |
| aeruginosa      |                  | 5            | 5                | 5             | 5            | 5                        | 40μg/ml  |
| (ATCC 27853)    |                  | 43           | 43               | 43            | 43           | 43                       | 84μg/ml  |
| Pseudomonas      |                  | 1            | 1                | 1             | 1            | 1                        | 2μg/ml  |
| aeruginosa      |                  | 43           | 43               | 43            | 43           | 43                       | 84μg/ml  |
| (IPM-S)         |                  |              |                  |              |              |                          |          |
| Acinetobacter    | PL-5             | 1            | 1                | 1             | 1            | 1                        | 2μg/ml  |
| baumannii       |                  | 5            | 5                | 5             | 5            | 5                        | 40μg/ml  |
| (ATCC 19606)    |                  | 43           | 43               | 43            | 43           | 43                       | 84μg/ml  |

The above results showed that antimicrobial peptide PL-5 had certain antibacterial and bactericidal effects on clinical isolates of IPM-R *Pseudomonas aeruginosa*, IPM-S *Pseudomonas aeruginosa*, IPM-R *Acinetobacter baumannii* and IPM-S *Acinetobacter baumannii*. Considering the content of pure peptide to be 84.34%, the MIC range and MBC range of IPM-R *Pseudomonas aeruginosa* were 1.6868→107.9552μg/ml and 3.3736→107.9552μg/ml respectively; MIC50 and MBC50 were 13.4944μg/ml and 53.9776μg/ml respectively; The MIC range and MBC range of IPM-S *Pseudomonas aeruginosa* were 3.3736→13.4944μg/ml and 3.3736→26.9888μg/ml respectively; The MIC50 and MBC50 were 13.4944μg/ml and 26.9888μg/ml respectively. The MIC range and MBC range of IPM-R *Acinetobacter baumannii* were 1.6868→53.9776μg/ml and 1.6868→53.9776μg/ml respectively; The MIC50 and MBC50 were 6.7472μg/ml and 13.4944μg/ml respectively. The MIC range and MBC range of IPM-S *Acinetobacter baumannii* were 1.6868→53.9776μg/ml and 1.6868→53.9776μg/ml respectively; The MIC50 and MBC50 were 6.7472μg/ml and 6.7472μg/ml respectively.
### Bactericidal effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Pseudomonas aeruginosa* in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (µg/ml) of tested drugs (%) | MBC<sub>50</sub>/ MBC<sub>90</sub> | MBC<sub>50</sub> (µg/ml) | MBC<sub>90</sub> (µg/ml) |
|--------|-----------------|------------------|-------------------------------------------------|--------------------|----------------|----------------|
| Acinetobacter baumannii ATCC 27853 | Pseudomonas aeruginosa (ATCC 27853) | 81 | 100 99 98 91 88 40 | 1 0 - - - | 2 4 |
|          | (IPM-S)         | 43  | 100 100 100 100 88 30 | 0 0 - - - | 1 2 |

### Cumulative antibacterial percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Pseudomonas aeruginosa* in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative antibacterial percentage of different concentrations (µg/ml) of tested drugs (%) | MBC<sub>50</sub>/ MBC<sub>90</sub> | MBC<sub>50</sub> (µg/ml) | MBC<sub>90</sub> (µg/ml) |
|--------|-----------------|------------------|-------------------------------------------------|--------------------|----------------|----------------|
| Acinetobacter baumannii ATCC 27853 | Pseudomonas aeruginosa (ATCC 27853) | 83 | 96 94 84 57 36 5 | 0 0 - - - | 2 4 |
|          | (IPM-S)         | 43  | 100 100 100 100 88 56 | 14 0 0 - - - | 1 2 |

### Cumulative bactericidal percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Pseudomonas aeruginosa* in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (µg/ml) of tested drugs (%) | MBC<sub>50</sub>/ MBC<sub>90</sub> | MBC<sub>50</sub> (µg/ml) | MBC<sub>90</sub> (µg/ml) |
|--------|-----------------|------------------|-------------------------------------------------|--------------------|----------------|----------------|
| Acinetobacter baumannii ATCC 27853 | Pseudomonas aeruginosa (ATCC 27853) | 87 | 86 86 85 84 71 60 | 8 0 - 0 0 87 | 2 4 8 |

## Antibacterial effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Acinetobacter baumannii* in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative antibacterial percentage of different concentrations (µg/ml) of tested drugs (%) | MBC<sub>50</sub>/ MBC<sub>90</sub> | MBC<sub>50</sub> (µg/ml) | MBC<sub>90</sub> (µg/ml) |
|--------|-----------------|------------------|-------------------------------------------------|--------------------|----------------|----------------|
| Acinetobacter baumannii ATCC 27853 | Pseudomonas aeruginosa (ATCC 27853) | 87 | 86 86 85 84 71 60 | 8 0 - 0 0 87 | 2 4 8 |

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (µg/ml) of tested drugs (%) | MBC<sub>50</sub>/ MBC<sub>90</sub> | MBC<sub>50</sub> (µg/ml) | MBC<sub>90</sub> (µg/ml) |
|--------|-----------------|------------------|-------------------------------------------------|--------------------|----------------|----------------|
| Acinetobacter baumannii ATCC 27853 | Pseudomonas aeruginosa (ATCC 27853) | 87 | 86 86 85 84 71 60 | 8 0 - 0 0 87 | 2 4 8 |

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (µg/ml) of tested drugs (%) | MBC<sub>50</sub>/ MBC<sub>90</sub> | MBC<sub>50</sub> (µg/ml) | MBC<sub>90</sub> (µg/ml) |
|--------|-----------------|------------------|-------------------------------------------------|--------------------|----------------|----------------|
| Acinetobacter baumannii ATCC 27853 | Pseudomonas aeruginosa (ATCC 27853) | 87 | 86 86 85 84 71 60 | 8 0 - 0 0 87 | 2 4 8 |
Cumulative antibacterial percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on Acinetobacter baumannii in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative antibacterial percentage of different concentrations (μg/ml) of tested drugs (%) | MBCw/ MICw | MBCw/ MICw |
|--------|-----------------|-------------------|-------------------------------------------------|-------------|-------------|
| PL-5   | Acinetobacter baumannii (ATCC 19606) | 87 | 128 64 32 16 8 4 2 1 0.5 0.25 0.125 | 2 | 2 |
|        | Acinetobacter baumannii (IPM-R)      | 32 | 99 98 97 82 46 0 0 | 1 | 2 |
|        | Acinetobacter baumannii (IPM-S)      | 33 | 97 94 91 70 0 0 | 1 | 1 |
|        | imipenem cilastatin sodium            | 100 | | | |
|        | Acinetobacter baumannii (IPM-S)      | 33 | 100 100 97 82 73 52 6 | 2 | 4 |

Cumulative bactericidal percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on Acinetobacter baumannii in vitro

| Sample | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | MBCw/ MICw |
|--------|-----------------|-------------------|-------------------------------------------------|-------------|
| PL-5   | Acinetobacter baumannii (IPM-R) | 87 | 128 64 32 16 8 4 2 1 0.5 0.25 0.125 | 2 |
|        | Acinetobacter baumannii (IPM-S) | 33 | 97 94 91 70 55 0 0 | 2 |
|        | imipenem cilastatin sodium        | 100 | 97 88 85 64 55 48 6 | 4 |

6. Antibacterial and bactericidal effects of antimicrobial peptide PL-5 on Enterobacter cloacae, Enterobacter aerogenes, Citrobacter Ferrandi and Proteus in vitro

The antibacterial effect and bactericidal efficacy of antimicrobial peptide PL-5 on clinical isolates of 40 strains of Enterobacter cloacae, 40 strains of Enterobacter aerogenes, 20 strains of Citrobacter Ferrandi and 20 strains of Proteus were tested in vitro using microdilution method and nutrient agar plate culture method, and Imipenem Cilastatin sodium was used as the control drug.

The results showed that (1) The MIC range of PL-5 to Enterobacter cloacae was 4–16μg/ml; the geometric mean of MIC was 7.09μg/ml; MIC50 were 8μg/ml and MIC90 were 16μg/ml respectively. According to the cumulative antibacterial percentage, at the concentration of 16μg/ml, PL-5 could inhibit the growth of Enterobacter cloacae by 100%. The MBC ranges was 4–64μg/ml; MBC50 was 8μg/ml, and MBC90 was 32 μg/ml. At the concentration of 64μg/ml, the cumulative bactericidal percentage of PL-5 on Enterobacter cloacae was 100%. (2) The MIC range of PL-5 to Enterobacter aerogenes was 2–> 64μg/ml; the geometric mean of MIC was 12.34μg/ml; MIC50 were 8μg/ml and MIC90 were >64μg/ml respectively. According to the cumulative antibacterial percentage, at the concentration of 64μg/ml, PL-5 could inhibit the growth of Enterobacter aerogenes by 70%. The MBC ranges was 2–64μg/ml; MBC50 was 8μg/ml, and MBC90 was >64μg/ml. At the concentration of 64μg/ml, the cumulative bactericidal percentage of PL-5 on Enterobacter aerogenes was 70%. (3) The MIC range of PL-5 to Citrobacter Ferrandi was 2–> 64μg/ml; the geometric mean of MIC was 7.21μg/ml; MIC50 were 8μg/ml and MIC90 were 16μg/ml respectively. According to the cumulative antibacterial percentage, at the concentration of 64μg/ml, PL-5 could inhibit the growth of Citrobacter Ferrandi by 90%. The MBC
ranges was $2-64\mu{g/ml}$; MBC$_{0}$ was $8\mu{g/ml}$, and MBC$_{90}$ was $>64\mu{g/ml}$. At the concentration of $64\mu{g/ml}$, the cumulative bactericidal percentage of PL-5 on *Citrobacter Ferrandi* was 85%. (4) PL-5 at the concentration of $64\mu{g/ml}$ and below had no antibacterial effects on 20 strains of *Proteus*.

The above results showed that antimicrobial peptide PL-5 had certain antibacterial and bactericidal effects on clinical isolates of *Enterobacter cloacae*, *Enterobacter aerogenes* and *Citrobacter Ferrandi*, while PL-5 at the concentration of $64\mu{g/ml}$ and below had no antibacterial effect on *Proteus*. Considering the content of pure peptide to be $84.34\%$, the MIC range and MBC range of *Enterobacter cloacae* were $3.3736-13.4944\mu{g/ml}$ and $3.3736-53.9776\mu{g/ml}$ respectively; MIC$_{0}$ and MBC$_{90}$ were $13.4944\mu{g/ml}$ and $26.9888\mu{g/ml}$ respectively. The MIC range and MBC range of *Enterobacter aerogenes* were $1.6868-53.9776\mu{g/ml}$ and $1.6868-53.9776\mu{g/ml}$ respectively; the MIC$_{0}$ and MBC$_{90}$ were $>53.9776\mu{g/ml}$ and $>53.9776\mu{g/ml}$ respectively. The MIC range and MBC range of *Citrobacter Ferrandi* were $1.6868-53.9776\mu{g/ml}$ and $1.6868-53.9776\mu{g/ml}$ respectively; the MIC$_{0}$ and MBC$_{90}$ were $13.4944\mu{g/ml}$ and $>53.9776\mu{g/ml}$ respectively.

### Antibacterial effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter Ferrandi* and *Proteus* in vitro

| Sample | Bacteria Strains | Number of Strains | Strains of bacteria not growing under different drug concentrations (μg/ml) | MIC range (μg/ml) | MBC$_{0}$ (μg/ml) | MBC$_{90}$ (μg/ml) | MIC Geometric mean (μg/ml) |
|--------|------------------|-------------------|---------------------------------------------------------------------|-----------------|----------------|-----------------|------------------------|
| PL-5   | *Enterobacter cloacae* (029M) | 1 | 1 1 1 1 1 1 0 0 0 - 0 1 4 4 4 4 |
|        | *Enterobacter cloacae* | 40 | 40 40 40 34 13 0 0 0 - 0 40 4 - 16 8 16 7.09 |
|        | *Enterobacter aerogenes* | 40 | 28 27 27 24 14 3 0 0 - 0 40 2 - >64 8 >64 12.34 |
|        | *Citrobacter Ferrandi* | 20 | 18 18 18 16 8 3 0 0 - 0 20 2 - >64 8 16 7.21 |
|        | *Proteus* | 20 | 0 0 0 0 0 0 0 0 - 0 20 >64 >64 >64 |
|        | imipenem cilastatin sodium | | | | | | |
|        | *Enterobacter cloacae* (029M) | 1 | 1 1 1 1 1 1 1 1 1 0 0 1 0.5 0.5 0.5 0.5 |
|        | *Enterobacter cloacae* | 40 | 39 37 37 37 36 34 24 10 1 0 40 <0.25 - >64 1 <4 1.54 |
|        | *Enterobacter aerogenes* | 40 | 39 37 37 37 36 34 30 14 3 0 40 <0.25 - >64 1 4 1.80 |
|        | *Citrobacter Ferrandi* | 20 | 19 18 18 17 17 16 10 6 2 0 20 <0.25 - >64 1 <6 1.87 |
|        | *Proteus* | 20 | 20 20 20 20 16 8 2 0 0 0 20 <0.25 - >64 4 8 3.25 |

### Bactericidal effect of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter Ferrandi* and *Proteus* in vitro

| Sample | Bacteria Strains | Number of Strains | Strains of bacteria not growing under different drug concentrations (μg/ml) | MBC range (μg/ml) | MBC$_{0}$ (μg/ml) | MBC$_{90}$ (μg/ml) |
|--------|------------------|-------------------|---------------------------------------------------------------------|-----------------|----------------|-----------------|
| PL-5   | *Enterobacter cloacae* (029M) | 1 | 1 1 1 1 1 1 0 0 0 - 0 1 4 4 4 8 |
|        | *Enterobacter cloacae* | 40 | 40 38 35 28 6 0 0 0 - 0 40 4 - >64 8 >64 32 |
|        | *Enterobacter aerogenes* | 40 | 28 27 25 20 11 3 0 0 - 0 40 2 - >64 8 >64 64 |
|        | *Citrobacter Ferrandi* | 20 | 17 17 17 11 6 1 0 0 - 0 20 2 - >64 8 >64 64 |
|        | imipenem cilastatin sodium | | | | | | |
|        | *Enterobacter cloacae* (029M) | 1 | 1 1 1 1 1 1 0 0 0 1 1 1 1 1 1 1 1 |
|        | *Enterobacter cloacae* | 40 | 39 34 27 13 9 6 3 1 0 0 40 0.5 - >64 16 >64 64 |
|        | *Enterobacter aerogenes* | 40 | 37 36 30 23 12 8 6 4 1 0 40 <0.25 - >64 8 >64 32 |
|        | *Citrobacter Ferrandi* | 20 | 17 16 14 14 9 6 5 3 2 0 20 <0.25 - >64 8 >64 64 |
|        | *Proteus* | 20 | 19 18 17 15 0 0 0 0 0 0 20 4 - >64 16 >64 32 |
Cumulative antibacterial percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Enterobacter cloacae, Enterobacter aerogenes, Citrobacter Ferrandi and Proteus in vitro*

| Sample          | Bacteria Strains | Number of Strains | Cumulative antibacterial percentage of different concentrations (μg/ml) of tested drugs (%) | MBC<sub>50</sub>/MIC<sub>50</sub> | MBC<sub>90</sub>/MIC<sub>90</sub> |
|-----------------|------------------|-------------------|-------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------|
| PL-5            | Enterobacter cloacae | 40               | 100 100 100 85 33 0 0 0 - 1 2                                                             |                                 |                                 |
|                 | Enterobacter aerogenes | 40               | 70 68 68 60 35 8 0 0 - 1 -                                                             |                                 |                                 |
|                 | Citrobacter Ferrandi | 20               | 90 90 90 80 40 15 0 0 - 1 >4                                                          |                                 |                                 |
|                 | Enterobacter cloacae | 20               | 0 0 0 0 0 0 0 0 - - -                                                                  |                                 |                                 |

Cumulative bactericidal percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Enterobacter cloacae, Enterobacter aerogenes, Citrobacter Ferrandi and Proteus in vitro*

| Sample          | Bacteria Strains | Number of Strains | Cumulative bactericidal percentage of different concentrations (μg/ml) of tested drugs (%) | 64 32 16 8 4 2 1 0.5 0.25 |
|-----------------|------------------|-------------------|-------------------------------------------------------------------------------------------------|--------------------------|
| PL-5            | Enterobacter cloacae | 40               | 100 95 88 70 15 0 0 0 -                                                                   | 0                        |
|                 | Enterobacter aerogenes | 40               | 70 68 63 50 28 8 0 0 -                                                                   | 0                        |
|                 | Citrobacter Ferrandi | 20               | 85 85 85 55 30 5 0 0 -                                                                   | 0                        |

Cumulative bactericidal percentage of antimicrobial peptide PL-5 and imipenem cilastatin sodium on *Enterobacter cloacae, Enterobacter aerogenes, Citrobacter Ferrandi and Proteus in vitro*
Overview of Preclinical Pharmacokinetic Study

Twenty-five healthy minipigs were divided into 5 groups, with 5 pigs in each group, 3 males and 2 females. The three groups were given 0.01, 0.05 and 0.25 mg/cm² PL-5 on an area of 3 cm × 3 cm. At 0.5, 1, 2 and 3 hours after application, wipe the skin of the administration site on the back of the pigs respectively, and scrape the cuticle skin of the administration site at the corresponding time point with a scalpel (scrape until the skin is obviously red but there is no bleeding). Wipe the skin of the administration site 3 hours after application, and scrape the cuticle skin of the administration site at the corresponding time point at 0.5, 1, 2, 3 and 4 h (i.e. 3.5, 4, 5, 6 and 7 hours after administration). At the same time, on each pig the control part was set to smear normal saline. The other two groups were smeared or injected with PL-5 with 20 mg/animal respectively. Blood was taken from the vein of pig forelimb at 15 and 30 min, 1, 1.5, 2, 3, 4 and 6 h after application or 5, 15, 30 min, 1, 1.5, 2, 3, 4 and 6 h after intravenous injection.

The results showed that the plasma concentration of PL-5 was lower than the lower limit of quantitation; suggesting that PL-5 could not enter the blood circulation when administered percutaneously on minipigs; After intravenous administration in minipigs, the blood drug concentration reached the peak instantaneously and then decreased. The elimination process accorded with the first-order kinetic elimination model. The blood drug concentration C₀ value was 14418.50 ± 5846.15 ng / ml, the plasma drug exposure level AUC (0-t) was 12307.64 ± 1916.28 h . ng . ml⁻¹, and the elimination phase half-life t¹/₂ was 0.66 ± 0.15 h.

270 BALB/c mice were randomly divided into 45 groups with 6 mice in each group. Every 9 groups were smeared with 0.01, 0.05 and 0.25 mg/cm² PL-5, on an area of 1 cm² × 2 cm. A group of mice were killed at each time point 0.5, 1, 2 and 3 hours after application. The skin of the administration site was wiped clean and the corresponding skin tissue was harvested. Wipe the skin of the administration site 3 hours after application, and take the skin tissue in the same way at 0.5, 1, 2, 3 and 4 hours (i.e. 3.5, 4, 5, 6 and 7 hours after administration). The remaining 8 groups of mice were smeared with 0.05 mg/cm² of PL-5. One group of mice were killed at 15 and 30 minutes, 1, 1.5, 2, 3, 4 and 6 hours after application, and the skin tissue was taken in the same way. Nine groups of mice were intraperitoneally injected with PL-5, 0.1 mg/each. One group of mice was injected at each time point. Blood was collected at 5, 15 and 30 minutes, 1, 1.5, 2, 3, 4 and 6 hours after intravenous injection. The remaining one group of mice was set as the blank control group, and blank blood samples and blank skin tissues were taken respectively.

The results showed that there was basically no detectable drug concentration in plasma after smear administration (lower limit of quantification: 25 ng/ml), and the plasma drug concentration slightly higher than the lower limit of quantification could only be detected in few individual mice; After intraperitoneal injection, the peak time of plasma concentration was 1~1.5 h, and the AUCₜ₀⁻₅ of plasma exposure level was 581.83 h . ng . ml⁻¹. The proportion of mice with drug entering the blood circulation after smear administration was about 3.4%, suggesting that the drug could barely enter the blood circulation after percutaneous administration of PL-5 in BALB/c mice.

The results of pharmacokinetic study showed that after PL-5 was applied to the skin of minipigs or BALB/c mice, most of the applied drugs stayed on the surface of the skin stratum corneum, and less than 1% of the drugs could enter the epidermis through the skin pores. In the two animals, with the increase of dose, the time point and individual of blood drug concentration increased, suggesting that the amount entering the epidermal layer is basically proportional to the dose. Individual differences are obvious. After PL-5 was applied to the skin of the two animals, the proportion of drug absorption into blood through the skin was very low.
Toxicity test

1. Acute toxicity test

1.1 Acute toxicity of single dose of PL-5 in SD rats

Sixty SD rats, half male and half female were divided into 3 groups (solvent control group, intact skin group and wounded skin group), with 20 rats in each group. The dosage of antimicrobial peptide PL-5 was 2000 mg / kg. The administration volume was 20 ml / kg. For transdermal administration, a layer of sulfuric acid paper is placed to cover the administration and fixed with non-irritating adhesive tape. After 4 hours of drug administration, the sulfuric acid paper is removed, and the residual solvent or test drug is removed with warm soapy water. After administration, the rats were kept in single cage for 14 days. The occurrence and duration of poisoning symptoms, the development of poisoning symptoms and the time of death were observed, and the dead animals were dissected.

On the day of administration, the behavior and physiological indexes of the animals in the solvent control group and intact skin group were normal, and no adverse reactions were found. In the wounded skin administration group, except for slight bleeding at the wounded skin, the behavior and physiological indexes of the animals were normal after administration, and there were no abnormal symptoms related to administration such as skin redness and swelling.

During the 14 day observation period, the weight of the animals in the solvent control group, intact skin group and wounded skin group all increased. There was no significant difference in body weight among intact skin group, wounded skin group and solvent control group. All animals had normal behavior and physiological indexes, no adverse reactions were observed and no death occurred for all the animals. In the wounded skin group, the wounded skin gradually crusted, and then the crusts gradually fell off, and the hair gradually grew and returned to normal. After the end of the observation period, no abnormality was found in dissections.

The results of the study showed the maximum tolerated dose of PL-5 was more than 2000 mg/kg.

1.2 Acute toxicity of single dose of antimicrobial peptide PL-5 in mini pigs

The experiment was divided into three groups: normal skin control group, normal skin administration group, and wounded skin administration group. The normal skin control group was given the solvent. For the normal skin administration group and the wounded skin administration group, antimicrobial peptide PL-5 solution was applied to the skin at a dose of 2000 mg per animal. There were 2 animals in each group, half male and half female. The animal behaviour and the skin site of drug administration were carefully observed and recorded. The blood samples were collected before and 14 days after the drug delivery, and the corresponding blood biochemical indexes and ECG were examined. At the end of the observation period, dissections of the mini pigs were conducted under anaesthesia.

From the day of drug administration to the end of the experiment, there was no toxic reaction related to the drug administration in all groups of animals. There were no significant changes in body temperature, body weight, blood tests and biochemical indexes before and after drug administration. At the end of the observation period, there was no obvious abnormality in the dissection findings of the animals in each group.

The results of the study showed that under the experimental conditions, for a single dose of antimicrobial peptide PL-5 given to mini pigs through normal skin and wounded skin at a dose of 2000 mg/pig, there was no obvious systemic toxicity observed.

2. Chronic toxicity test

2.1 Study on toxicity and toxicokinetics of antimicrobial peptide PL-5 after repeated administration for 30 days in SD rats

A total of 190 SD rats, half male and half female, were randomly divided into three groups, including solvent control group, low, medium and high dose of PL-5 groups (0.25, 0.75 and 1.50 mg / cm², respectively) and wounded skin group (PL-5 was given at 1.50 mg / cm²), with 15 male and 15 female rats in each group. In all PL-5 groups, there were 5 more animals used for blood collection of toxicokinetic experiment. The shaving range of rats was about 5 × 5 cm². The wounded skin was damaged to the extent of slight bleeding. The rats were smeared with 1 ml of drug through the intact skin or wounded skin, once a day for 30 days, and the recovery
period was 14 days. At the end of administration (D31), 10 animals per sex were killed in each group, and at the end of recovery period (D45), 5 animals per sex were killed in each group. The examined indexes included general physiological indexes, body weight, food intake, haematology, coagulation, serum biochemistry, electrolytes, urine indexes and histopathological examination.

During the transdermal delivery of PL-5 at 0.25, 0.75, 1.50 mg / cm² respectively in normal skin group and 1.50 mg / cm² in wounded skin group for 30 days, there was no toxic reaction related to the administration observed. There was no irritating effect on normal intact skin or wounded skin. The no observed adverse effect level (NOAEL) was 1.50 mg / cm² (the area of administration was 5 × 5cm²).

After 2 weeks of drug withdrawal, no delayed or cumulative toxicity was found. There was a detectable level of PL-5 in the plasma after repeated application of the drug to the skin of rats, indicating plasma exposure. The study results indicate: a) The level of PL-5 exposure in plasma was positively correlated with administration dose; b) There was a similar degree of drug accumulation in each dose group after repeated administration, and the accumulation rate was between 4.1 and 5.4. c) There was no significant difference in drug absorption between intact skin and wounded skin under the experimental conditions.

2.2 Study on the toxicity and toxicokinetics of antimicrobial peptide PL-5 after repeated administration for 30 days in mini pigs

The doses of antimicrobial peptide PL-5 were given as 20, 60, 200, 200 mg / animal respectively for low, medium, high and wounded skin high dose groups. Another solvent control group was given solvent only. There were 10 animals in each group, with half male and half female. Drug/solvent were administered to the animals by smearing onto the skin for 30 continuous days, and the observed recovery period was 30 days. The detection indexes included: general condition, food intake, body weight, body temperature, electrocardiogram, haematological indexes, serum biochemical indexes, urine indexes and histopathological examination. The haematological and biochemical indexes were detected twice in quarantine period, 1 time at the end of administration and 1 time at the end of recovery period. At the end of administration and recovery period, the corresponding animals were killed for urine index and gross anatomy observation, organ weighing and histopathological examination. At the same time, the concomitant toxicokinetics analysis was carried out after drug administration. The blood sampling points of toxicokinetics were before administration, 1 h, 2 h, 4 h, 8 h and 24 h after administration on the first day; On the 30th day before administration, 1h, 2h, 4h, 8h and 24h after administration.

Under the experimental conditions, mini pigs were smeared with antimicrobial peptide PL-5 through the skin at a dose of 20, 60, 200mg / animal for 30 days. No toxic pathological changes clearly related to the administration were found in the main organs and tissues of animals in each administration group, and there was no obvious irritation to the integrity of the administration site and wounded skin. After 30 days of withdrawal, there was no delayed or cumulative toxicity associated with administration. There was no detectable level of PL-5 in the plasma of miniature pigs after the drug was smeared on the skin repeatedly. In this experiment, the NOAEL of antimicrobial peptide PL-5 was 200 mg / pig, and the MTD was more than 200 mg / pig.

2.3 Study on toxicity and toxicokinetics of antimicrobial peptide PL given intravenously for 4 weeks in Beagle Dogs

The experiment was designed to set up four dose groups: solvent control group, low dose group, medium dose group and high dose group, with the dosage of 0, 0.5, 1 and 2 mg/kg respectively. The trial was designed with repeated administration for 4 weeks, and the actual continuous drug administration period was 16 days (the blood was collected on the 15th day of clinical pathology examination. The drug was administered for another 2 days and the animal was killed on D17), and recovery period was 28 days. The test indexes include general animal condition, food intake, body weight, body temperature, electrocardiogram, ophthalmology, haematology, coagulation, serum biochemistry, electrolyte, urine index, toxicokinetics and histopathology examination. The body weight was measured for 3 times in quarantine period. The body temperature and food intake was tested twice in quarantine period. The above tests were conducted once a week during administration and recovery period. ECG examination, haematology, coagulation, biochemistry, electrolyte index detection were performed twice for quarantine period, one time on D15 and at the end of recovery period. Ophthalmic examination was conducted before drug administration, on D15, and after the end of recovery period. The urine indexes and general anatomy were observed by killing the animals at the end of administration and recovery period. The blood was collected for toxicokinetics on the first day of drug administration and on D14.
On the fourth day of drug administration, swelling appeared in the administration site of individual animals in low, medium and high dose groups. One week after administration, swelling appeared in the administration site of most experimental animals, and gradually returned to normal in the recovery period. The body weight, body temperature, food intake, haematology, serum biochemistry, electrolyte indexes, coagulation indexes, ECG indexes, organ weight and organ coefficient, and ophthalmic examination all indicated: no abnormalities with toxicological significance were found in each dose group at the end of administration and recovery period.

Toxicokinetics
Under the condition of this experiment, after repeated intravenous administration of 0.5, 1 and 2mg/kg of PL-5 for 2 weeks, accumulation of drug was absent on D14 compared with the first day of administration. The exposure increased with the increase of drug dosages, while without significant gender differences.

Histopathology
Under the condition of this experiment, Beagle dogs were repeatedly injected with 0.5 mg / kg, 1 mg / kg and 2 mg / kg of antimicrobial peptide PL-5 intravenously for 16 days, and the drug induced certain irritation reaction to the skin of the administration site, and caused subcutaneous edema of the local skin. No toxic pathological changes related to the administration were found in other organs and tissues. After 4 weeks of drug withdrawal, the subcutaneous edema of local skin partially recovered, and no delayed or accumulated toxic pathological changes were found in other organs and tissues.

The experiment showed that under the conditions of this experiment, Beagle dogs received toxicity test through repeated intravenous injection of antimicrobial peptide PL-5 for 16 days, and no obvious toxic reaction was found with the dose of 2 mg / kg.
Allergy test

Skin allergy test of guinea pigs after percutaneous administration of antimicrobial peptide PL-5

40 guinea pigs were selected by Buehler test, with half male and half female. The animals were divided into negative control group, positive control group and PL-5 spray group. There are 10 animals in both negative control group and positive control group, and 20 animals in PL-5 spray group. The negative control group was given 0.5ml of 0.9% Sodium Chloride Injection each for sensitization, and 0.5ml of PL-5 spray was given for stimulation. For drug delivery group, 0.5ml of PL-5 was given for both sensitization and stimulation. For the positive control group, 0.2ml of 1% 2,4-dinitrochlorobenzene was given to each animal during sensitization, and 0.2ml of 0.1% 2,4-dinitrochlorobenzene was given to each animal for stimulation. During sensitization, the drug was administered on D0, 7 and 14. For stimulation, drug was applied to the back of the non-administration side 14 days after the last sensitization. Observe and score the erythema and edema of the local skin at 1 hour and 24 hours after each sensitization application, as well as 24 hours and 48 hours after removal of stimulation patch to evaluate the intensity of allergic reaction.

During the experiment, the animals in the treatment group and the control group had normal water drinking, food intake and behavioural activities, and were all in good health; The animals showed no abnormal symptoms related to the drug administration.

There was no significant difference in body weight between male and female animals in the first sensitization group, the last sensitization group and the positive control group and the negative control group.

After first sensitization, 1 hour and 24 hours after the sensitization patch was removed, abnormal skin reactions were not observed in the control group and PL spray group.

One hour and 24 hours after the removal of sensitization patch of second sensitization, abnormal skin reactions were not observed in the negative control group and PL spray group, while mild erythema appeared on the local skin surface of some animals in the positive control group.

Twenty-four hours after the stimulation, the patches were removed. Abnormal local skin reactions were not observed in the negative control group and the PL-5 spray group. Seven of the 10 animals in positive control groups showed mild erythema on the local skin, and no erythema was found in the other 3 animals. No edema reaction was found in the local skin of all tested animals.

The patches were removed 48 hours after the stimulation, No abnormal local skin reactions were observed in the negative control group and the PL-5 spray group, nine of the 10 animals in positive control groups showed mild erythema on the local skin, and no erythema was found in the remaining 1 animal. No edema reaction was found in the local skin of all tested animals. After all animals were stimulated, the blocking patches were removed for 24 hours and 48 hours for observation. The sensitization rate of the positive control group was 90%, and the allergic reaction grade was grade V, which was extremely sensitized; The sensitization rate of the negative control group and the PL-5 spray group was 0, and there was no allergenic effect.

Under the experimental conditions, PL-5 Spray did not cause skin allergic reactions in guinea pigs.
Irritation test

Six Japanese white rabbits with half male and half female were used in this experiment. The experiment was carried out by self-comparison on the left and right sides of the same body. There were intact skin administration group, control group, wounded skin administration group and control group respectively. All 6 animals had intact skin area on the cephalic side and wounded skin area on the caudal side. The left side was the administration side and the right side was the control side. The control group was given 0.9% sodium chloride injection. Transdermal drug administration was adopted in the experiment. The dosage was 0.5ml/administration area, once a day for 7 consecutive days. During the administration period and 1 hour, 24 hours, 48 hours and 72 hours after the last administration, the stimulation response score was given and the stimulation response intensity was evaluated. During this test, 6 animals drank and ate normally, gained weight and were in good health. No abnormality was found in general observation.

During the administration period, the skin irritation reaction was observed on the intact skin administration side and the wounded skin administration side of all test animals 1 hour after each removal of the drug and before re-administration. There was no abnormality in the local skin of the test animals, and the irritation reaction score was 0.

At 1 hour, 24 hours, 48 hours and 72 hours after the last administration, no abnormality was found in the local skin of the test animals, and the irritation response score was 0. Under the experimental conditions, rabbit skin transdermal PL-5 spray had no irritation on skin tissue.

Under the experimental conditions, PL-5 spray transdermal administration had no irritation on rabbit skin tissues.
Phase I clinical trial

1. Clinical study on single dose tolerance of antimicrobial peptide PL-5 spray to human body

From September 2016 to March 2017, the clinical study on the single dose tolerance of antimicrobial peptide PL-5 Spray was conducted in the Beijing You An hospital affiliated to Capital Medical University. A total of 40 healthy subjects were enrolled. The dose group and the number of cases in each group were as follows:

| Groups | 1       | 2       | 3       | 4       | 5       |
|--------|---------|---------|---------|---------|---------|
| Dosages| 0.003125mg/cm² (0.5‰) | 0.00625mg/cm² (1‰) | 0.0125mg/cm² (2‰) | 0.025mg/cm² (4‰) | 0.0375mg/cm² (6‰) |
| NO. of Subjects | 6+2 | 6+2 | 6+2 | 6+2 | 6+2 |

Safety

There were no serious adverse reactions in this study. The age, body mass index, gender and other general data of all subjects met the inclusion criteria, and the vital signs, physical examination and skin status of all subjects were normal during the screening period.

In terms of safety, the vital signs, physical examination and laboratory examination of the subjects were normal, except for few subjects had abnormal laboratory examination indexes and ECG examination during the trial, which had no clinical significance.

During the clinical study, there were 13 subjects having a total of 18 adverse events, all of which were abnormal laboratory examinations, including abnormal ECG (5/13, 38.46%), positive urine occult blood (2/13, 15.38%), increased creatinine (2/13, 15.38%), increased leukocyte (2/13, 15.38%), increased neutrophils (2/13, 15.38%), decreased neutrophils (1/13, 7.69%), increased monocytes (1/13, 7.69%), increased lymphocytes (1/13, 7.69%), increased blood bilirubin (1/13, 7.69%) and positive fecal occult blood (1/13, 7.69%). No SAE occurred during the clinical study.

Pharmacokinetics

In the clinical study of single dose tolerance of antimicrobial peptide PL-5 spray, with different dosages given to human body, all samples were below the lower limit of quantification, and no plasma concentration was detected.

2. Clinical study on the safety and tolerance of repeated application of antimicrobial peptide PL-5 sprays in human body

From November 2017 to December 2017, the clinical study on the safety and tolerance of repeated application of antimicrobial peptide PL-5 Spray was conducted in the Beijing You An hospital affiliated to Capital Medical University. A total of 24 healthy subjects were enrolled. The dose group and the number of cases in each group were as follows:

| Groups | A       | B       |
|--------|---------|---------|
| Dosages| 0.025mg/cm² (4‰) | 0.0375mg/cm² (6‰) |
| NO. of Subjects | 10+2 | 10+2 |

Safety

There were no serious adverse reactions in this study. The age, body mass index, gender and other general data of all subjects met the inclusion criteria, and the vital signs, physical examination and skin status of all subjects were normal during the screening period.
In terms of safety, the vital signs, physical examination and laboratory examination of the subjects were normal, except for few subjects had abnormal laboratory examination indexes and ECG examination during the trial, which had no clinical significance.

During the clinical study, there were 7 subjects having a total of 8 adverse events, all of which were abnormal laboratory examinations, including increased leukocyte (2/7, 28.57%), increased total bilirubin (2/7, 28.57%), increased lymphocytes (1/7, 14.29%), abnormal ECG (1/7, 14.29%), urinary protein 1+ (1/7, 14.29%), increased alanine aminotransferase (1/7, 14.29%). No SAE occurred during the clinical study.

Pharmacokinetics
In the clinical study of the safety and tolerance of repeated application of antimicrobial peptide PL-5 sprays in healthy subjects, the drug concentrations of all the blood samples of the 0.0375mg/cm² (6‰) dose group were lower than the lower limit of quantification, no plasma concentration was detected, which confirmed that the drugs did not enter the blood circulation system.
Phase IIa clinical trial

An open-label, randomized, controlled, dose-exploration phase IIa clinical trial of antimicrobial peptide PL-5 spray for treating wound infections

From July 2018 to September 2019, an open-label, randomized, controlled, dose-exploration phase IIa clinical trial of antimicrobial peptide PL-5 spray for treating wound infections was conducted in 4 research centres including Jinan Central People's Hospital, Zhengzhou first people's Hospital, Guangzhou Red Cross Hospital and Yifu Hospital Affiliated to Nanjing Medical University. A total of 40 subjects were enrolled.

The characteristics of demographic data and baseline (overall wound infection, wound area and positive rate of bacteriological examination) were similar in each group, and the proportion between groups was relatively balanced. The number of cases in the dose group and the control group was as follows:

| Groups                  | 0.00625 mg/cm² (1% PL-5) | 0.025 mg/cm² (4% PL-5) | 0.0375 mg/cm² (6% PL-5) | Positive control |
|-------------------------|---------------------------|------------------------|-------------------------|------------------|
| NO. of Subjects         | 10                        | 10                     | 10                      | 10               |

Safety
There were no serious adverse reactions in this study. The age, body mass index, gender and other general data of all subjects met the inclusion criteria, and the vital signs and physical examination of all subjects were normal during the screening period.

In terms of safety, except that some subjects had cough, expectoration, hemorrhoids (previous history of hemorrhoids), scar skin pruritus, constipation, rash and abnormal laboratory examination results, other subjects had normal vital signs, physical examination and laboratory examinations during medication.

During the trial, 10 subjects had a total of 15 adverse events (AE), and the incidence of AE was 25%. AE grading showed that 86.7% were grade 1 adverse events and 13.3% were grade 2 adverse events; The correlation analysis between AEs and drugs showed that 20% were possibly related and 80% were possibly or definitely unrelated. All AEs have disappeared or been relieved after the trial. There were no serious adverse events/adverse reactions, adverse events/adverse reactions leading to drug discontinuation, and adverse events/adverse reactions leading to withdrawal from the trial during the study period.

The adverse events were listed as follows according to their incidence: occult blood in routine urine (2/15, 13.33%), increased crystallinity in routine urine (2/15, 13.33%), leukocyte lower than the lower limit of normal value (1/15, 6.67%), neutrophil lower than the lower limit of normal value (1/15, 6.67%), allergic reaction (1/15, 6.67%), hemorrhoids (1/15, 6.67%), cough (1/15, 6.67%), expectoration (1/15, 6.67%), scar skin itching (1/15, 6.67%), increased blood urea (1/15, 6.67%), increased platelet count (1/15, 6.67%), constipation (1/15, 6.67%), and rash (1/15, 6.67%).

Efficacy
Clinical efficacy evaluation: On the first day after treatment's end, the clinical cure rates of subjects in the control group (SSD Cream), 0.00625mg/cm² group (1% PL-5), 0.025mg/cm² group (4% PL-5) and 0.0375mg/cm² group (6% PL-5) were 70% (7/10), 70% (7/10), 90% (9/10) and 100% (10/10) respectively.

PL-5 sprays of all dosage groups have showed good clinical effects. On the first day after treatment's end, the cure rates in each dose group were all above 70%, while 0.025mg/cm² group (4% PL-5) and 0.0375mg/cm² group (6% PL-5) reached 90% and 100% respectively, which were superior to those of the positive control SSD Cream.

Evaluation of microbiological efficacy: On the third day of administration, the bacterial clearance rates of the control group, 0.00625mg/cm² (1%), 0.025mg/cm² (4%) and 0.0375mg/cm² (6%) groups were 20% (1/5), 0 (0/3), 25% (1/4) and 0 (0/6) respectively. On the fifth day of administration, the clearance rates of each group were 60.0% (3/5), 33.3% (1/3), 25.0% (1/4) and 0 (0/6), respectively. On the first day after treatment's end, the clearance rates of each group were 60.0% (3/5), 0 (0/3), 50% (2/4) and 16.7% (1/6), respectively.

Due to the relatively small sample size and the low positive rate of microbial detection in the screening period, there is no statistical difference in the bacterial clearance rate among different groups. However, the existing data
have showed that the bacterial clearance rate of the 0.025mg/cm² (4‰ PL-5) group is equivalent to that of the control group on the third day of administration and the first day after treatment's end. It is necessary to increase the sample size in the later stage and improve the positive rate in the screening period to obtain more accurate evaluation results.

The results of drug sensitivity test of clinical strains showed that PL-5 had strong antibacterial effect on clinically isolated Staphylococcus, Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae and Streptococcus parahaemolyticus in vitro, and the MIC range was 8~32μ g/ml, which was better than the positive control drug SSD (MIC range 16~128μ g/ml). The antibacterial effect of PL-5 on Acinetobacter and Pseudomonas aeruginosa (MIC range 16~64μ g/ml was close to the positive control drug SSD (MIC range 4~16μ g/ml). PL-5 has relatively poor antibacterial effect on Enterococcus faecalis, Proteus mirabilis, Providencia and Mycobacterium abscessum in vitro. The results of sensitivity test in this study are in good agreement with the results of preclinical study.

Comprehensive efficacy analysis: on the third day of administration, the comprehensive cure rates of the control group, 0.00625mg/cm² (1‰ PL-5), 0.025mg/cm² (4‰ PL-5) and 0.0375mg/cm² (6‰ PL-5) groups were 20% (1/5), 0 (0/3), 0 (0/4) and 0 (0/6) respectively. On the first day after treatment's end, the comprehensive cure rates of the control group, 0.00625mg/cm² (1‰ PL-5), 0.025mg/cm² (4‰ PL-5) and 0.0375mg/cm² (6‰ PL-5) groups were 40% (2/5), 0 (0/3), 50% (2/4) and 16.7% (1/6) respectively.

Due to the insufficient microbiological efficacy data, there are few available data for comprehensive efficacy evaluation. Therefore, it is necessary to increase the sample size in the later studies. However, the existing results suggest that the 0.025mg/cm² (4‰ PL-5) group has better comprehensive curative effect and cure rate than the control group on the first day after treatment's end. Antimicrobial peptide PL-5 spray has shown promising efficacy in fighting against bacterial infection and promoting wound healing.
Table S1. The Skin Infection Rating Scales (SIRS)

| Signs/Symptom          | Score | Definition                                                                 |
|------------------------|-------|----------------------------------------------------------------------------|
| Exudate/pus            | 0=Absent | No evidence of exudates or pus                                             |
|                        | 1=mild | Small amounts of fluid/pus coming from the lesions                        |
|                        | 2=Moderate | Exudate/pus infected area is moderate                                      |
|                        | 3=Severe | Extensive area of skin lesion is infected and there is draining exudate    |
| Crusting               | 0=Absent | No evidence of crusting                                                   |
|                        | 1=mild | A few areas have some evidence of crusting lesions                         |
|                        | 2=Moderate | Crusting is present throughout the infected area                           |
|                        | 3=Severe | Thick crusting appears over the entire infected area                      |
| Erythema/inflammation  | 0=Absent | Skin tone and color are normal; no signs of erythema or inflammation      |
|                        | 1=mild | Skin is pink with minimal signs of inflammation                           |
|                        | 2=Moderate | Skin is red with definite signs of inflammation                           |
|                        | 3=Severe | Skin is red and severe inflammation is present                            |
| Tissue edema           | 0=Absent | No evidence of tissue edema                                                |
|                        | 1=mild | Tissue has mild edema                                                     |
|                        | 2=Moderate | Tissue has moderate edema                                                 |
|                        | 3=Severe | Tissue has severe edema                                                   |
| Tissue warmth          | 0=Absent | No evidence of tissue warmth                                               |
|                        | 1=mild | Tissue has mild warmth                                                    |
|                        | 2=Moderate | Tissue has moderate warmth                                                |
|                        | 3=Severe | Tissue has severe warmth                                                   |
| Itching                | 0=Absent | No itching                                                                 |
|                        | 1=Mild | Some evidence of scratching or rubbing the area is evident and subject reports minor discomfort |
|                        | 2=Moderate | Evidence of scratching and subject reports bothersome itching             |
|                        | 3=Severe | Evidence of extensive scratching and subject reports itching interferes with daily activities or sleep |
| Pain                   | 0=Absent | No pain                                                                   |
|                        | 1=mild | Slight pain; not bothersome; no analgesics being taken                     |
|                        | 2=Moderate | Definite pain; subject reports bothersome pain, without loss of sleep, mild analgesic may be taken |
|                        | 3=Severe | Intense pain that that interferes with daily activities or sleep; medication required to control pain |
Inclusion and Exclusion Criteria of the phase IIb clinical trial

**Inclusion Criteria (subjects should meet all of the following criteria):**

1. All genders, aged between 18 to 75 years old with both ends included.
2. Having been diagnosed with secondary open wound infections caused by burns, abrasions, scratches, lacerations, stitched wounds, trauma ulcers, pressure ulcers, venous ulcers or Wagner grade 2 diabetic foot ulcers, and fulfilling the following condition:
   a) The length of scratch, tear or suture wound should be no more than 10 cm, the peripheral erythema should be no more than 2 cm, and the area of burn, abrasion, ulcer and pressure ulcer should not be less than 4 cm²;
   b) The clinical symptoms of wound infection meet the following conditions: Skin Infection Rating Scales (SIRS) Scores were no less than 8 points. The infection did not involve deep soft tissues including deep facia and muscles, and topical antibiotics were needed;
3. Positive microscopic bacteria examinations;
4. Male subjects or female subjects of childbearing age agreed to take effective and safe contraceptive measures during and within one week after the treatment (female of childbearing age is defined as a woman who is able to conceive anatomically and physiologically after menarche and before menopause without sterilization);
5. Subjects agree to comply with the prohibitions and restrictions in the protocol, understand and sign the informed consent

**Exclusion criteria (subjects meet any one of the following criteria should be excluded from the trial):**

1. No bacterial culture was performed before entering the group;
2. Patients who used systemic antibiotics within 72 hours before entering the group or used topical antibiotics for the studied wound should be excluded from the trial. However, if the signs of infection didn’t improve after those previous treatments, the patients could still be included through decisions made by the investigators;
3. Mild skin infections that only need simple surgical or wound care procedures, such as furuncle, acne, folliculitis, pustules, etc;
4. Patients had one of the following wound infections:
   a) The wound infection was known or suspected to be caused by fungi, parasites or viruses;
   b) The infected wound developed rapid tissue necrosis, such as necrotizing fasciitis;
   c) The patient had skin inflammatory diseases that may affect the efficacy and safety evaluation, such as atopic dermatitis or eczema;
   d) The wounds were covered with artificial materials that could not be removed, such as central venous catheter, battery pack of permanent pacemaker, or artificial joint;
   e) The studied wound belonged to multiple ulcers of the extremities;
   f) Diabetic foot ulcers of Wagner grade 0 to grade 1, grade 3 and above;
   g) Gangrene of any causes;
5. The treatment of the wound included amputations;
6. The patient was allergic to the studied drug, or any ingredients of the studied drugs, including Antimicrobial Peptide PL-5 Spray and SSD cream; The patient was allergic to sulfonamides or silver salts, or was suffering from any allergic diseases;
7. The female patient was pregnant or was in breast-feeding period; However, if the patient agreed to stop breast-feeding during the treatment period and one week after treatment end, they could still be included.
8. Patients with the following abnormal lab tests should be excluded:
   a) The patient had abnormal hepatic function, including total bilirubin reached 3 times above the normal upper limits, or Alanine aminotransferase (ALT), or aspartate aminotransferase (AST) reached 5 times above the normal upper limits;
   b) The patient had or suspected to have a history of severe renal impairment (Serum creatinine reached 5 times above the normal upper limits) or had any types of dialysis;
   c) The patient had neutropenia (absolute neutrophil count ≤1.5x10⁹/L) or platelet count≤50x10⁹/L;
9. Patient with grade III or grade IV abnormal heart function according NYHA criteria;
10. Patient with immune deficiency, such as congenital immune deficiency, being treated with immunosuppressant or HIV positive;
11. Patient who was using tumor chemotherapy drugs;
12. Patient with mental disorders or epilepsy;
13. Patient who participated in clinical trials of any drugs or devices within 3 months before screening;
14. The patient had any situations that the researchers considered that may increase the risk of the subjects or affect the evaluation of the efficacy.
Table S3. Demographic and Clinical Characteristics of the Patients at Baseline

|                      | 1% PL-5 (N=59) | 2% PL-5 (N=61) | 4% PL-5 (N=60) | 1% SD-Ag (N=40) | Total (N=220) |
|----------------------|----------------|----------------|----------------|-----------------|---------------|
| **Age - yr**         |                |                |                |                 |               |
| Mean (SD)            | 49.2 (12.94)   | 50.1 (13.89)   | 48.9 (15.63)   | 51.0 (15.80)    | 49.7 (14.42)  |
| Median               | 51.0           | 51.0           | 49.0           | 53.5            | 50.5          |
| Interquartile Range  | 41.0, 58.0     | 43.0, 61.0     | 36.5, 61.5     | 40.5, 66.5      | 40.0, 61.0    |
| Range                | 25-72          | 18-74          | 19-74          | 22-73           | 18-74         |
| **Sex**              |                |                |                |                 |               |
| Male                 | 43 (72.9)      | 39 (63.9)      | 39 (65.0)      | 23 (57.5)       | 144 (65.5)    |
| Female               | 16 (27.1)      | 22 (36.1)      | 21 (35.0)      | 17 (42.5)       | 76 (34.5)     |
| **Types of wound – no. (%)** |            |                |                |                 |               |
| Wagner grade 2 diabetic foot ulcer |           |                |                |                 |               |
| Abrasion             | 0 (0.0)        | 3 (4.9)        | 0 (0.0)        | 5 (12.5)        | 8 (3.6)       |
| Stitched Wound       | 1 (1.7)        | 0 (0.0)        | 0 (0.0)        | 2 (5.0)         | 3 (1.4)       |
| Scratches            | 1 (1.7)        | 0 (0.0)        | 0 (0.0)        | 0 (0.0)         | 1 (0.5)       |
| Venous Ulcer         | 3 (5.1)        | 7 (11.5)       | 6 (10.0)       | 1 (2.5)         | 17 (7.7)      |
| Burns                | 18 (30.5)      | 20 (32.8)      | 14 (23.3)      | 10 (25.0)       | 62 (28.2)     |
| Lacerations          | 0 (0.0)        | 1 (1.6)        | 1 (1.7)        | 1 (2.5)         | 3 (1.4)       |
| Trauma Ulcer         | 6 (10.2)       | 7 (11.5)       | 8 (13.3)       | 6 (15.0)        | 27 (12.3)     |
| Pressure Ulcer       | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (2.5)         | 1 (0.5)       |
| Others               | 29 (49.2)      | 21 (34.4)      | 29 (48.3)      | 14 (35.0)       | 93 (42.3)     |
| **Wound Area cm²**   |                |                |                |                 |               |
| Mean (SD)            | 35.789 (43.3819) | 38.307 (51.877) | 33.567 (42.7419) | 35.212 (42.9645) | 35.779 (45.3995) |
| Median               | 18.000         | 18.000         | 18.000         | 16.500          | 18.000        |
| Interquartile Range  | 10.500-48.000  | 10.000-40.000  | 8.200-40.000   | 8.000-42.000    | 8.000-41.500  |
| Range                | 4.00-224.000   | 4.25-224.000   | 4.10-201.50    | 0.00-178.50     | 0.00-224.000  |
| **Wound SIRS scores**|                |                |                |                 |               |
| Mean (SD)            | 10.6 (2.15)    | 10.6 (2.18)    | 10.2 (1.89)    | 10.4 (2.07)     | 10.5 (2.07)   |
| Median               | 10.0           | 11.0           | 10.0           | 10.0            | 10.0          |
| Interquartile Range  | 9.0, 12.0      | 9.0, 12.0      | 9.0, 11.5      | 9.0, 12.0       | 9.0, 12.0     |
| Range                | 8-17           | 8-19           | 8-16           | 8-15            | 8-19          |
| Table S4. The SIRS evaluations before and after interventions |
|-------------------------------------------------------------|
|                | 1 % PL-5 (N=59) | 2 % PL-5 (N=61) | 4 % PL-5 (N=60) | 1% SSD (N=40) |
|                | n (%)            | n (%)            | n (%)            | n (%)         |
| Exudate/pus    |                 |                 |                 |               |
| Baseline       | 0=AAbsent       | 1 (1.7)         | 0 (0.0)         | 0 (0.0)       |
|                | 1=Mild          | 7 (11.9)        | 15 (24.6)       | 18 (30.0)     |
|                | 2= Moderate      | 31 (52.5)       | 34 (55.7)       | 24 (40.0)     |
|                | 3=Severe        | 20 (33.9)       | 11 (18.0)       | 18 (30.0)     |
| D5             | 0=AAbsent       | 5 (8.5)         | 4 (6.6)         | 6 (10.0)      |
|                | 1=Mild          | 42 (71.2)       | 43 (70.5)       | 39 (65.0)     |
|                | 2= Moderate      | 11 (18.6)       | 11 (18.0)       | 13 (21.7)     |
|                | 3=Severe        | 1 (1.7)         | 0 (0.0)         | 1 (1.7)       |
| D5 vs Baseline | Increased       | 0 (0.0)         | 2 (3.3)         | 1 (1.7)       |
|                | Unchanged       | 12 (20.3)       | 19 (31.1)       | 19 (31.7)     |
|                | Decreased       | 47 (79.7)       | 37 (60.7)       | 39 (65.0)     |
| D8             | 0=AAbsent       | 12 (20.3)       | 8 (13.1)        | 13 (21.7)     |
|                | 1=Mild          | 42 (71.2)       | 46 (75.4)       | 40 (66.7)     |
|                | 2= Moderate      | 5 (8.5)         | 5 (8.2)         | 6 (10.0)      |
|                | 3=Severe        | 0 (0.0)         | 0 (0.0)         | 0 (0.0)       |
| D8 vs Baseline | Increased       | 0 (0.0)         | 2 (3.3)         | 1 (1.7)       |
|                | Unchanged       | 6 (10.2)        | 14 (23.0)       | 13 (21.7)     |
|                | Decreased       | 53 (89.8)       | 43 (70.5)       | 45 (75.0)     |
| Crusting       | 0=AAbsent       | 17 (28.8)       | 18 (29.5)       | 18 (30.0)     |
|                | 1=Mild          | 22 (37.3)       | 16 (26.2)       | 18 (30.0)     |
|                | 2= Moderate      | 10 (16.9)       | 15 (24.6)       | 16 (26.7)     |
|                | 3=Severe        | 10 (16.9)       | 12 (19.7)       | 8 (13.3)      |
| D5             | 0=AAbsent       | 33 (55.9)       | 41 (67.2)       | 33 (55.0)     |
|                | 1=Mild          | 20 (33.9)       | 12 (19.7)       | 14 (23.3)     |
|                | 2= Moderate      | 6 (10.2)        | 4 (6.6)         | 10 (16.7)     |
|                | 3=Severe        | 0 (0.0)         | 1 (1.6)         | 2 (3.3)       |
| D5 vs Baseline | Increased       | 4 (6.8)         | 2 (3.3)         | 5 (8.3)       |
|                | Unchanged       | 24 (40.7)       | 25 (41.0)       | 24 (40.0)     |
|                | Decreased       | 31 (52.5)       | 31 (50.8)       | 30 (50.0)     |
| D8             | 0=AAbsent       | 39 (66.1)       | 42 (68.9)       | 40 (66.7)     |
|                | 1=Mild          | 19 (32.2)       | 13 (21.3)       | 11 (18.3)     |
|                | 2= Moderate      | 1 (1.7)         | 3 (4.9)         | 5 (8.3)       |
|                | 3=Severe        | 0 (0.0)         | 1 (1.6)         | 3 (5.0)       |
| D8 vs Baseline | Increased       | 2 (3.4)         | 3 (4.9)         | 4 (6.7)       |
|                | Unchanged       | 22 (37.3)       | 23 (37.3)       | 21 (35.0)     |
|                | Decreased       | 35 (59.3)       | 33 (54.1)       | 34 (56.7)     |
| Erythema/inflammation | 0=AAbsent       | 1 (1.7)         | 2 (3.3)         | 3 (5.0)       |
|                | 1=Mild          | 18 (30.5)       | 21 (34.4)       | 21 (35.0)     |
|                | 2= Moderate      | 35 (59.3)       | 31 (50.8)       | 29 (48.3)     |
|                | 3=Severe        | 5 (8.5)         | 7 (11.5)        | 7 (11.7)      |
| D5             | 0=AAbsent       | 12 (20.3)       | 20 (32.8)       | 19 (31.7)     |
|                | 1=Mild          | 42 (71.2)       | 29 (47.5)       | 33 (55.0)     |
|                | 2= Moderate      | 5 (8.5)         | 9 (14.8)        | 7 (11.7)      |
|                | 3=Severe        | 0 (0.7)         | 3 (4.9)         | 1 (1.7)       |
| D5 vs Baseline | Increased       | 0 (0.0)         | 0 (0.0)         | 2 (3.3)       |
|                | Unchanged       | 17 (28.8)       | 16 (26.2)       | 18 (30.0)     |
|                | Decreased       | 42 (71.2)       | 42 (68.9)       | 39 (65.0)     |
| D8             | 0=AAbsent       | 34 (57.6)       | 32 (52.5)       | 30 (50.0)     |
|                | 1=Mild          | 25 (42.4)       | 26 (42.6)       | 26 (43.3)     |
|                | 2= Moderate      | 0 (0.0)         | 1 (1.6)         | 3 (5.0)       |
|                | 3=Severe        | 0 (0.0)         | 0 (0.0)         | 0 (0.0)       |
| D8 vs Baseline | Increased       | 0 (0.0)         | 0 (0.0)         | 3 (5.0)       |
|                | Unchanged       | 7 (11.9)        | 9 (14.8)        | 8 (13.3)      |
|                | Decreased       | 52 (88.1)       | 50 (82.0)       | 48 (80.0)     |
| Tissue edema | Baseline | 0=A bsent | 6 (10.2) | 6 (9.8) | 4 (6.7) | 1 (2.5) |
|--------------|----------|-----------|----------|----------|----------|----------|
|              | 1=Mild   | 20 (33.9) | 28 (45.9) | 27 (45.0) | 18 (45.0) |
|              | 2=Moderate | 31 (52.5) | 23 (37.7) | 23 (38.3) | 17 (42.5) |
|              | 3=Severe | 2 (3.4) | 4 (6.6) | 6 (10.0) | 4 (10.0) |
| D5          | 0=A bsent | 20 (33.9) | 24 (39.3) | 23 (38.3) | 8 (20.0) |
|              | 1=Mild   | 35 (59.3) | 28 (45.9) | 31 (51.7) | 20 (50.0) |
|              | 2=Moderate | 4 (6.8) | 6 (9.8) | 5 (8.3) | 8 (20.0) |
|              | 3=Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.5) |
| D5 vs Baseline | Increased | 0 (0.0) | 1 (1.6) | 2 (3.3) | 3 (7.5) |
|              | Unchanged | 23 (39.0) | 21 (34.4) | 20 (33.3) | 16 (40.0) |
|              | Decreased | 36 (61.0) | 36 (59.0) | 37 (61.7) | 18 (45.0) |
| D8          | 0=A bsent | 36 (61.0) | 37 (60.7) | 34 (56.7) | 17 (42.5) |
|              | 1=Mild   | 22 (37.3) | 21 (34.4) | 23 (38.3) | 16 (40.0) |
|              | 2=Moderate | 1 (1.7) | 0 (0.0) | 2 (3.3) | 4 (10.0) |
|              | 3=Severe | 0 (0.0) | 1 (1.6) | 0 (0.0) | 0 (0.0) |
| D8 vs Baseline | Increased | 0 (0.0) | 0 (0.0) | 1 (1.7) | 0 (0.0) |
|              | Unchanged | 14 (23.7) | 17 (27.9) | 13 (21.7) | 11 (27.5) |
|              | Decreased | 45 (76.3) | 42 (68.9) | 45 (75.0) | 26 (65.0) |
| Tissue warmth | Baseline | 0=A bsent | 5 (8.5) | 5 (8.2) | 8 (13.3) | 5 (12.5) |
|              | 1=Mild   | 28 (47.5) | 23 (37.7) | 32 (53.3) | 22 (55.0) |
|              | 2=Moderate | 22 (37.3) | 27 (44.3) | 18 (30.0) | 8 (20.0) |
|              | 3=Severe | 4 (6.8) | 6 (9.8) | 2 (3.3) | 5 (12.5) |
| D5          | 0=A bsent | 37 (62.7) | 30 (49.2) | 39 (65.0) | 18 (45.0) |
|              | 1=Mild   | 21 (35.6) | 25 (41.0) | 20 (33.3) | 15 (37.5) |
|              | 2=Moderate | 1 (1.7) | 2 (3.3) | 0 (0.0) | 3 (7.5) |
|              | 3=Severe | 0 (0.0) | 1 (1.6) | 0 (0.0) | 1 (2.5) |
| D5 vs Baseline | Increased | 0 (0.0) | 2 (3.3) | 0 (0.0) | 1 (2.5) |
|              | Unchanged | 17 (28.8) | 13 (21.3) | 18 (30.0) | 16 (40.0) |
|              | Decreased | 42 (71.2) | 43 (70.5) | 41 (68.3) | 20 (50.0) |
| D8          | 0=A bsent | 51 (86.4) | 45 (73.8) | 47 (78.3) | 24 (60.0) |
|              | 1=Mild   | 8 (13.6) | 13 (21.3) | 12 (20.0) | 10 (25.0) |
|              | 2=Moderate | 0 (0.0) | 1 (1.6) | 0 (0.0) | 3 (7.5) |
|              | 3=Severe | 0 (0.0) | 2 (3.3) | 1 (1.7) | 3 (7.5) |
| D8 vs Baseline | Increased | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
|              | Unchanged | 7 (11.9) | 8 (13.1) | 16 (26.7) | 13 (32.5) |
|              | Decreased | 52 (88.1) | 51 (83.6) | 43 (71.7) | 24 (60.0) |
| Itching      | Baseline | 0=A bsent | 18 (30.5) | 16 (26.2) | 19 (31.7) | 9 (22.5) |
|              | 1=Mild   | 33 (55.9) | 28 (45.9) | 29 (48.3) | 28 (70.0) |
|              | 2=Moderate | 7 (11.9) | 15 (24.6) | 11 (18.3) | 2 (5.0) |
|              | 3=Severe | 1 (1.7) | 2 (3.3) | 1 (1.7) | 1 (2.5) |
| D5          | 0=A bsent | 33 (55.9) | 31 (50.8) | 33 (55.0) | 12 (30.0) |
|              | 1=Mild   | 22 (37.3) | 26 (42.6) | 21 (35.0) | 24 (60.0) |
|              | 2=Moderate | 4 (6.8) | 1 (1.6) | 2 (3.3) | 1 (2.5) |
|              | 3=Severe | 0 (0.0) | 0 (0.0) | 3 (5.0) | 0 (0.0) |
| D5 vs Baseline | Increased | 5 (8.5) | 7 (11.5) | 7 (11.7) | 4 (10.0) |
|              | Unchanged | 30 (50.8) | 24 (39.3) | 29 (48.3) | 22 (55.0) |
|              | Decreased | 24 (40.7) | 27 (44.3) | 23 (38.3) | 11 (27.5) |
| D8          | 0=A bsent | 40 (67.8) | 32 (52.5) | 36 (60.0) | 21 (52.5) |
|              | 1=Mild   | 17 (28.8) | 26 (42.6) | 20 (33.3) | 16 (40.0) |
|              | 2=Moderate | 2 (3.4) | 1 (1.6) | 2 (3.3) | 0 (0.0) |
|              | 3=Severe | 0 (0.0) | 0 (0.0) | 1 (1.7) | 0 (0.0) |
| D8 vs Baseline | Increased | 3 (5.1) | 5 (8.2) | 6 (10.0) | 2 (5.0) |
|              | Unchanged | 27 (45.8) | 24 (39.3) | 26 (43.3) | 18 (45.0) |
|              | Decreased | 29 (49.2) | 30 (49.2) | 27 (45.0) | 17 (42.5) |
| Pain        | Baseline | 0=A bsent | 3 (5.1) | 2 (3.3) | 2 (3.3) | 1 (2.5) |
|              | 1=Mild   | 23 (39.0) | 24 (39.3) | 23 (38.3) | 12 (30.0) |
|              | 2=Moderate | 25 (42.4) | 27 (44.3) | 28 (46.7) | 21 (52.5) |
|              | 3=Severe | 8 (13.6) | 8 (13.1) | 7 (11.7) | 6 (15.0) |
|        | 0=Absent | 1=Mild | 2=Moderate | 3=Severe |
|--------|----------|--------|------------|----------|
| D5     | 17 (28.8) | 31 (52.5) | 8 (13.6) | 3 (5.1) |
|        | 15 (24.6) | 34 (55.7) | 8 (13.1) | 1 (1.6) |
|        | 17 (28.3) | 35 (58.3) | 7 (11.7) | 0 (0.0) |
|        | 12 (30.0) | 18 (45.0) | 7 (17.5) | 0 (0.0) |
| D5 vs Baseline | 2 (3.4) | 3 (4.9) | 1 (1.7) | 0 (0.0) |
|        | 22 (37.3) | 19 (31.1) | 19 (31.7) | 15 (37.5) |
|        | 35 (59.3) | 36 (59.0) | 39 (65.0) | 22 (55.0) |
| D8     | 24 (40.7) | 30 (50.8) | 4 (6.8) | 1 (1.7) |
|        | 28 (45.9) | 29 (47.5) | 2 (3.3) | 0 (0.0) |
|        | 32 (53.3) | 25 (41.7) | 2 (3.3) | 0 (0.0) |
|        | 15 (37.5) | 19 (47.5) | 3 (7.5) | 0 (0.0) |
| D8 vs Baseline | 0 (0.0) | 14 (23.7) | 45 (76.3) | 8 (13.1) |
|        | 1 (1.6) | 12 (20.0) | 50 (82.0) | 0 (0.0) |
|        | 0 (0.0) | 7 (17.5) | 47 (78.3) | 0 (0.0) |
|        | 0 (0.0) | 30 (75.0) | 30 (75.0) | 15 (37.5) |
| Positive bacteria cultures after debridment | 1% PL-5 (N=59) | 2% PL-5 (N=61) | 4% PL-5 (N=60) | 1% SD-Ag (N=40) | Total (N=220) |
|--------------------------------------------|----------------|----------------|----------------|-----------------|---------------|
| **Staphylococcus aureus**                  | 17 (28.8)      | 17 (27.9)      | 27 (45.0)      | 17 (42.5)       | 78 (35.5)     |
| **Acinetobacter baumannii**                | 5 (8.5)        | 1 (1.6)        | 0 (0.0)        | 1 (2.5)         | 7 (3.2)       |
| **Escherichia coli**                       | 1 (1.7)        | 3 (4.9)        | 3 (5.0)        | 1 (2.5)         | 8 (3.6)       |
| **Klebsiella pneumoniae**                  | 5 (8.5)        | 2 (3.3)        | 8 (13.3)       | 1 (2.5)         | 16 (7.3)      |
| **Pseudomonas aeruginosa**                 | 3 (5.1)        | 13 (21.3)      | 2 (3.3)        | 6 (15.0)        | 24 (10.9)     |
| **Enterobacter cloacae**                   | 3 (5.1)        | 3 (4.9)        | 7 (11.7)       | 3 (7.5)         | 16 (7.3)      |
| **Citrobacter ferrandi**                   | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (2.5)         | 1 (0.5)       |
| **Streptococcus pyogenes**                 | 1 (1.7)        | 0 (0.0)        | 2 (3.3)        | 0 (0.0)         | 3 (1.4)       |
| **Enterococcus faecalis**                  | 2 (3.4)        | 1 (1.6)        | 4 (6.7)        | 1 (2.5)         | 8 (3.6)       |
| **Enterococcus faecium**                   | 0 (0.0)        | 1 (1.6)        | 0 (0.0)        | 0 (0.0)         | 1 (0.5)       |
| **Enterobacter aerogenes**                 | 1 (1.7)        | 0 (0.0)        | 1 (1.7)        | 0 (0.0)         | 2 (0.9)       |
| **Others**                                 | 22 (37.3)      | 23 (37.7)      | 15 (25.0)      | 11 (27.5)       | 71 (32.3)     |
### Table S6. The results of adverse event

| Adverse Event (AE)                | 1 % PL-5 | 2 % PL-5 | 4 % PL-5 | 1% SSD |
|-----------------------------------|----------|----------|----------|--------|
| Adverse Drug Reaction (ADR)       | 10 (16.9)| 12 (19.7)| 4 (6.7)  | 3 (7.9) |
| Severe Adverse Event (SAE)        | 1 (1.7)  | 1 (1.6)  | 0 (0.0)  | 1 (2.6) |
| Severe ADR                        | 1 (1.7)  | 0 (0.0)  | 0 (0.0)  | 0 (0.0) |
| Adverse event resulting in death  | 0 (0.0)  | 0 (0.0)  | 0 (0.0)  | 0 (0.0) |
| AE resulting in discontinuation of drug | 0 (0.0) | 0 (0.0)  | 0 (0.0)  | 1 (2.6) |
| ADR resulting in discontinuation of drug | 0 (0.0) | 0 (0.0)  | 0 (0.0)  | 0 (0.0) |
| Important AE                      | 6 (10.2) | 9 (14.8) | 7 (11.7) | 6 (15.8) |
| Important ADR                     | 5 (8.5)  | 2 (3.3)  | 2 (3.3)  | 1 (2.6) |