Increased intracellular action of MP1102 and NZ2114 against *Staphylococcus aureus in vitro* and *in vivo*

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SUPPORTING INFORMATION

Supplementary 1: Materials and Methods

Antimicrobial susceptibility testing of *S. aureus*. Antimicrobial susceptibility testing of *S. aureus* (ATCC43300 and CVCC546) was performed by Kirby Bauer disc diffusion method according to the standards procedures recommended by Clinical Laboratory Standards Institute (CLSI 2012)[1,2]. The used 22 antibiotics included: 1) aminoglycosides (amikacin, neomycin, gentamicin, kanamycin, and streptomycin), 2) quinolones (ciprofloxacin, norfloxacin), 3) sulfonamides (sulfisoxazole), 4) tetracyclines (tetracycline), 5) polypeptides (bacitracin and polymyxin B), 6) penicillins (penicillin, amoxicillin, ampicillin, oxacillin), 7) lincosamides (lincomycin), 8) macrolides (erythromycin and azithromycin), 9) amphenicols (chloramphenicol), 10) glycopeptides (vancomycin), and 11) cephalosporins (cefazolin and cefotaxime) (Qianshun Bio-tech, Beijing).

SCC*mec* and *spa* typing of *S. aureus*. For rapid DNA extraction, one to five bacterial colonies were

suspended in 50 μl of sterile distilled water and heated at 100°C for 10 min. Then the solution was frozen in -80°C for 30 min and heated at 100°C for 10 min. After centrifugation at 5000 rpm/min for 2 min. A total of 2 μl of the supernatant was used as a template in a 50-μl PCR. The SCC*mec* and x region of the *spa* gene was amplified by PCR with primers in Supplementary Table 1[3-5]. The x region of the spa genes was sequenced by Sangon Biotech (Shanghai, China) and the types were determined with the database accessible via http://spa.ridom.de/spatypes.shtml.

Growth curves of *S. aureus*. The *S. aureus* ATCC25923, ATCC43300 and CVCC546 strains were used to evaluate growth rate in Mueller – Hinton broth (MHB)[6]. Briefly, overnight cultures in MHB was inoculated into 50 ml of fresh MHB (with the inoculum size of 1% (v/v))[7]. All flasks were incubated at 37°C for 15 h with shaking (250 rpm). Aliquots (2 ml) were taken out per hour to determine the culture turbidity at 600 nm in a TU-1810 ultraviolet visible spectrophotometer. It was performed for three times.

FITC-labeled peptides. The FITC-labeled peptides were carried out in ChinaPeptides Co., Ltd. (Shanghai, China) as previously described[8]. The peptides (MP1102 or NZ2114) were dissolved in 0.2 M PBS (pH 8.4) containing 1.1 equivalent of FITC. The mixture was stirred at room temperature for 12 h to complete the reaction. The reaction product was then dialyzed in dialysis tubing MD18 (with a molecular weight cut-off 2.0 kDa) at 4°C for 48 h with an exchange of ddH_2O per 12 h to remove free FITC. The fluorescence intensity of FITC in dialyzed ddH_2O was monitored to assure the complete removal of free FITC. Finally, the product was lyophilized in a freezer dryer for 36 h. The procedure was kept away from light.

References:

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Region, Ethiopia. *Arch Public Health* **72**, *16* (2014).

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Supplementary 2: Tables

Table 1 Primers used in SCC*mec* and *spa* typing of *S. aureus*

Name	Primer (5'→3')	Amplicon size (bp)	Reference
SCC*mec* type			
SCCmecI-F	GCTTTAAAGAGTGTCGTTACAGG	613	3, 4
SCCmec Ⅰ-R	GTTCTCTCATAGTATGACGTCC		
SCCmec Ⅱ-F	CGTTGAAGATGATGAAGCG	398	
SCCmec Ⅱ-R	CGAAATCAATGGTTAATGGACC		
SCCmecⅢ-F	CCATATTGTGTACGATGCG	280	
SCCmecⅢ-R	CCTTAGTTGTCGTAACAGATCG		
SCCmecⅣa-F	GCCTTATTCGAAGAAACCG	776	
SCCmecⅣa-R	CTACTCTTCTGAAAAGCGTCG		
SCCmecⅣb-F	TCTGGAATTACTTCAGCTGC	493	
SCCmecⅣb-R	AAACAATATTGCTCTCCCTC		
SCCmecⅣc-F	ACAATATTTGTATTATCGGAGAGC	200	
SCCmecⅣc-R	TTGGTATGAGGTATTGCTGG		
SCCmecⅣd-F	CTCAAAATACGGACCCCAATACA	881	
SCCmecⅣd-R	TGCTCCAGTAATTGCTAAAG		
SCCmec Ⅴ-F	GAACATTGTTACTTAAATGAGCG	325	
SCCmec Ⅴ-R	TGAAAGTTGTACCCTTGACACC		
***mecA* gene**			
mecA-F	GTGAAGATATACCAAGTGATT	147	3
mecA-R	ATGCGCTATAGATTGAAAGGAT		
mecI-F	CCCTTTTTATACAATCTCGTT	146	
mecI-R	ATATCATCTGCAGAATGGG		
***Spa* type**			
Spa-x-F	TAAAGACGATCCTTCGGTGAGC	-	5
Spa-x-R	CAGCAGTAGTGCCGTTTGCTT		

-: no data. The primers were synthesized by Sangon Biotech (Shanghai, China).

Table 2 Antimicrobial susceptibility patterns of *S. aureus* ATCC43300 and CVCC546

Antibiotics	***S. aureus* ATCC43300**	***S. aureus* CVCC546**
Vancomycin	S	S
Lincomycin	R	I
Amoxicillin	R	S
Ciprofloxacin	S	S
Amikacin	R	S
Ampicillin	R	S
Oxacillin	R	S
Erythromycin	R	I
Tetracycline	I	R
Bacitracin	I	R
Norfloxacin	S	S
Polymyxin B	R	R
Sulfisoxazole	R	R
Neomycin	R	I
Azithromycin	R	S
Kanamycin	R	I
Streptomycin	I	S
Cefotaxime	I	I
Gentamicin	R	S
Chloramphenicol	S	S
Cefazolin	S	S
Penicillin	R	I

R: resistant; I: Intermediate; S: susceptible.

89 **Table 3** Sources, types and susceptibilities of different *S. aureus* strains

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S. aureus	**Source**	**SCC*mec* type**	***Spa* type**	**Antibiotic resistance profile**	**Biosafety level**
Methicillin-susceptible ATCC25923	Clinical isolate	-	-	-	II
Methicillin-resistant ATCC43300	Clinical isolate	II	t007	Methicillin, oxacillin[a]	II
Highly virulent CVCC546	Isolation from a pig in Bejing, China	-	t034	Tetracycline, bacitracin, polymyxin B and sulfisoxazole[b]	III

91 -: negative. a: the ATCC website. b: in this study.

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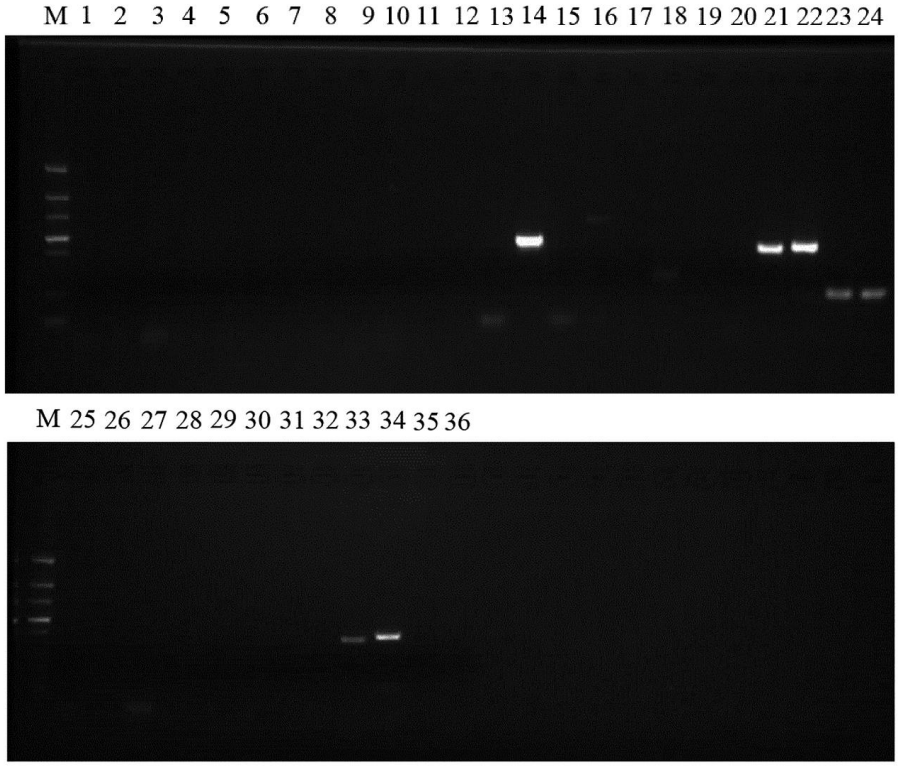
Table 4 Effect of cathepsin B on the MICs of MP1102, NZ2114 against *S. aureus* resistant ATCC43300

	MIC (μg/ml)	
	Buffer + AMP	Buffer + cathepsin B + AMP
MP1102	0.25	0.25
NZ2114	0.5	0.5

Supplementary 3: Figures

A



B

S. aureus ATCC43300

TAATTAAAGACGATCCTTCGGTGAGCAAAGAAATTTTAGCAGAAGCTAAAAAGCTAAA
CGATGCTCAAGCACCAAAAGAGGAAGACAACAACAAGCCTGGCAAAGAAGACAACA
ACAAGCCTGGTAAAGAAGACGGCAACAAACCTGGTAAAGAAGACGGCAACAAACCT
GGTAAAGAAGACGGCAACAAACCTGGTAAAGAAGACGGCAACAAACCTGGTAAAGA
AGACAACAAAAAACCTGGCAAAGAAGATGGCAACAAACCTGGTAAAGAAGACGGCA
ACAAGCCTGGTAAAGAAGACGGCAACGGAGTACATGTCGTTAAACCTGGTGATACAGT
AAATGACATTGCAAAAGCAAACGGCACTACTGCTG
t007,15-12-16-16-16-16-02-25-17

S. aureus CVCC546

TAAAGACGATCCTTCGGTGAGCAAAGAAATTTTAGCAGAAGCTAAAAAGCTAAACGAT
GCTCAAGCACCAAAAGAGGAAGACAACAACAAGCCTGGTAAAGAAGACGGCAACAA
ACCTGGTAAAGAAGACAACAAAAAACCTGGCAAAGAAGATGGCAACAAACCTGGTA
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AACAAAAAACCTGGTAAAGAAGATGGCAACAAGCCTGGTAAAGAAGATGGCAACAA
ACCTGGTAAAGAAGACGGCAACGGAATACATGTCGTTAAACCTGGTGATACAGTAAAT
GACATTGCAAAAGCAAACGGCACTACTGCTG
t034,08-16-02-25-02-25-34-24-25

Fig. S1

Figure 1 PCR assay identifies SCC*mec* and *spa* types and subtypes and simultaneously detects the

methicillin resistance (*mecA* gene). **(A)** Gel electrophoresis. M: DNA Marker II; 1-12: *S. aureus* ATCC25923; 13-24: *S. aureus* ATCC43300; 25-36: *S. aureus* CVCC546. 1, 13, 25: SCC*mec* I; 2, 14, 26: SCC*mec* Ⅱ; 3, 15, 27: SCC*mec* Ⅲ; 4, 16, 28: SCC*mec* Ⅳe; 5, 17, 29: SCC*mec* Ⅳe; 6, 18, 30: SCC*mec* Ⅳc; 7, 19, 31: SCC*mec* Ⅳe; 8, 20, 32: SCC*mec* Ⅴ; 9, 21, 33: spa-x-1; 10, 22, 34: spa-x-2; 11, 23, 35: *mecA*; 12, 24, 36: *mec* I. **(B)** The x region of the *spa* gene sequence.

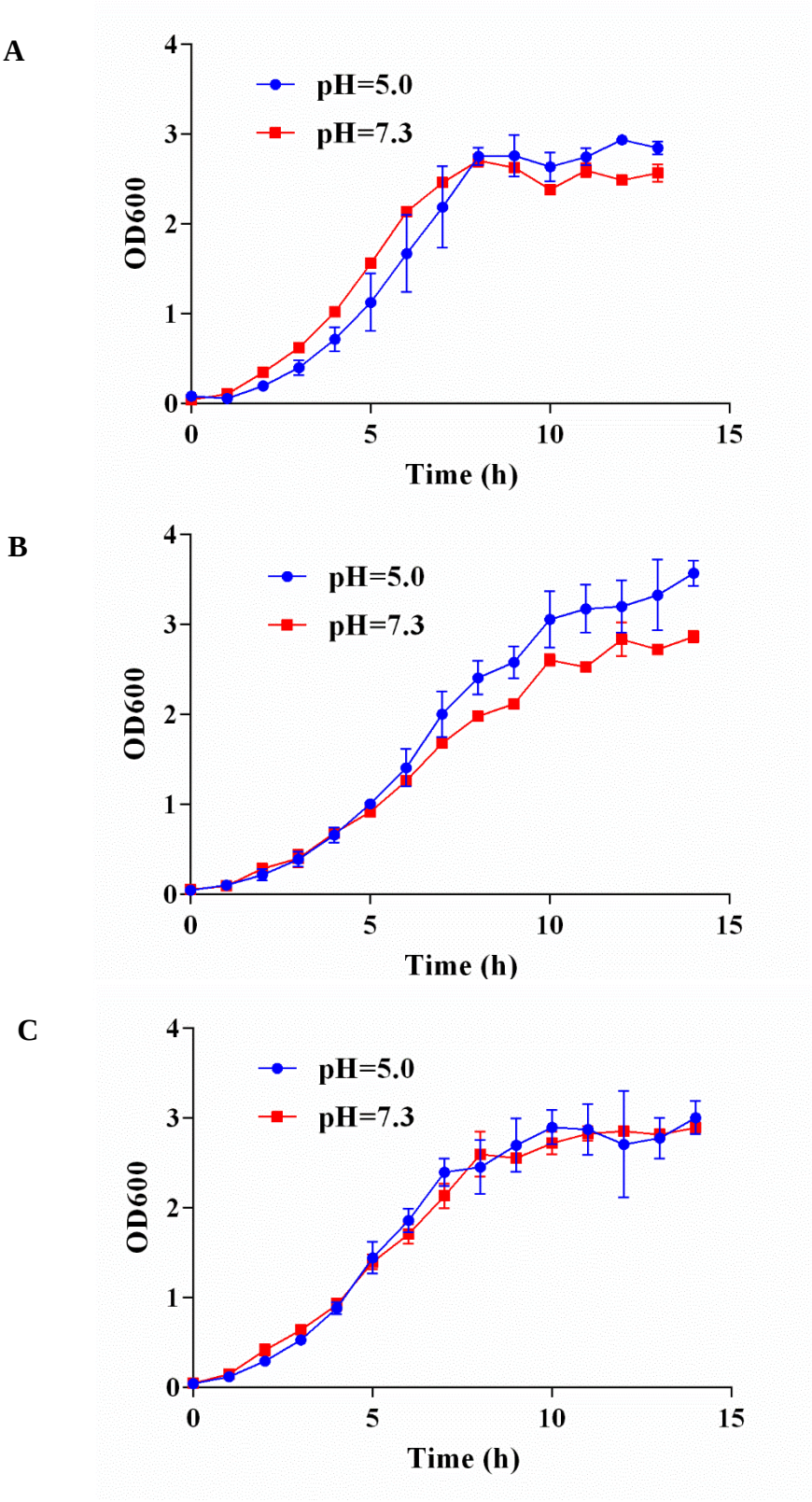
A
pH=5.0
pH=7.3
OD600
Time (h)
B
pH=5.0
pH=7.3
OD600
Time (h)
C
pH=5.0
pH=7.3
OD600
Time (h)

Fig. S2

Figure 2 Growth curves of *S. aureus* ATCC25923 **(A)**, ATCC43300 **(B)** and CVCC546 **(C)** at pH 5.0 and pH 7.3.

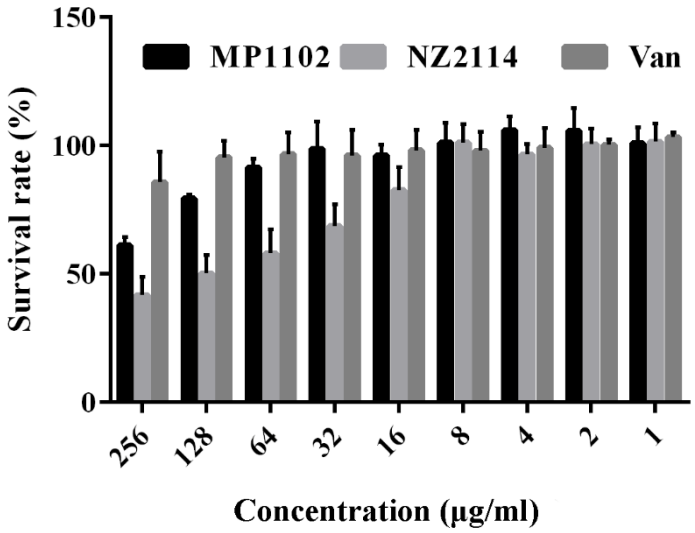

Fig. S3

Figure 3 Viability of RAW 264.7 cells incubated with NZ2114 and MP1102 for 24 h. RAW 264.7 cells were incubated with 1-256 μg/ml for 24 h and subsequently cell viability was determined using the MTT assay. Results are means ± S.D. for three experiments.

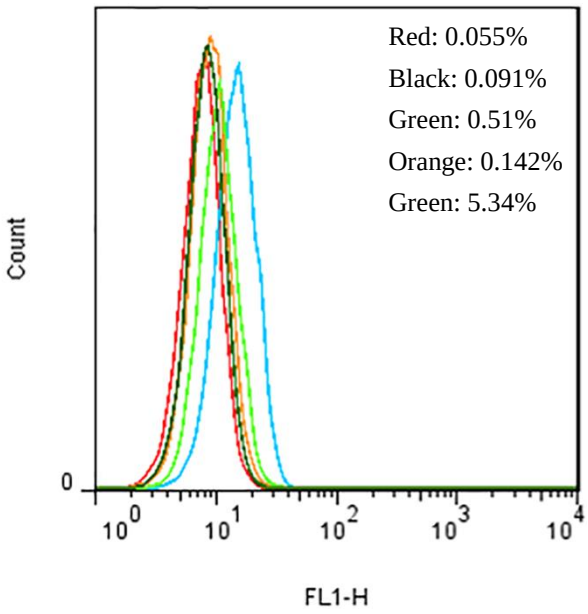

Fig. S4

Figure 4 Effect of MP1102 and NZ2114 on the cell membrane of RAW 264.7 cells. Cells were incubated with different concentrations of MP1102 and NZ2114 for 24 h prior to staining with 5 μg/ml PI at 37°C for 10 min. PI fluorescence in RAW 264.7 cells was measured by flow cytometry. Red line: control; black line: 25 μg/ml MP1102; green line: 250 μg/ml MP1102; orange line: 25 μg/ml NZ2114; green line: 250 μg/ml NZ2114.