

# A Novel $\beta$ -Hairpin Peptide Z-d14CFR Enhances Multidrug-Resistant Bacterial Clearance in a Murine Model of Mastitis

**Table S1 Information of bacterial strains used in this study**

Bacteria	Genotype	Source
<i>Escherichia coli</i> ATCC <sup>1</sup> 25922		Purchased from China Institute of Veterinary Drug Center
<i>Escherichia coli</i> CVCC <sup>2</sup> 1450		Purchased from China Institute of Veterinary Drug Center
<i>Escherichia coli</i> CAU 201919	( <i>tet(A)</i> , <i>bla</i> CTX-M-65, <i>aac</i> (3)-IIId, <i>aph</i> (3'')-Ib, <i>aph</i> (3')-IIa, <i>aph</i> (6)-Id, <i>formA</i> , <i>mdf(A)</i> , <i>sul2</i> )	Isolated from unpasteurized milk
<i>Escherichia coli</i> CAU 201920	( <i>tet(A)</i> , <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>mdf(A)</i> , <i>bla</i> TEM-1, <i>sul2</i> )	Isolated from unpasteurized milk
<i>Salmonella typhimurium</i> ATCC14028		Purchased from China Institute of Veterinary Drug Center
<i>Klebsiella pneumoniae</i> CAU202084	( <i>tet(A)</i> , <i>oqxAB</i> , <i>bla</i> CTX-M-3, <i>fosA5</i> )	Isolated from unpasteurized milk
<i>Proteus vulgaris</i> CVCC1971		Purchased from China Institute of Veterinary Drug Center
<i>Staphylococcus aureus</i> ATCC29213		Purchased from China Institute of Veterinary Drug Center
<i>Staphylococcus haemolyticus</i> CAU202078	( <i>tet(M)</i> , <i>drf(A)</i> , <i>ermC</i> , <i>blaZ</i> )	Isolated from unpasteurized milk
<i>Bacillus cereus</i> CAU 202020	( <i>tet(M)</i> , <i>bla</i> TEM-1, <i>sul2</i> )	Isolated from unpasteurized milk
MRSA <sup>3</sup> ATCC33591		Purchased from China Institute of Veterinary Drug Center
<i>Streptococcus suis</i> CVCC3307		Purchased from China Institute of Veterinary Drug Center

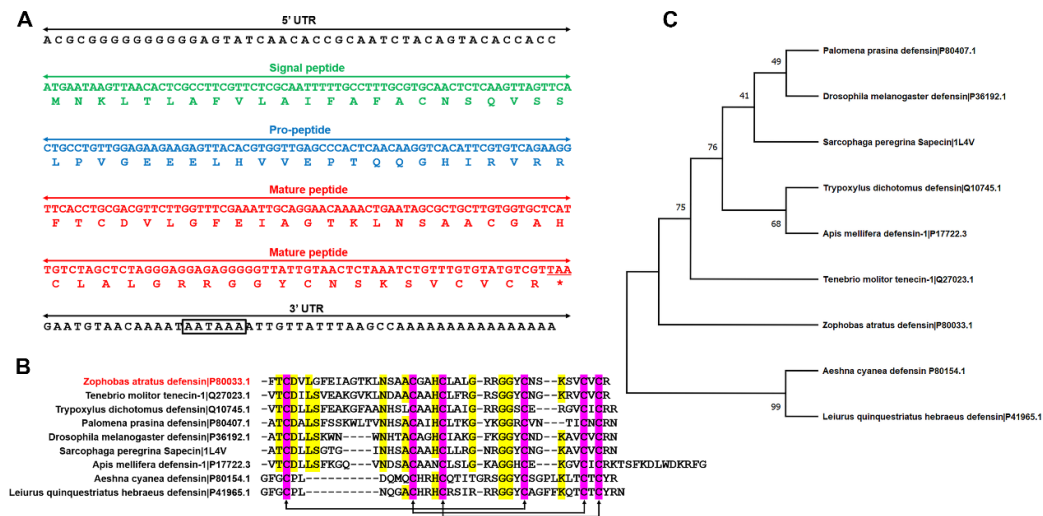
<sup>1</sup> American Type Culture Collection; <sup>2</sup> China Veterinary Culture Collection Center. <sup>3</sup> Methicillin-resistant *S. aureus*.

**Table S2 Primers used for Real-Time PCR**

Gene	Accession NO.	Prime sequence (5'-3')
$\beta$ -actin	NM_007393.5	f- GCTCTTTTCCAGCCTTCCTT

IL1β NM\_008361.4  
TNF-α XM\_006504297.5

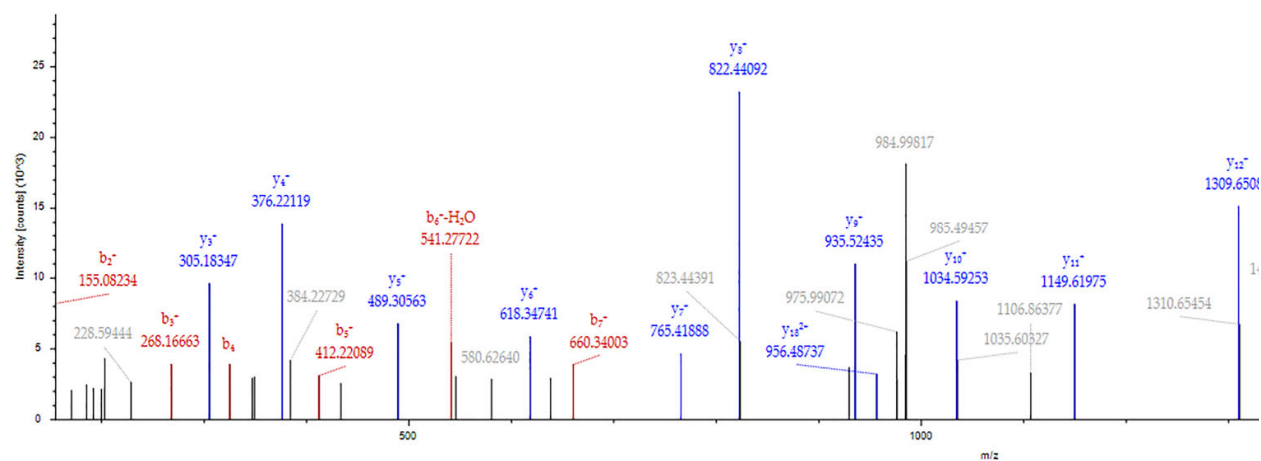
r- GATGTCAACGTCACACTT  
f- TGCCACCTTTTGACAGTGATG  
r- AAGGTCCACGGGAAAGACAC  
f- ACGGCATGGATCTCAAAGAC  
r-AGATAGCAAATCGGCTGACG



**Figure S1** Amplification and analysis of *Z. atratus* defensin. (A) Analysis of *Z. atratus* defensin transcript, the 5' UTR and 3' UTR are marked with black, the putative polyA additional signal at 3' UTR (AATAAA) is boxed. The signal peptide, pro-peptide and mature peptide of *Z. atratus* defensin and their corresponding nucleotide sequence are respectively marked with green, blue and red. (B) Multiple sequence alignment of *Zophobas atratus* (*Z. atratus*) defensin. *Z. atratus* defensin (ZA-defensin) has six conserved cysteine residues like other insect defensins, and forms three disulfide bonds between Cys1-Cys4, Cys2-Cys5, Cys3-Cys6 (cysteines are shadowed in yellow and other identical residues are shadowed in grey). (C) A neighbor-joining distance tree

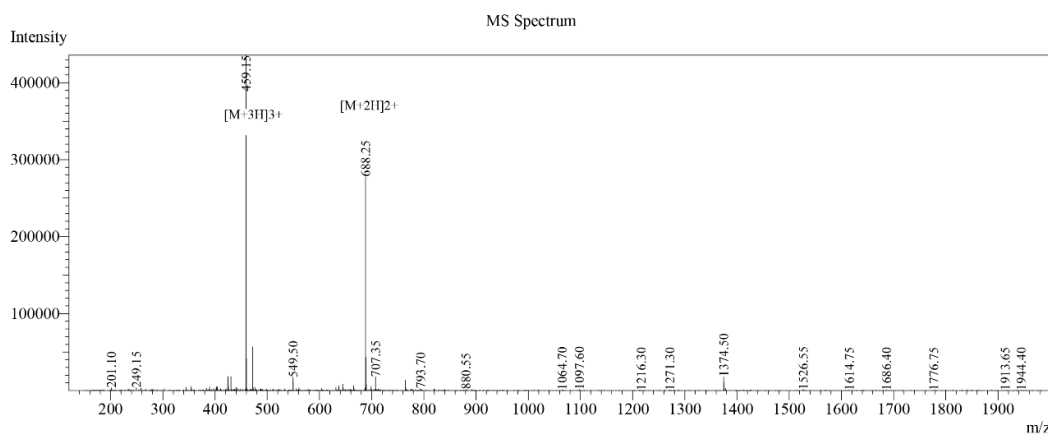
constructs from the alignment of defensin sequences presented in (B), numbers on

interior branches represent bootstrap values

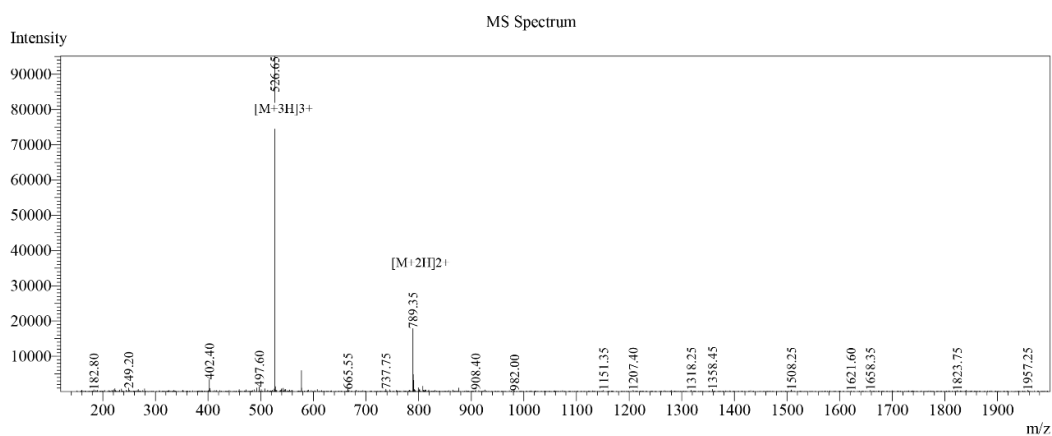


**Figure S2** Mass spectrometry of recombinant *Z. atratus* defensin.

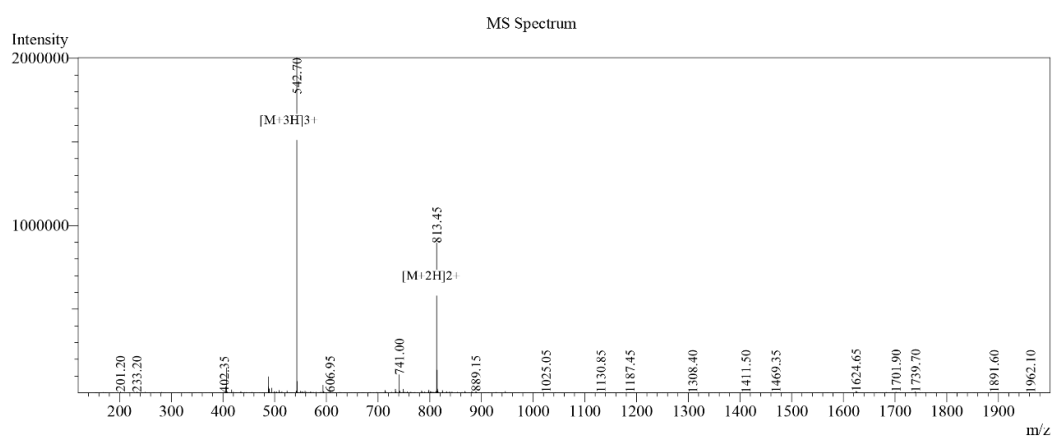
Z-d13



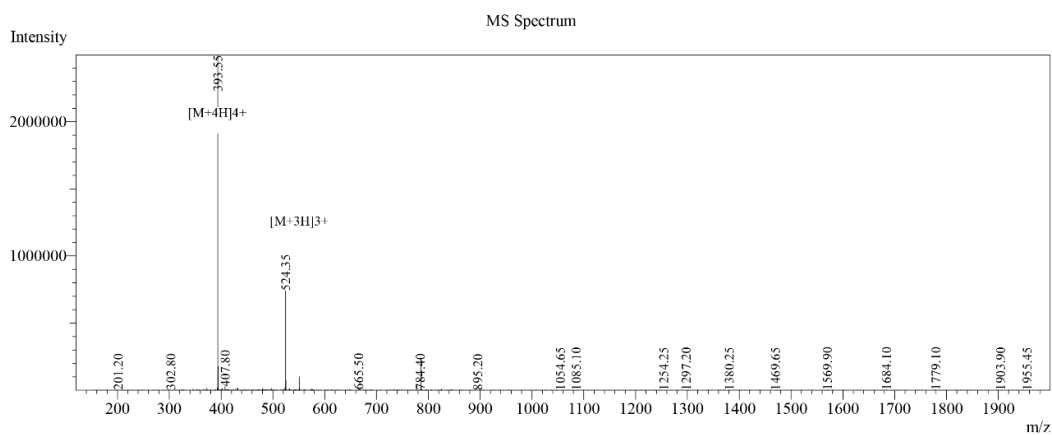
Z-d114C



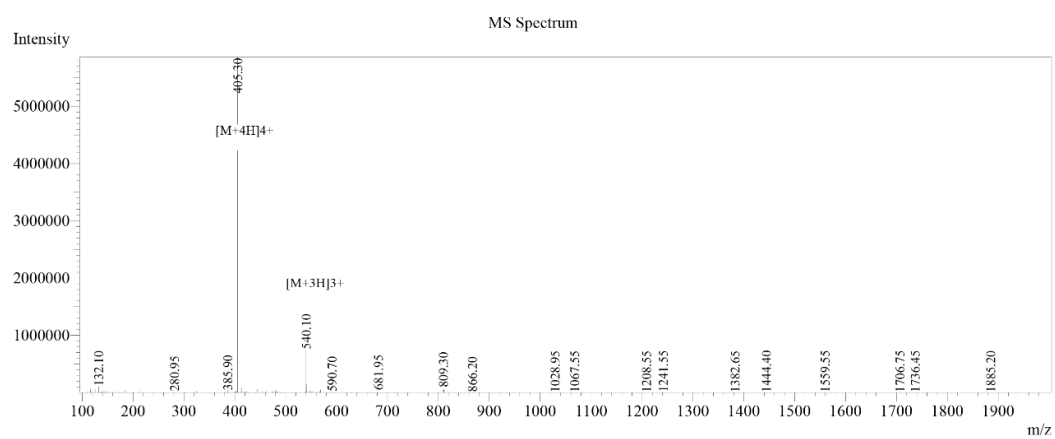
## Z-d114CF



## Z-d114CR



## Z-d114CFR



**Figure S3** Mass spectrometry of the synthesized peptides.