

Supplementary Information

Antibacterial potential analysis of novel α -helix peptides in the Chinese wolf spider *Lycosa sinensis*

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A

Peptide name	Homology AMPs			
	Name	APD ID	Similarity percentage	Organism
LS-AMP-E1	Uperin 2.7	AP00321	45.83%	Toad
LS-AMP-F1	GLK-19	AP00142	45.45%	Synthetic
LS-AMP-G1	Latarcin 3b	AP01013	50.00%	Spider

B

LS-AMP-E1	---AGMKNIIDAIKKKLGGKL-----	18
Uperin 2.7	-----GIIDIAKKLVGGIRNVLGI-----	19
LS-AMP-F1	---TGLGKIGYLMKKLLSKAKV-----	19
GLK-19	-----GLKKLLGKLLKKLGKLLK-----	19
LS-AMP-G1	SWAVMLPDLVRQ---KLKQRW-----	18
Latarcin 3b	SWASMAKKLKEYMEKLKQRA-----	20

Figure S1. The sequence homology matching by the Antimicrobial Peptide Database (A). Multiple sequence alignment of LS-AMPs with homology AMPs (B).

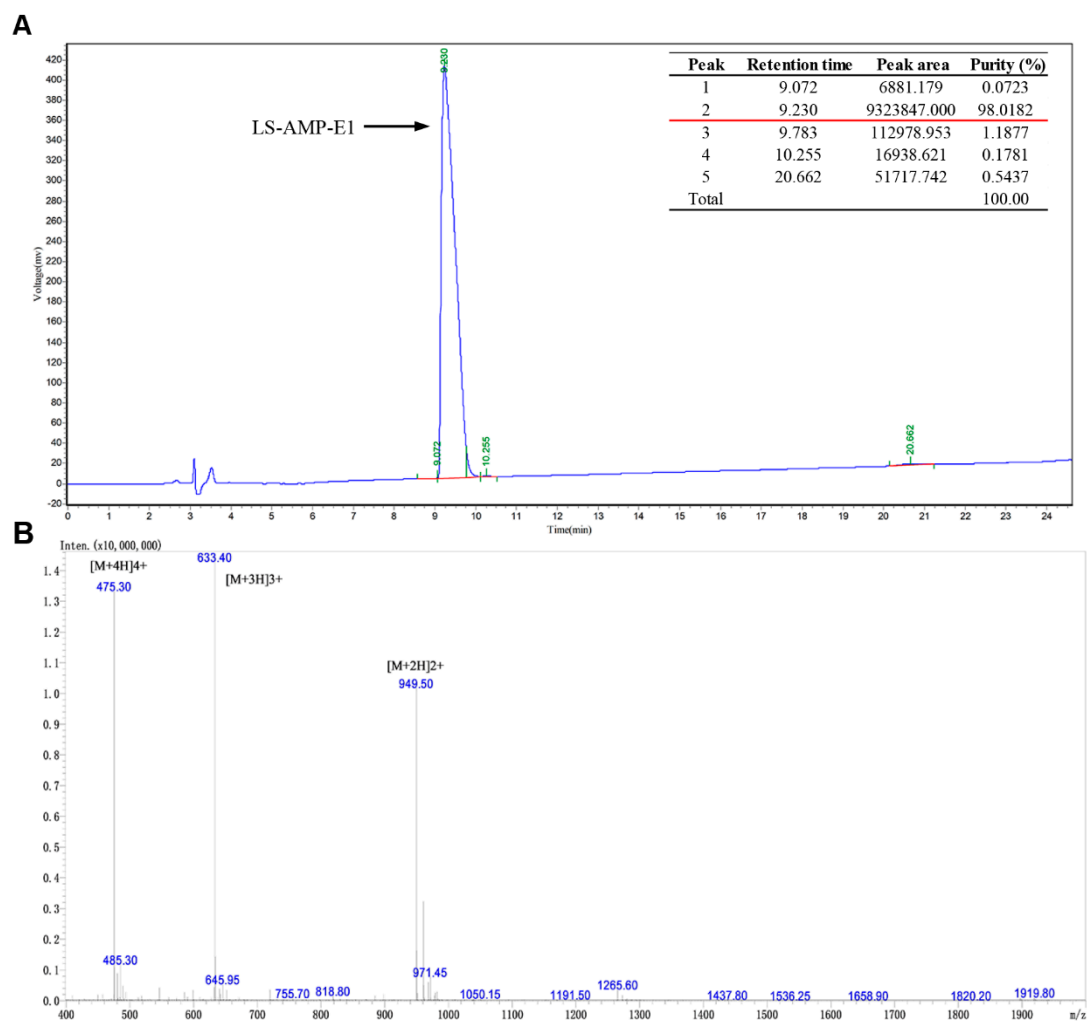


Figure S2. The High-performance liquid chromatography (HPLC) (A) and Mass spectrometry (MS) (B) profiles of LS-AMP-E1.

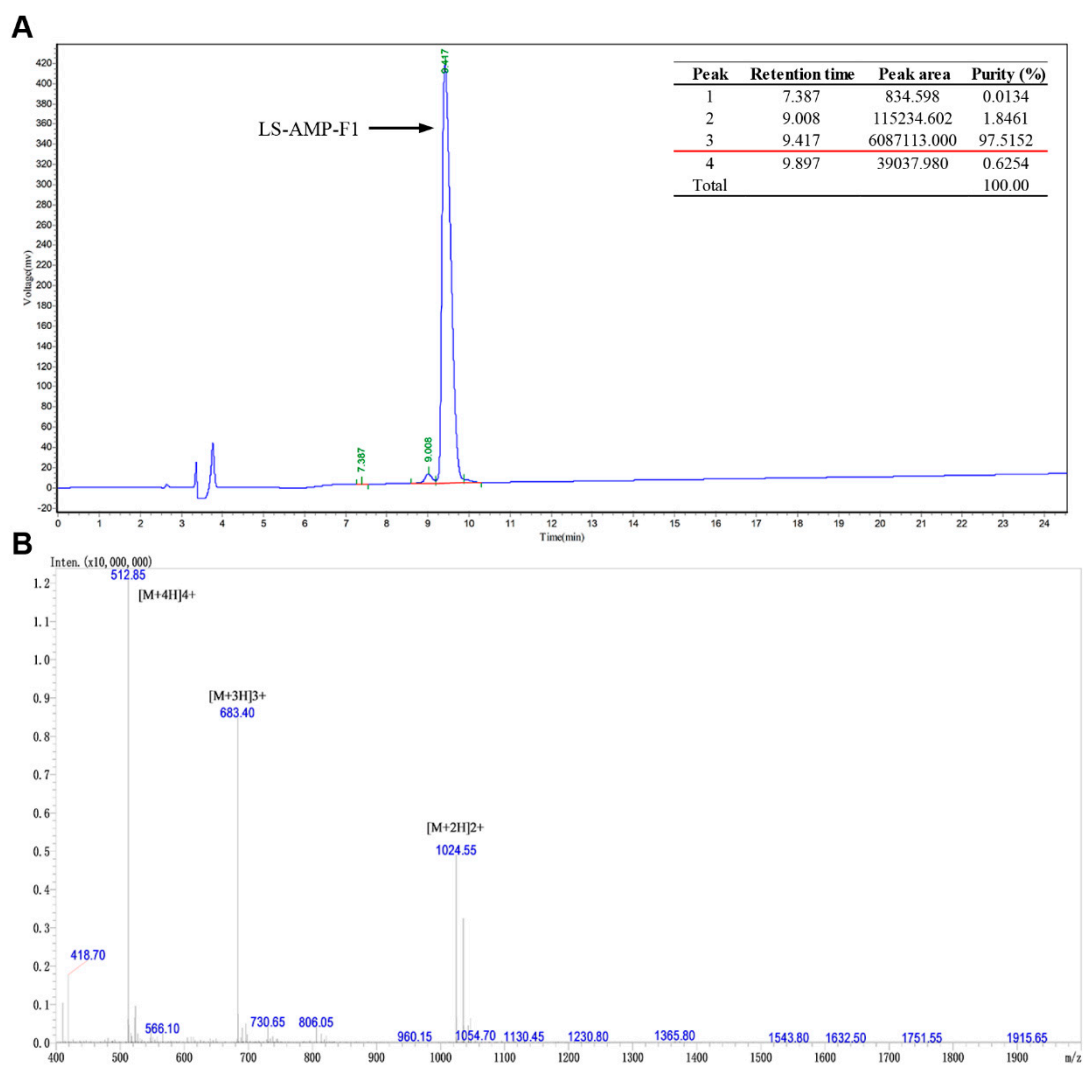


Figure S3. The HPLC (A) and MS (B) profiles of LS-AMP-F1.

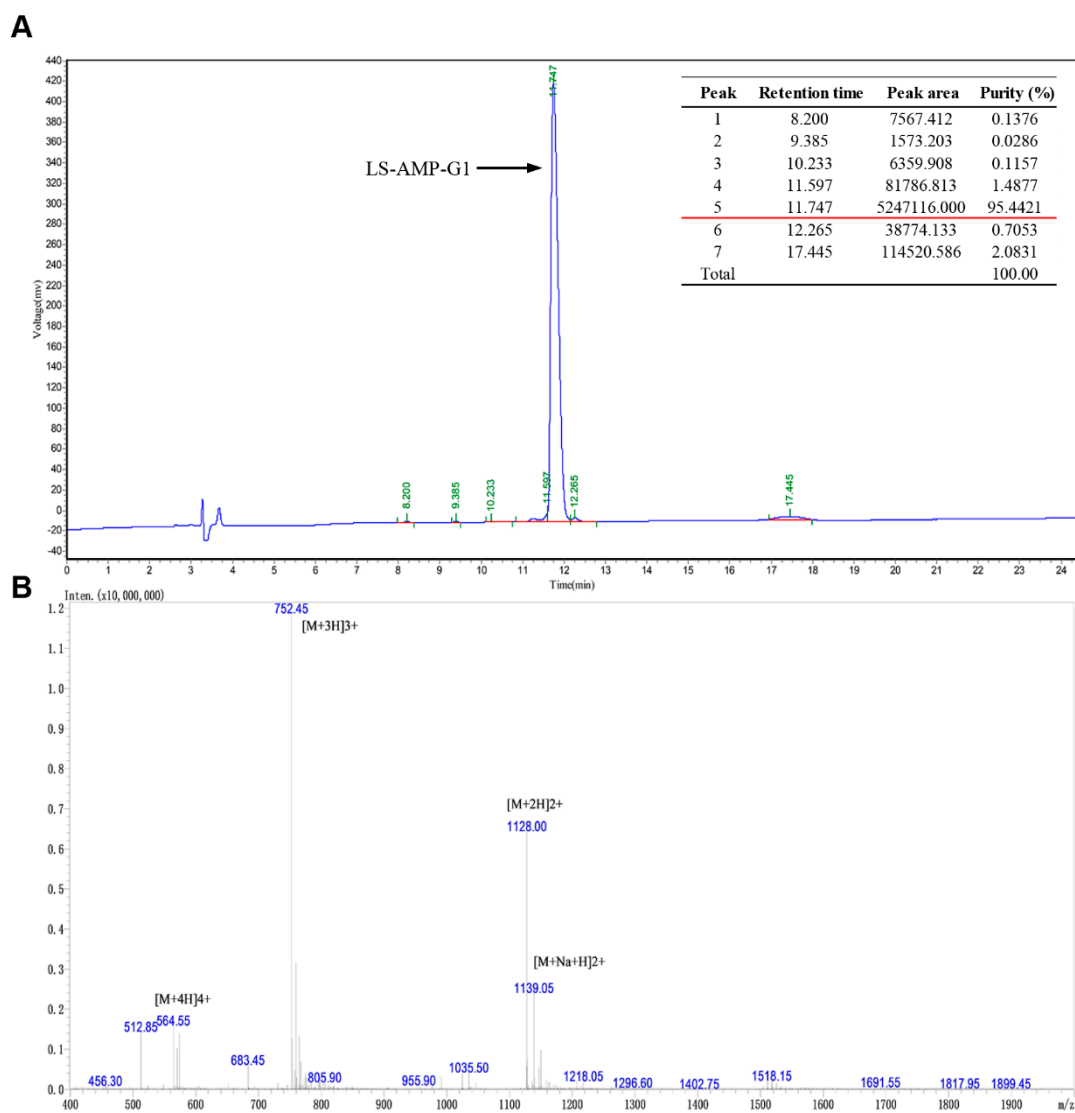


Figure S4. The HPLC (A) and MS (B) profiles of LS-AMP-G1.

Table S1. The clinical profiles of MDR strains isolated from 30 cases.

Species	Number	Resistant type	Age range	Disease	Specimen type	Sample number
<i>E. faecium</i>	1359	None	80-90	UTI	urine	20220505117
	1320	VRE	20-30	AP	blood	20220405146
<i>S.aureus</i>	1090	MRSA	40-50	CI	urine	20211101018
	1022	MRSA	60-70	injury	sputum	20210930047
	1065	MRSA	50-60	diabetic foot	excretion	20211020125
	1081	MRSA	70-80	head trauma	sputum	20211027035
	1049	CR	40-50	open injuries	excretion	20211017048
<i>K.pneumoniae</i>	1096	None	60-70	brainstem infarction	blood	20211105003
	1041	CR	0-10	fever	urine	20211013047
	1044	CR	50-60	HE	blood	20211014205
<i>A.baumannii</i>	1055	CR	50-60	buring	excretion	20211018150
	1023	CR	60-70	stroke	sputum	20211005014
	1025	CR	70-80	abdominal pain	sputum	20211008184
	1032	CR	70-80	abdominal pain	urine	20211009174
	1038	CR	70-80	abdominal pain	blood	20211012080
	1040	CR	60-70	gout	BALF	20211013103
	1121	CR	50-60	aneurysm	sputum	20211115011
<i>P.aeruginosa</i>	1088	CR	80-90	pneumonia	sputum	20211031041
	1099	CR	80-90	pneumonia	sputum	20211104085
	1095	CR	60-70	COPD	sputum	20211104106
<i>E.coli</i>	1270	CR	70-80	prostatic hyperplasia	urine	20220223151
	1172	CR	70-80	hematencephalon	urine	20211215062
	1188	CR	70-80	hematencephalon	urine	20211224059
	1064	ESBLs	70-80	notalgia	urine	20211019073
	1010	ESBLs	70-80	pancreatitis	blood	20210915118
	1278	ESBLs	70-80	abdominal pain	excretion	20220301065
	1165	ESBLs	60-70	abdominal pain	urine	20211212047
	1288	ESBLs	50-60	ureteral stones	blood	20220304189
	1170	ESBLs	60-70	hematencephalon	sputum	20211214079
	1156	ESBLs	70-80	UTI	blood	20211206179

VRE: Vancomycin-resistant enterococcus; MRSA: Methicillin-resistant Staphylococcus aureus; CR: Carbapenem-Resistant; ESBLs: extended spectrum beta-lactamases; UTI: urinary tract infection; AP: acute pancreatitis; CI: cerebral infarction; HT: Hepatic Encephalopathy; COPD: chronic obstructive pulmonary disease; BALF: bronchoalveolar lavage fluid.

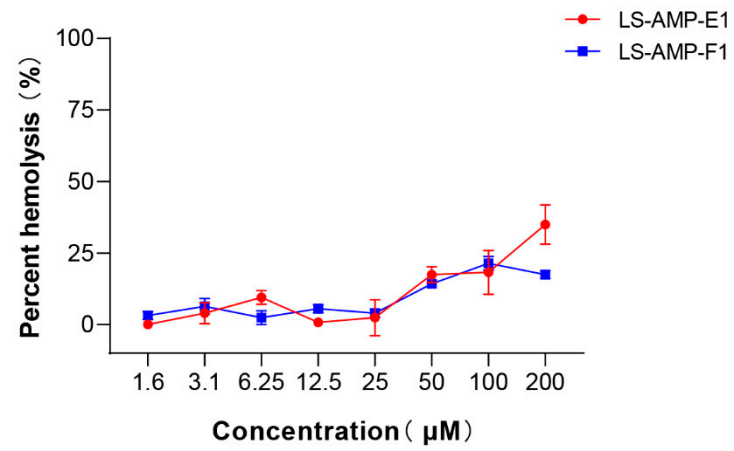


Figure S5. The hemolytic effects of LS-AMP-F1 and LS-AMP-E1 on mouse erythrocytes. Data represent means \pm SD of 3 individual experiments.

Table S2. The MICs of the checkerboard test for standard strains.

Peptides/Antibiotics		Strains	
		<i>E. coli</i> CCTCC AB 2018675	<i>S. albus</i> CGMCC 1.3374
MIC LS-AMP-E1 (μ M)	Alone	6.25	12.5
	with AZI	0.3906	0.3906
MIC AZI (μ g/mL)	Alone	0.05	3.1
	with LS-AMP-E1	0.025	0.775
MIC LS-AMP-E1 (μ M)	Alone	6.25	12.5
	with ERY	1.5625	0.3906
MIC ERY (μ g/mL)	Alone	6.25	6.25
	with LS-AMP-E1	1.5625	1.5625
MIC LS-AMP-E1 (μ M)	Alone	6.25	12.5
	with DOX	0.1953	12.5
MIC DOX (μ g/mL)	Alone	1.6	0.025
	with LS-AMP-E1	0.4	0.0008
MIC LS-AMP-F1 (μ M)	Alone	12.5	6.25
	with AZI	3.125	3.125
MIC AZI (μ g/mL)	Alone	0.05	3.1
	with LS-AMP-F1	0.0016	0.003
MIC LS-AMP-F1 (μ M)	Alone	12.5	6.25
	with ERY	3.125	0.1953
MIC ERY (μ g/mL)	Alone	6.25	6.25
	with LS-AMP-F1	0.0977	1.5625
MIC LS-AMP-F1 (μ M)	Alone	12.5	6.25
	with DOX	3.125	1.5625
MIC DOX (μ g/mL)	Alone	1.6	0.025
	with LS-AMP-F1	0.2	0.00625
MIC Lycosin-I (μ M)	Alone	3.1	1.6
	with AZI	0.775	0.05
MIC AZI (μ g/mL)	Alone	0.05	3.1
	with Lycosin-I	0.025	0.775
MIC Lycosin-I (μ M)	Alone	3.1	1.6
	with ERY	0.0969	0.05
MIC ERY (μ g/mL)	Alone	6.25	6.25
	with Lycosin-I	3.125	1.5625
MIC Lycosin-I (μ M)	Alone	3.1	1.6
	with DOX	0.0484	0.025
MIC DOX (μ g/mL)	Alone	1.6	0.025
	with Lycosin-I	0.4	0.025

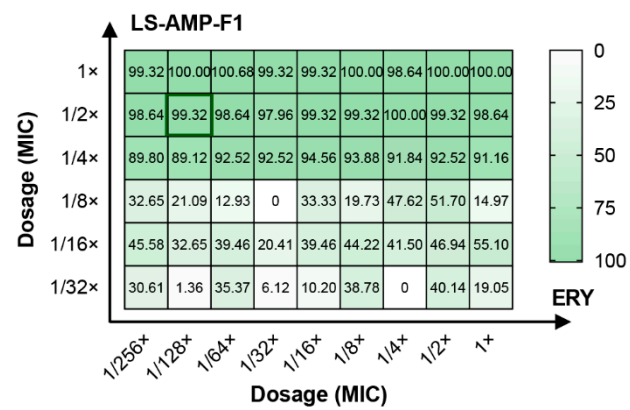


Figure S6. Inhibition rate of the checkerboard test for MDR *E. faecium* (1320).

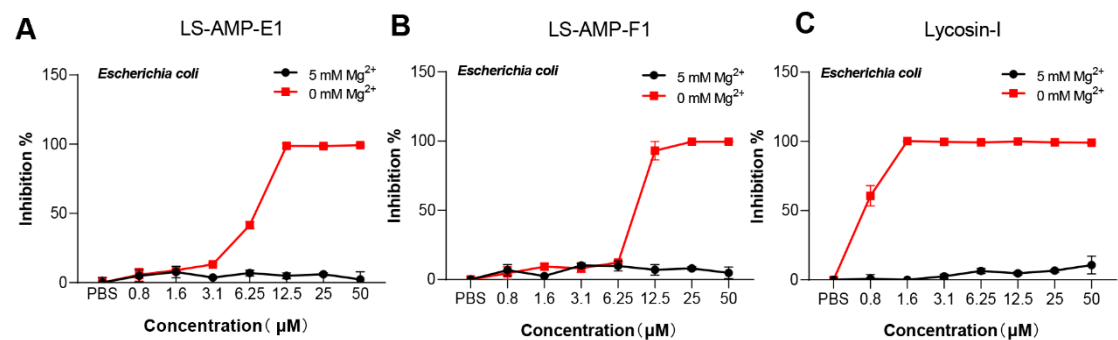


Figure S7. Effect of Mg²⁺ on the antimicrobial activity of LS-AMP-E1 (A), LS-AMP-F1 (B) and Lycosin-I (C) against a strain of *E. coli*.