Additional file

A Potential Strategy against Clinical Carbapenem-Resistant *Enterobacteriaceae*: Antimicrobial Activity Study of Sweetener-Decorated Gold Nanoparticles *in Vitro* and *in Vivo*

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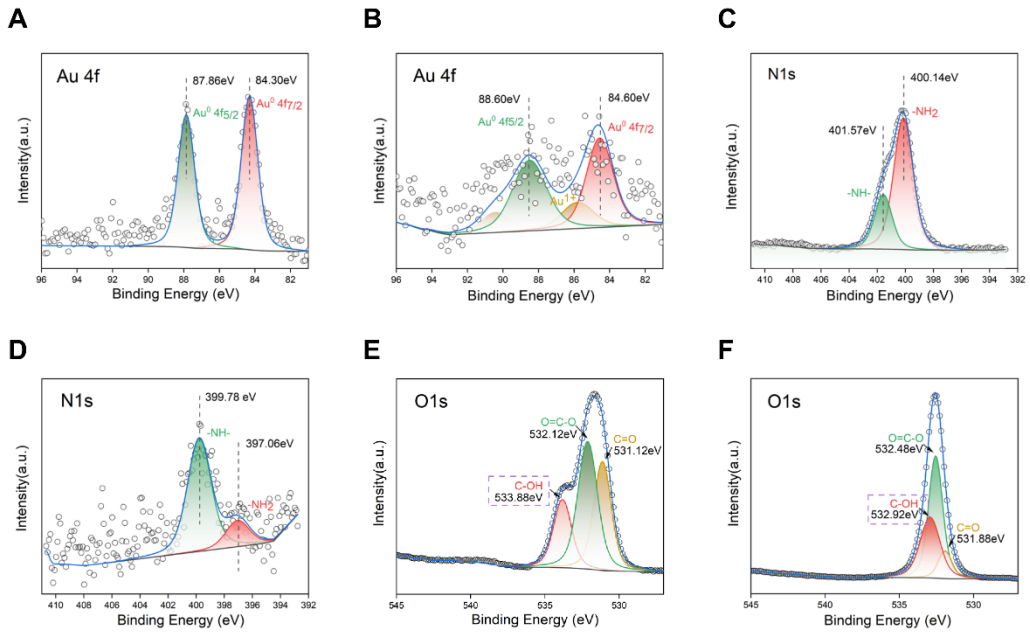

Figure S1. XPS analysis of Au 4f, N1s, and O1s for ASP, Au NPs, and ASP_Au NPs. (A) Au 4f spectrum analysis of Au NPs. (B) Au 4f spectrum analysis of ASP_Au NPs. (C) N1s spectrum analysis of ASP. (D) N1s spectrum analysis of ASP_Au NPs. (E) O1s spectrum analysis of ASP. (F) O1s spectrum analysis of ASP_Au NPs. Hollow dots represent raw data, blue curves represent the overall fitting curve of the data, and black curves represent the baseline. Colored curves and their corresponding peak labels are shown in the figure.

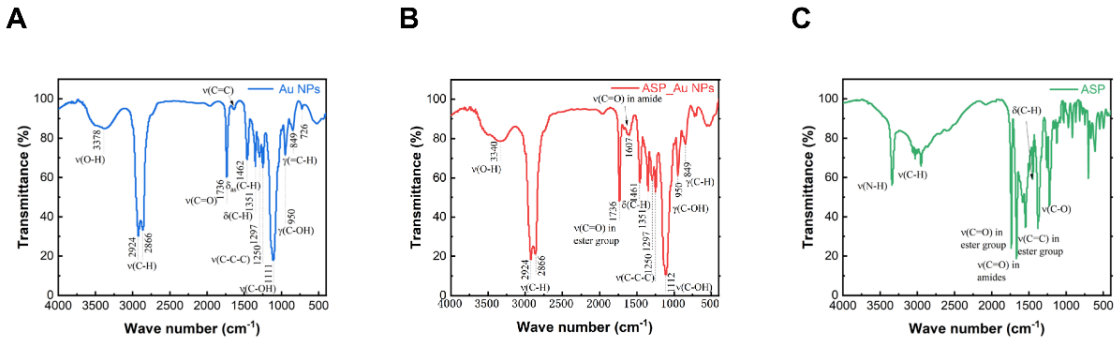

Figure S2. FTIR peaks of Au NPs, ASP, and ASP_Au NPs.

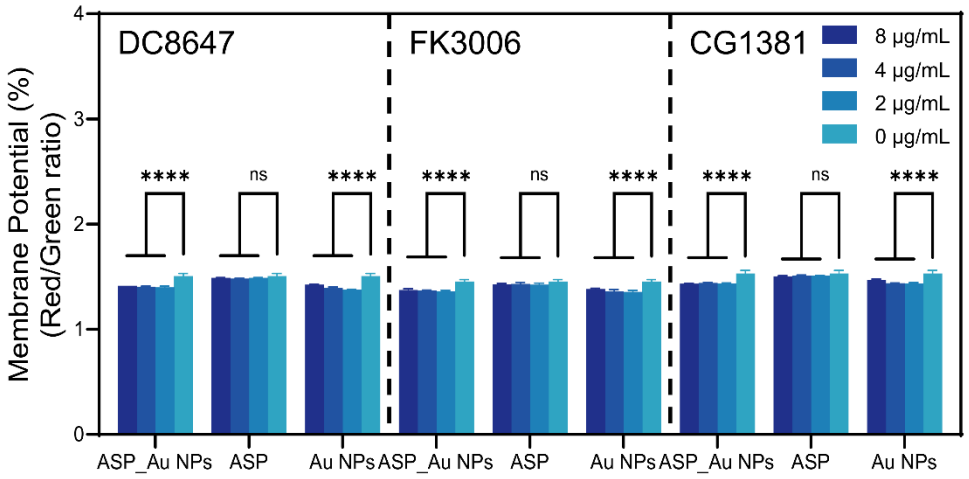

Figure S3. Changes in bacterial membrane potential after treatment with different concentrations of ASP, Au NPs, and ASP_Au NPs in clinical CRE strains. The statistical difference in fluorescence intensity compared to the PBS group (represented in the figure as 0 μg/mL) indicates membrane depolarization.

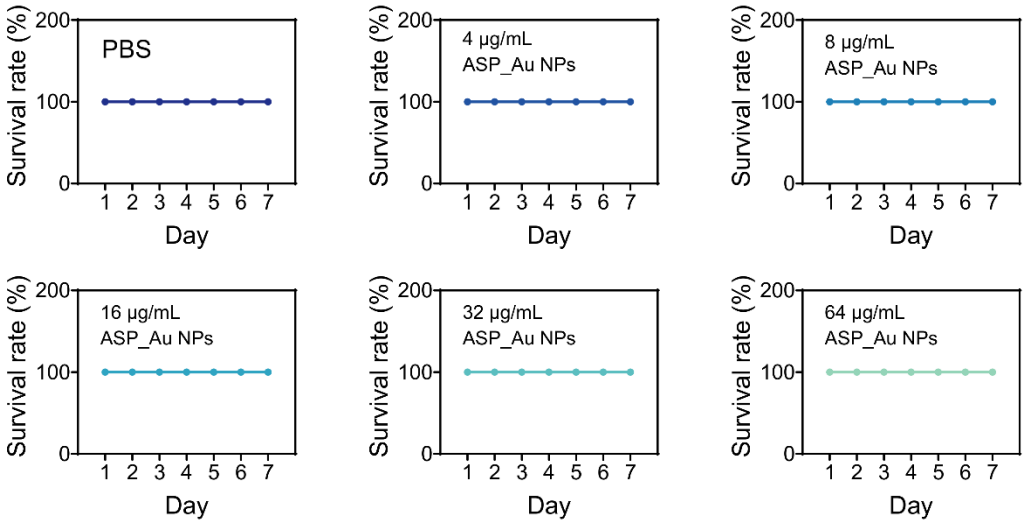

Figure S4. Toxicity experiment in *Galleria mellonella* larvae. Survival of *Galleria mellonella* larvae (10 per group) after injection with different concentrations of ASP_Au NPs.

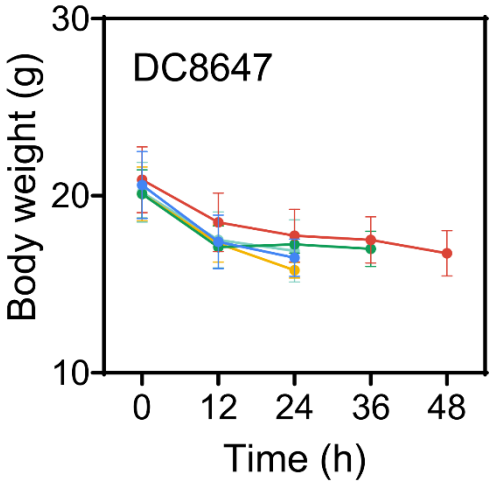

Figure S5. Weight variation curve of mice infected with clinical CRE and treated with different formulations over time.

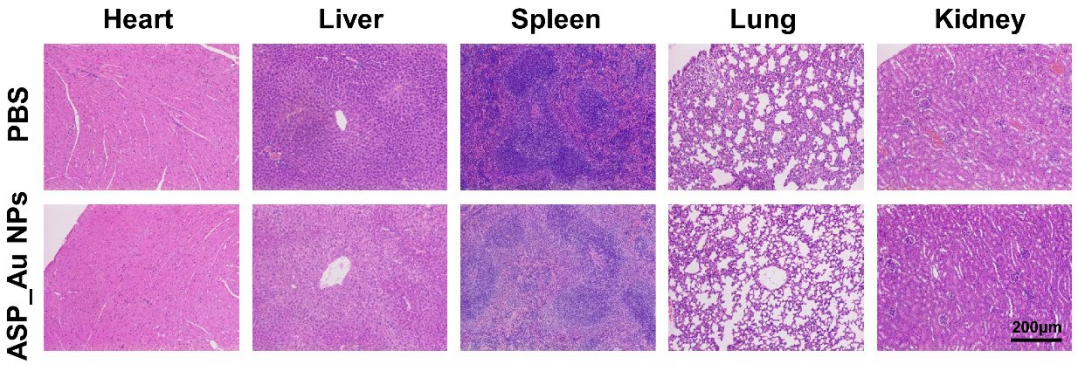

Figure S6. Histopathological sections of major organ tissues (heart, liver, spleen, lung, kidney) in mice injected with PBS (represented in the figure as 0 μg/mL) or ASP_Au NPs. The sections were stained with H&E and observed under a microscope. The injection dosage and time correspond to the acute intraperitoneal infection model in mice.

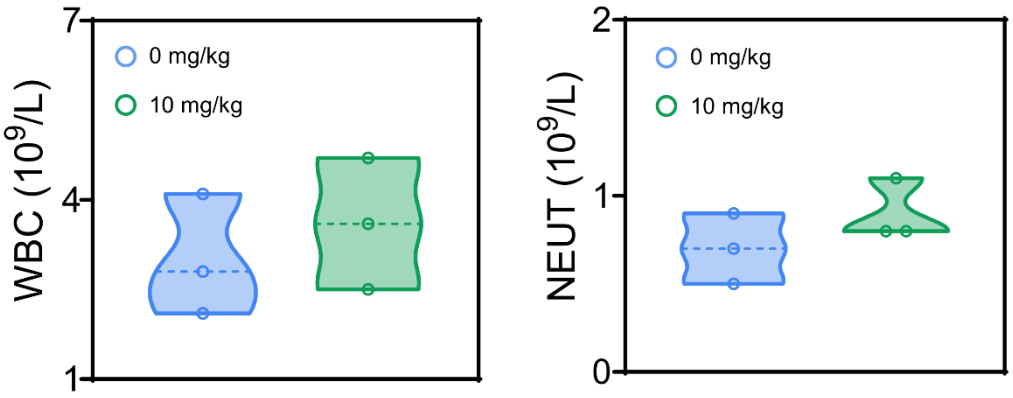

Figure S7. Hematological analysis of mice injected with PBS (represented in the figure as 0 μg/mL) or ASP_Au NPs, reflecting changes in major inflammatory cells in the blood. WBC represents white blood cell count, and NEUT represents neutrophil count.

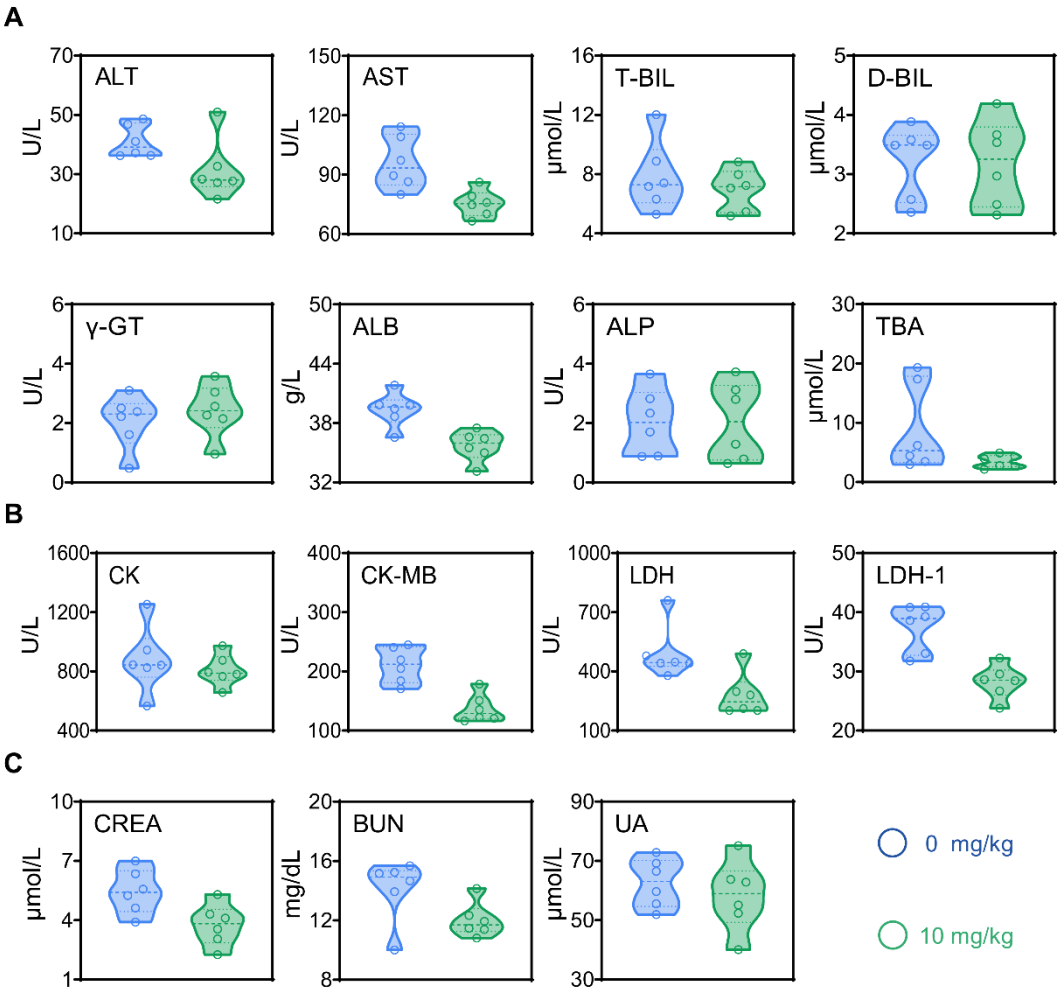

Figure S8. Biochemical analysis of mice injected with PBS (represented in the figure as 0 μg/mL) or ASP_Au NPs. (A) Reflects liver function with 8 parameters. (B) Reflects cardiac enzyme profile with 4 parameters. (C) Reflects renal function with 3 parameters. The abbreviations in the figure are as follows. ALT: alanine aminotransferase; AST: aspartate aminotransferase; T-BIL: total bilirubin; D-BIL: direct bilirubin; ALB: albumin; ALP: alkaline phosphatase; γ-GT: γ-glutamyl transpeptidase; TBA: total bile acid; BUN: blood urea nitrogen; CREA: serum creatinine; UA: uric acid; CK: creatine kinase; CK-MB: creatine kinase-MB; LDH: lactate dehydrogenase; LDH-1: lactate dehydrogenase-1.

Table S1. Mechanism of carbapenem resistance and antimicrobial susceptibility of the IPM, MEM and ETP against 32 strains used in this study.

Species	Strains	Antimicrobial resistance mechanism	MIC (μg/mL)		
			IPM	MEM	ETP
E. coli	DC2003	CTX-M-1, CTX-M-9, OmpF	32	64	256
	DC5113	KPC-2, TEM, CTX-M-1, CTX-M-9	8	16	128
	DC5128	CTX-M-1, CTX-M-9,OmpC, OmpF, NDM	32	64	256
	DC5293	TEM, SHV	0.25	64	128
	DC6856	KPC-2, CTX-M-1, CTX-M-9, OmpF	8	16	128
	DC7114	NDM, TEM, CTX-M-1, OmpC	16	32	128
	DC7706	NDM, TEM, CTX-M-1, CTX-M-9, OmpF	32	64	128
	DC8647	NDM-1	16	32	128
	DC10694	CTX-M-1, CTX-M-9, TEM, SHV	0.5	0.5	256
	DC11722	KPC-2, OXA-1, CMY-42	32	128	512
	ATCC25922	β-lactamase negative	<0.25	<0.25	<0.25
K. pneumoniae	FK2836	KPC-2, IMP	128	64	256
	FK3006	KPC-2, IMP	32	128	256
	FK3020	IMP	32	128	256
	FK6709	KPC-2, OXA-23	32	64	256
	FK6724	KPC-2, OXA-23	32	16	128
	FK7079	CTX-M-9, SHV, TEM	8	256	128
	FK7112	KPC-2, OmpK37mutation	64	128	512
	FK7513	NDM-5	32	64	128
	FK8696	KPC-33, CTX-M-9, SHV,TEM	0.25	2	128
	FK9283	NDM-1	8	128	64
	ATCC700603	SHV-18, OXA-2, OmpK35 and OmpK37 mutations, TEM-1	0.25	0.25	0.5
E. cloacae	CG648	KPC-2, AmpC	16	32	32

CG1038	KPC-2, AmpC	8	32	128
CG1181	SHV, TEM, CTX-M-1, CTX-M-9, CTX-M-14, impermeability	2	<0.25	128
CG1212	TEM, CTX-M-14, impermeability, efflux pump	1	1	64
CG1249	AmpC, impermeability	0.5	1	128
CG1257	IMP	32	4	64
CG1330	NDM-1	8	16	128
CG1381	OXA-23	32	32	64
CG1737	NDM-5	32	64	128
CG1813	IMP, efflux pump	8	16	128

Table S2. Antimicrobial susceptibility of ASP and Au NPs single or in combination against the 6 clinical isolates used in this study.

Strains	Monotherapy (MIC, μg/mL)		Combination (MIC, μg/mL)	
	ASP	Au NPs	ASP	Au NPs
DC8647	≥256	≥256	≥256	≥256
DC11722	≥256	≥256	≥256	≥256
FK3006	≥256	≥256	≥256	≥256
FK7513	≥256	≥256	≥256	≥256
CG1330	≥256	≥256	≥256	≥256
CG1381	≥256	≥256	≥256	≥256

Table S3. Antimicrobial susceptibility of ASP_Au NPs before and after using quercetin against the 6 clinical isolates used in this study.

Strains	ASP_Au NPs (MIC, μg/mL)	
	Before	After
DC8647	8	≥256
DC11722	16	≥256
FK3006	8	≥256
FK7513	8	≥256
CG1330	4	≥256
CG1381	8	≥256

Table S4. Reference range of biochemical indicators in mice.

Testing items	Indicators	Reference range	Unit
Liver function	ALT	10.06-96.47	U/L
	AST	36.31-235.48	U/L
	T-BIL	6.09-53.06	μmol/L
	D-BIL	0.45-33.89	μmol/L
	ALB	21.22-39.15	g/L
	ALP	22.52-474.35	U/L
	γ-GT	0-7.78	U/L
	TBA	0-8.51	μmol/L
Renal function	BUN	10.81-34.74	mg/dL
	CREA	10.91-85.09	μmol/L
	UA	44.42-224.77	μmol/L
Myocardial enzymes	CK	0-2070.55	U/L
	CK-MB	0-1500	U/L
	LDH	157.41-899.72	U/L
	LDH-1	0-37.07	U/L

Table S5. Primers used to amplify mRNAs via RT-qPCR.

Gene	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
TNF-α	GAGTCCGGGCAGGTCTACTTT	CAGGTCACTGTCCCAGCATCT
β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA

Table S6. Main materials used in this study and the corresponding manufacturers.

Materials	**Manufacturer**
$HAuCl_4 \cdot 3H_2O$ (48~50% Au basis)	Macklin (Shanghai, China)
Tween 80	Solarbio (Beijing, China)
Triethylamine	Wenzhou Jinshan Chemical Reagent Instrument Co., Ltd. (Zhejiang, China)
Dialysis filter membranes	Solarbio (Beijing, China)
SAC	Macklin (Shanghai, China)
SUC	Macklin (Shanghai, China)
ACE	Macklin (Shanghai, China)
ASP	Macklin (Shanghai, China)
Quercetin	MedChemExpress (China)
Glycerol	Thermo Fisher Scientific (America)
$DiOC_2(3)$	Maokang Biology (Shanghai)
$NaBH_4$	Macklin (Shanghai, China)
CCK-8	Solarbio (Beijing, China)
ROS assay kit	Beyotime (Shanghai, China)
Protein detection kits	Boyun (Shanghai, China)
RevertAid First Strand cDNA Synthesis Kit	Thermo Fisher Scientific (America)
Tli RNaseH Plus	Takara (Japan)
Cyclophosphamide	Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China)
2.5% glutaraldehyde	Servicebio (Hubei, China)