Supporting Information

Label-free identification Carbapenem-Resistant *Escherichia coli* based on surface-enhanced resonance Raman scattering

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Experiemental Section

Materials and chemicals

Silver nitrate ($AgNO_3$), chlorauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$) and sodium borohydride ($NaBH_4$) were purchased from Sinopharm Chemical Reagent Co. Ascorbic acid (AA), hexadecyltrimethylammonium bromide (CTAB) were purchased from Sigma–Aldrich Chemicals Co. All aqueous solutions purified with a Milli-Q system (18.2 MΩ cm^{-1}). All bacteria strains included in this study were prospectively collected from Affiliated Hospital of Xuzhou Medical University, and they have been identified using VITEK2 COMPACT high intelligent automatic microbial identification system (bioMérieux, La Balme-les-Grottes, France).

Instruments

Transmission electron microscopy (TEM) images were obtained from a Hitachi H-7650 microscope operating at an accelerating voltage of 80 kV. High-resolution TEM (HRTEM) images and EDX spectra were obtained using a JEM-2010F microscope at an accelerating voltage of 200 kV (JEOL Ltd, Japan). UV-Vis spectra were recorded with a Shimadzu 2600 spectrometer. All Raman spectra were recorded with a portable Raman system (B&W Tek, i-Raman Plus BWS465-785H spectrometer) with a back-illuminated CCD as the detector. SERS measurements were measured using a 785 nm excitation laser, 20 × microscope objective, and spectral resolution of 1 cm^{-1}. The laser beam spot size was around 105 μm at a working distance of 8.8 mm. For each

sample, five spectra from different sites were collected and averaged to ensure the reproducibility.

Preparation of plasmonic Au@Ag core-shell NRs

Au NRs were synthesized following a previously reported seeding growth method with some modifications (*Chem. Mater.* 2003, 15, 1957). In brief, the seed solution was prepared by adding 2.5 mL of $HAuCl_4$ (0.5 mM) into 2.5 mL of CTAB solution (0.2 M), and 300 μL of freshly prepared $NaBH_4$ (0.01 M) was then added under vigorous stirring (500 rpm, 30 °C) for 1 min. After the stirring was stopped, the resulting seed solution was kept at 30 °C for 1 h before use. To synthesize Au NRs with longitudinal plasmon resonance peak located at 785 nm, 20 mL of $HAuCl_4$ (1 mM) was added into 20 mL of CTAB solution (0.2 M); after gentle mixing of the solution, 0.4 mL of $AgNO_3$ solution (0.01 M) was added and mixed. Then, 0.38 mL of HCl (2 M) was added and mixed. Subsequently, 0.32 mL of AA (0.1 M) was mixed with the solution before the seed solution (140 μL) was added. Finally, the mixture was placed into a water bath and kept undisturbed at 30 °C for 12 h without any further stirring until the color change was complete. The obtained AuNRs were purified three times through centrifugation at 7500 rpm for 6 min to remove any excess reagents.

To prepare Au@Ag core-shell NRs, 2 mL of the as-prepared Au NRs was added to 4 mL of CTAB solution (0.04 M) with vigorous stirring at 30 °C. Then 40 μL of freshly prepared ascorbic acid (0.1 M), 20 μL of $AgNO_3$ (1 mM), and 240 μL of NaOH (0.1 M) were added sequentially. After 2 min, the color change was complete, and the solution was centrifuged twice at 7500 rpm for 6 min to remove the excess reagents. The final precipitate was redispersed in 6 mL of deionized water for further use.

Preparation of 60 nm Au@Ag core-shell NPs

Au NPs (50 nm) were synthesized through a previously reported sodium citrate reduction method (*Nature protocols.* 2013, 8, 52–65). The Au@Ag core–shell NPs were prepared by coating the Ag shell on the Au NPs. Briefly, 100 mL of as-prepared Au NPs solution was double diluted with deionized water, heated to boiling, and then vigorously stirred. Subsequently, 2 mL of trisodium citrate (1%, w/v) was added to the suspension and used as the reducing agent. Finally, 1 mL of silver nitrate (10 mM) was dropwise added to the above suspension and the mixture was then maintained at 100°C to keep boiling state for 45 min, yielding the 60 nm Au@Ag core–shell NPs.

SERS measurements

5 μL of *E. coli* solution (approximately 1×10^8 cells/mL) was mixed with 5 μL of 10-fold concentrated Au@Ag NRs, and then vortexed to ensure the homogeneity of the hybrid mixture. The mixture was incubated for 5 min at room temperature and transferred onto a Si chip. Subsequently, it was dried at room temperature for SERS measurement. Five spectra from different sites on the coffee-ring zone of each sample were collected and averaged to represent the

SERS result. Before analysis, spectral data were treated with baseline subtraction, smoothing, and area normalization to remove the fluorescence background and enhance signal-to-noise ratio effectively.

Statistical analysis

Raman spectral data analysis was performed based on SIMCA 14.0 software (Umetrics, Umea, Sweden). OPLS-DA models were developed on the basis of PLS-DA models. The most important feature of the method is that it can separate predictive from non-predictive (orthogonal) variation and it is very applicable to classify data that have multi-collinear and noisy variables, which is common for many types of biological data. Classification of bacteria was directly reflected by the principal component scores of OPLS-DA models, and the robustness of these models was assessed based on the following parameters: $R^2X(cum)$, cumulative sum of squares (SS) of all x-variables explained by all extracted components; $R^2Y(cum)$, cumulative SS of all y-variables explained by all extracted component; and $Q^2(cum)$, fraction of all x-variables (PC) and y-variables that can be predicted for the extracted component. In addition, ten-fold cross validation was used to evaluate the classifier and the capability of the OPLS-DA models to predict the classification of unknown samples. In brief, the spectral data were divided into 10 subgroups randomly, and each subgroup (test set) was tested by a training set model built with the remaining subgroups. The average accuracy rates of the training and test sets after 10 operations were then obtained.

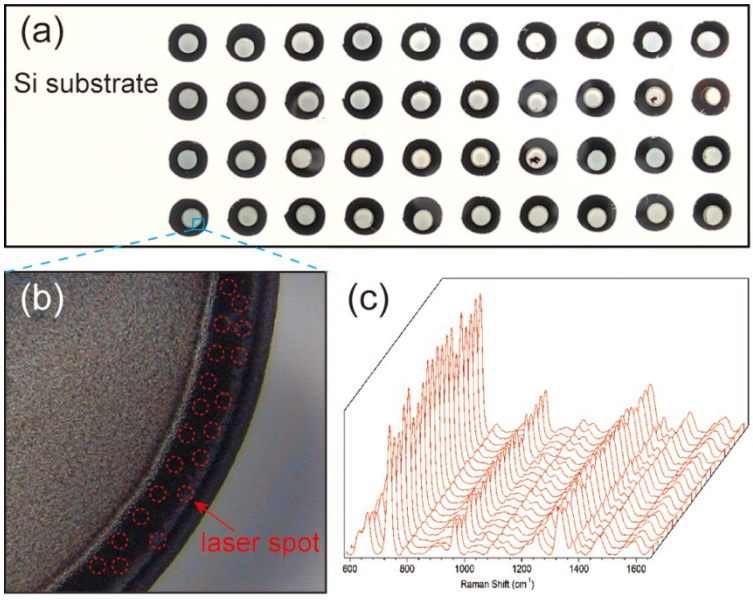

Fig. S1 Photographs (a), representative light microscopy images (b), and the SERS spectra collected from 20 randomly selected spots on the coffee-ring zone (c) of the *E. coli*/Au@Ag NRs complexes on the Si substrate.

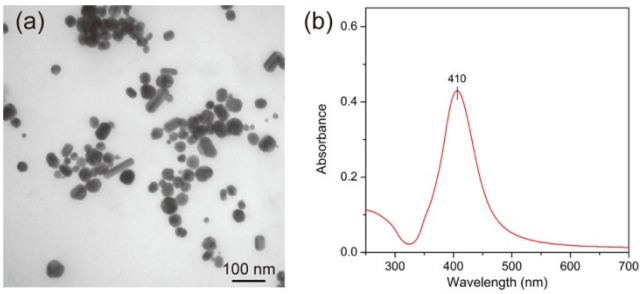

Fig. S2 TEM image (a) and UV–vis absorbance spectra (b) of Ag NPs that prepared by $AgNO_3$ reduction directly.

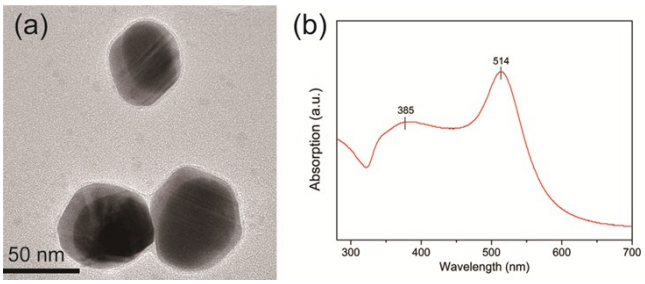

Fig. S3 TEM image (a) and UV–vis absorbance spectra (b) of 60 nm Au@Ag NPs.

Table S1 Minimal inhibitory concentration (MIC) and antimicrobial susceptibility testing of imipenem in 89 clinical isolates of *E. coli*.

Isolate	Phenotype		Isolate	Phenotype	
	MIC(mg/L)	Category[a]		MIC(mg/L)	Category[a]
1E	>=16	R	46E	8	R
2E	>=16	R	47E	>=16	R
3E	>=16	R	48E	<=1	R
4E	>=16	R	49E	>=16	R
5E	>=16	R	50E	>=16	R
6E	>=16	R	51E	8	R
7E	8	R	52E	8	R
8E	>=16	R	53E	>=16	R
9E	<=1	S	54E	<=1	R
10E	>=16	R	55E	>=16	R
11E	>=16	R	56E	>=16	R
12E	8	R	57E	8	R
13E	<=1	S	58E	>=16	R
14E	<=1	S	59E	<=1	R
15E	<=1	S	60E	>=16	R
16E	<=1	S	61E	>=16	R

17E	<=1	S	62E	<=1	S
18E	<=1	S	63E	<=1	S
19E	<=1	S	64E	<=1	S
20E	>=16	R	65E	<=1	S
21E	>=16	R	66E	<=1	S
22E	>=16	R	67E	<=1	S
23E	>=16	R	68E	<=1	S
24E	4	R	69E	<=1	S
25E	>=16	R	70E	<=1	S
26E	>=16	R	71E	<=1	S
27E	<=1	S	72E	<=1	S
28E	<=1	S	73E	<=1	S
29E	<=1	S	74E	<=1	S
30E	<=1	S	75E	<=1	S
31E	<=1	S	76E	<=1	S
32E	<=1	S	77E	<=1	S
33E	<=1	S	78E	<=1	S
34E	<=1	S	79E	<=1	S
35E	<=1	S	80E	<=1	S
36E	<=1	S	81E	<=1	S
37E	>=16	R	82E	<=1	S
38E	>=16	R	83E	<=1	S
39E	>=16	R	84E	<=1	S
40E	>=16	R	85E	>=16	R
41E	4	R	86E	>=16	R
42E	>=16	R	87E	>=16	R
43E	>=16	R	88E	>=16	R
44E	4	R	89E	>=16	R
45E	>=16	R			

[a] The MIC-based category was assessed according to the CLSI M100, S 27 (S, ≤1 mg/L; R, ≥4 mg/L)

Table S2 Result of the ten-fold cross validation of the model built with carbapenem-resistant Escherichia coli (CREC) and carbapenem-sensitive Escherichia coli (CSEC).

Number of tests	**training set accuracy**	**test set accuracy**
1	100%	100%
2	100%	100%
3	100%	100%
4	100%	100%
5	100%	100%

6	100%	100%
7	100%	100%
8	100%	100%
9	100%	94.3%
10	100%	100%
average value	100%	99.4%