Supporting Information

Metallic Nanoparticles Bioassay for *Enterobacter cloacae* P99 β-Lactamase Activity and Inhibitor Screening

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Identification of Class C *E. cloacae* P99 β-lactamase. The purity and isoform components of Class C *E. cloacae* P99 β-lactamase was analyzed by SDS-PAGE. As shown in Figure S1, the purified *E. cloacae* P99 β-lactamase exhibited one band with molecular weight of 39 KDa which indicated the higher molecular weight than that of transformed TEM-1 β-lactamase (29 KDa). The result is consistent with the previous report.1

![Figure S1](image)

**Figure S1.** SDS-PAGE analysis of *E. cloacae* P99 class C Bla (Lane 1, 12 µM) with molecular weight of 39 KDa and transformed TEM-1 class A Bla (Lane 2, 12 µM) with molecular weight of 29 KDa.

Colorimetric inhibition assay by using Nitrocefin: Class C *E. cloacae* P99 Bla was initially incubated with different inhibitors in phosphate buffer saline (PBS buffer, pH 7.4) for 10 min and subsequently β-lactam substrate was added for additional 20 min incubation. Then, the aliquot of above mixture solution was transferred into nitrocefin solution affording 5 µM of substrate, 5 µM of inhibitor and 3 nM of *E. cloacae* P99 Bla.

![Figure S2](image)

**Figure S2.** Colorimetric inhibition assay for *E. cloacae* P99 Bla in 96-well microplate with four kinds of inhibitors.
Figure S3. Absorbance change of nitrocefin at 486nm as a function of time in the presence of *E. cloacaе* P99 Bla inhibitors.

As shown in Figure S4, IC$_{50}$ values of ATM, TZB, SUL and CA was evaluated with nitrocefin to be 3.0 nM, 165.9 nM, 5.5 µM and 1.12 mM, respectively. This result is consistent with the IC$_{50}$ values recognized by using AgNPs and AuNPs.

Figure S4. IC$_{50}$ graph of ATM, TZB, SUL and CA by using absorbance change of nitrocefin at 486 nm.

Reference: