Supplementary Text S2 ROS and cell membrane permeability detection

The donor and recipient strains were cultured separately overnight at 30°C in LB medium. They were then centrifuged at 5000 rpm for 5 min, washed twice, and resuspended in PBS. According to the kit instructions, the cells were collected by centrifugation, followed by the addition of 10 μM DCFH-DA solution to achieve a cell concentration of 10^6–10^7 CFU/mL. The mixture was incubated at 37 °C for 20 min with intermittent mixing. After incubation, the cells were washed three times with PBS and resuspended in PBS. A 495 μL aliquot of the probe-loaded bacterial suspension was mixed with tetracycline, ampicillin, kanamycin, and streptomycin at final concentrations of 0.005, 0.05, 0.5, 5, and 50 mg/L, respectively, bringing the total volume to 500 μL. Positive and negative controls were included. After vortex mixing, the mixture was incubated in the dark at 25°C for 20-30 minutes. A defined volume of each was transferred into a 96-well plate, and the fluorescence intensity was measured using a microplate reader (excitation: 488 nm; emission: 525 nm). The fluorescence intensity was used to quantify intracellular ROS levels. All experiments were performed in triplicate.

For membrane permeability analysis, cells were cultured, washed, and resuspended in PBS to achieve a concentration of 10^6 CFU/mL. A defined volume of the bacterial suspension was added to tetracycline, ampicillin, kanamycin, and streptomycin at final concentrations of 0.005, 0.05, 0.5, 5, and 50 mg/L in a volume of 500 μL. The positive control consisted of heat-treated cells (100 °C, water), while the negative controls included anhydrous ethanol or ultrapure water. After vortexing, 100 μL of each bacterial mixture was transferred to a 96-well plate. Then, 1 μL of PI staining solution (final concentration: 20 μg/L) was added to each well. The samples were incubated in the dark at 37 °C for 30 min. Fluorescence intensity was measured using a microplate reader (excitation: 488 nm; emission: 630 nm). The fluorescence signal was used to represent cell membrane permeability. All experiments were conducted in triplicate.