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High-contrast imaging of cellular non-repetitive drug-resistant gene via *in situ* dead Cas12a-labeled PCR

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Experimental Procedures

Materials and reagents

All the oligonucleotides were synthesized from Sango Biotech (Shanghai, China). The oligonucleotide sequences were purified by polyacrylamide gel electrophoresis (Table S1 and S2). Biological reagents including 4% paraformaldehyde fix solution, Triton X-100, 1× PBS, 50× TAE buffer, agarose, LB broth, imidazole, and tetrazolium red were also purchased from Sango Biotech (Shanghai, China). Precast protein plus gel, ampicillin sodium, chloramphenicol, kanamycin, and Tris-HCl were purchased from Yeasen Biotech (Shanghai, China). Protease K and 2× Easy PCR super mix was purchased from Tiangen Biotech (Beijing, China). Triton X-100 was purchased from Solarbio Biotech (Beijing, China). 1000× GelRed dye and 6× loading buffer were purchased from Tsingke Biotechnology (Beijing, China). DH5α chemically competent cell and DE3 chemically competent cell were purchased from Tsingke Biotech (Beijing, China). T7 RNA polymerase, phi29 DNA polymerase, DNase I, dNTPS, and rNTPs were purchased from New England Biolabs (Beijing, China). The quinolone-sensitive *S.enterica* was purchased from China Center of Industrial Culture Collection (ATCC 14028) and the quinolone-resistant *S. enterica* was isolated from a poultry farm.

Plasmid Construction and Protein Purification of dFnCas12a-GFP

The sequences of dFnCas12a of dFnCas12a-PET28a plasmid and GFP of GFP/RNase H1 D210N plasmid were cloned in order into the pET28 vector to obtain dFnCas12a-PET28a-GFP plasmid (Fig. S1). The *E. coli* expression plasmids of the dFnCas12a-GFP were transformed in *Escherichia coli* Rosseta cells. Protein expressions were inducted with 0.1 mM IPTG for 16 h. Cells were resuspended in lysis buffer (20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 5 mM $MgCl_2$, 5% (v/v) glycerol) and disrupted by Hydraulic Breaker. Cell debris and insoluble particles were removed by centrifugation at 11,200 rpm at 4°C. The lysate was filtered through 0.8 μm filters and applied to a nickel column, and then eluted with a gradient of imidazole. The fusion protein with 25 mM, 50 mM and 100 mM imidazole elution concentration were purified and concentrated in the next step (Fig. S2). Fractions containing protein of the expected size were pooled and applied onto a HiTrap Heparin HP column equilibrated with buffer L (20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT,

500 mM NaCl, 5% (v/v) glycerol). The protein was eluted with a linear gradient of 0-100% buffer H (20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 1.5 M NaCl, 5% (v/v) glycerol). The protein peaks were dialyzed with buffer L and concentrated. Then, the proteins can be either used directly for biochemical assays or frozen at -80°C.

Preparation of CRISPR RNA (crRNA)

The initial step for synthesizing double-stranded DNA templates was conducted at 30°C for 30 min within 20 μL of reaction volume. The reaction mixture included 1× phi29 DNA polymerase buffer (33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate, 1 mM dithiothreitol (DTT), and 0.1% (v/v) Tween 20, pH 7.9), the T7 promoter (1 μM) and L-gRNA (1 μM), 4 U of phi29 DNA polymerase and 0.5 mM of dNTPs. Following the DNA template synthesis, the transcription of crRNAs took place at 37°C for 3 h in a 40 μL reaction volume. This mixture consisted of 20 μL of the previously synthesized DNA template, 1× transcription buffer, 20 U of T7 RNA polymerase, and 0.5 mM of rNTPs. Finally, 1 μL of DNase I (20 U/μL) was added to degrade any remaining DNA templates, and the mixture was incubated at 37°C for 2 h, followed by heating at 75°C for 10 min.

Extraction of Bacterial Genomic DNA

According to the manufacturer's instructions of the Ezup column-type bacterial genomic DNA extraction kit (Sangon Biotech) to extract bacterial genomic DNA. First, 1 mL of cultured bacterial suspension was centrifuged overnight at 8,000 rpm for 1 min at room temperature and the supernatant was discarded the supernatant. Then, 180 μL Buffer Digestion and 20 μL proteinase K solution was added to the precipitate, subsequently, was incubated at 56°C for 1 h until the bacterial cells were completely lysed. 200 μL of Buffer BD was added and mixed thoroughly, and the mixture was incubated at 70°C for 10 min. Next, 200 μL pf anhydrous ethanol was added. The mixture was added to the adsorption column and was centrifuged at 12,000 rpm for 1 min at room temperature, then the flowing fluid was removed. 500 μL of PW Solution and Wash Solution were added to the collection column and the mixture was centrifuged at 10,000 rpm for 30 s. The adsorption column was added to a new centrifuge tube and the mixture was centrifuged at 12000 rpm for 2 min. Finally, 50 μL of H_2O was added to dissolve DNA. The DNA concentration was determined by a microplate reader Synergy H1 (BioTek, Winooski, USA). The bacterial genomic

DNA was identified by PCR and agarose gel electrophoresis.

Bacterial Pretreatment

The bacteria used were incubated in LB at 37°C for 16 h. First, the 1 mL of bacterial solution was centrifuged at 6,000 rpm for 5 min to enrich bacteria, and the supernatant was discarded. Then, 200 μL of 4% paraformaldehyde was added to fix the bacteria for 1 h, Subsequently, the mixture was centrifuged at 6,000 rpm for 3 min, and the supernatant was discarded. the bacteria incubated with 200 μL of proteinase K solution (50 μg/mL) at 37°C for 10 min, and then treated with 5‰ Triton X-100 at 37 °C for 5 min. Then, the mixture was also centrifuged at 6,000 rpm for 3 min, and the supernatant was discarded. Finally, the bacterial precipitate was washed three times by centrifugation with 1× PBS buffer.

Gel Electrophoresis Analysis

The nondenaturing gel electrophoresis was conducted on a prepared gel in TAE buffer. The loading mixture for the gel consisted of 5 μL of oligonucleotide solution combined with 1 μL of gel loading buffer. Then, the electrophoresis was performed on a 3% agarose gel in 1× TAE buffer containing 1× GelRed dye at 150 V for 25 min. The results were photographed and documented using a Gel Doc XR+system (Bio-Rad, Winooski, VT, USA).

Bacteria Imaging

Before the imaging, i*n situ* PCR procedure was performed. The PCR reaction was carried out in a total volume of 30 μL, which contains 1 μL of bacteria solution after pretreatment, 1 μL of forward primers (100 μM), 1 μL of reverse primers (100 μM), 15 μL of KOD Mix, and 12 μL of H_2O. The cycling procedure involved the pre-denaturation at 95°C for 5 min; denaturation at 95°C for 15 s, followed by 30 cycles at 60°C for 15 s, 72°C for 45 s, and 72°C for 5 min. The process was performed in a ProFlex 3×32 - well PCR System (Thermo Fisher Scientific, USA). After *in situ* PCR amplification, the supernatant was removed by centrifugation at 6,000 rpm for 3 min, and 10 μL of dCas12a-GFP/crRNA reaction system was mixed with the bacterial precipitate. The dCas12a-GFP/crRNA reaction system was obtained by premixing crRNA and dCas12a-GFP fusion protein. The 10 μL premixed system consisted of 0.42 μL dCas12a-GFP protein (30 μM), 0.8 μL crRNA (10

μM), and 8.78 μL buffer, and was incubated at 37°C for 10 min. Finally, the dCas12a-GFP/crRNA premix, bacterial precipitate, and 30 μL of H_2O were incubated at 37°C for 1.5 h to image. The cellular images were acquired using a 60× objective. The glass slide was modified with poly-L-lysine. A 488 nm laser was used as the excitation source, and the emission wavelength ranged from 515 to 555 nm. The outline of bacterial cells was determined by the bright field images and the fluorescence intensity was measured by ImageJ. Then, the gray value was calculated as the mean fluorescence intensity of each bacterial cell. In each experimental group, we measured the fluorescence intensity of cells, with each dot representing the average fluorescence intensity of a single cell.

Table S1. The sequences of *in situ* PCR primers

Name	Oligonucleotides	Sequences (5' to 3')
	oqxB-F	ATGGACTTTTCCCGCTTTTTTA
	oqxB-R	CGCCACGGTCTCGGCAATCAC
P1	F1	ATATCGAAGAGGGGCTTGCG
	R1	GTCCCTGATAGCCGTTCGAG
P2	F2	AAAGTGATTGCCGAGACCGT
	R2	GGAGAACAGATGCACCACCA
P3	F3	GGCTATCGGTATCGTGGTGG
	R3	TTGTAGAACTGGCCGGTGAC
P4	F4	CTCGAACGGCTATCAGGGAC
	R4	CGATCTCGCTCATTTTGCGG
P5	F5	CCATCTCGACGGTGATCTCG
	R5	CGTTTTGCCTACCAGTCCCT
P6	F6	CGGGATGAATACCGAAGGGG
	R6	CGCGATTTTGGCGTTGATCT
P7	F7	TGGTGGTGCATCTGTTCTCC
	R7	CGGGTGACTGGAAGATACCG
P8	F8	CGGTATCTTCCAGTCACCCG
	R8	CCACCACGATACCGATAGCC
P9	F9	CCTGGTGGTGCATCTGTTCT
	R9	TCCCGACCGCATCCTTATTG
P10	F10	AGGGACTGGTAGGCAAAACG
	R10	ACCCAGACCTAAAATCGGCG

Table S2. The sequences of crRNAs targeting different locations of the *in situ* PCR amplicon

Oligonucleotides	Sequences (5' to 3')
crRNA1	GCGCCGCGGCAAGCGGCGAUCUACACUUAGUAGAAAUUACCCUAUAGUGAGUCGUAUUA
crRNA2	UCGCCUGAUGCGCCGCGGAUCUACACUUAGUAGAAAUUACCCUAUAGUGAGUCGUAUUA
crRNA3	GCACCAGCGCAAUGGCGAAUCUACACUUAGUAGAAAUUACCCUAUAGUGAGUCGUAUUA
crRNA4	CCGCACACAGCACCAGCGAUCUACACUUAGUAGAAAUUACCCUAUAGUGAGUCGUAUUA
crRNA5	GAGAAACGCCAUCGGCACAUCUACACUUAGUAGAAAUUACCCUAUAGUGAGUCGUAUUA

Table S3. The sequencing result of oqxB gene of the *S. enterica.*

Gene	Sequences (5’ to 3’)
oqxB	ATGGACTTTTCCCGCTTTTTTATCGACAGGCCGATTTTCGCCGCGGTGCTGTCG ATTTTAATTTTTATCACCGGGTTAATCGCTATCCCACTGCTGCCGGTGAGCGAAT ATCCGGATGTCGTCCCGCCGAGCGTCCAGGTGCGCGGAGTATCCCGGCGC CAACCCGAAAGTGATTGCCGAGACCGTGGCGACGCCGCTGGAGGAAGCGATCA ACGGCGTTGAAAACATGATGTACATGAAATCGGTCGCCGGCTCCGACGGCGTG CTGGTCACCACCGTCACCTTCCGCCCGGGTACCGACCCGGATCAGGCGCAGGT TCAGGTGCAGAACCGCGTCGCGCAGGCCGAAGCGCGTCTGCCGGAGGATGTA CGCCGTCTGGGGATCACCACCCAGAAGCAGTCTCCGACGCTGACCCTGGTGGT GCATCTGTTCTCCCCCGGCGGGAAGTACGACTCGCTGTATATGCGCAACTACGC CACGCTGAAAGTGAAGGATGAGCTGGCGCGCCTGCCCGGCGTCGGCCAGATC CAGATTTTTGGCTCCGGTGAATATGCGATGCGCGTCTGGCTGGATCCCAATAAG GTCGCTGCCCGCGGTCTGACGGCCTCGGATGTGGTGACGGCGATGCAGGAGC AAAACGTCCAGGTGTCTGCCGGACAGCTTGGCGCCGAGCCGCTGCCGCAGGA GAGCGATTTCCTGATCTCCATTAACGCCCAGGGCCGTCTGCATACCGAAGAAGA GTTTGGCAATATCATTCTGAAAACGGCGCAGGATGGCTCGCTGGTCCGCCTGCG CGACGTGGCGCGCATCGAGATGGGTTCCGGTAGCTATGCGCTGCGCTCCCAGC TCAACAATAAGGATGCGGTCGGGATCGGTATCTTCCAGTCACCCGGCGCTAACG CCATCGATCTGTCGAACGCGGTACGCGCCAAAATGGCCGAGCTGGCCACCCGC TTCCCGGAAGATATGCAATGGGCGGCGCCGTACGACCCGACGGTTTTCGTCCG CGACTCCATCCGCGCGGTGGTGCAGACGCTGCTGGAGGCGGTAGTGCTGGTG GTGCTGGTAGTGATCCTGTTCCTGCAGACCTGGCGCGCGTCGATTATCCCGTTG ATCGCTGTGCCGGTATCGGTGGTGGGTACCTTCAGCATTCTCTATCTGCTGGGC TTCTCGCTGAATACCCTGAGCCTGTTCGGGCTGGTACTGGCTATCGGTATCGTG GTGGACGACGCCATCGTGGTGGTGGAGAACGTCGAGCGTAATATCGAAGAGGG GCTTGCGCCGCTTGCCGCGGCGCATCAGGCGATGCGTGAGGTCTCCGGGCCG ATTATCGCCATTGCGCTGGTGCTGTGTGCGGTGTTCGTGCCGATGGCGTTTCTC TCCGGGGTCACCGGCCAGTTCTACAAACAGTTCGCGGTGACCATCGCCATCTC GACGGTGATCTCGGCCATCAACTCGCTGACGCTCTCCCCGGCGCTGGCGGCCC TGCTGTTAAAGCCGCACGGCGCGAAGAAAGACCTCCCTACCCGGCTGATCGAT CGCCTGTTTGGCTGGATTTTCCGTCCGTTTAACCGCTTTTTCCTGCGCAGCTCGA ACGGCTATCAGGGACTGGTAGGCAAAACGCTTGGACGCCGTGGCGCAGTGTTT GCGGTGTACCTGCTGCTGCTCTGCGCCGCTGGGGTGATGTTTAAAGTCGTCCC CGGCGGGTTTATTCCCACCCAGGATAAGCTGTATCTCATTGGCGGCGTGAAGAT GCCGGAAGGGTCGTCGCTGGCGCGCACCGACGCGGTGATCCGCAAAATGAGC GAGATCGGGATGAATACCGAAGGGGTCGACTATGCGGTCGCTTTCCCGGGGCT TAACGCGCTGCAGTTCACCAACACGCCGAATACCGGGACGGTCTTTTTTGGCCT GAAACCGTTCGACCAGCGCAAACACACGGCGGCGGAAATTAACGCGGAGATCA ACGCCAAAATCGCGCAAATCCAGCAGGGCTTTGGCTTTTCCATCCTGCCGCCGC CGATTTTAGGTCTGGGTCA

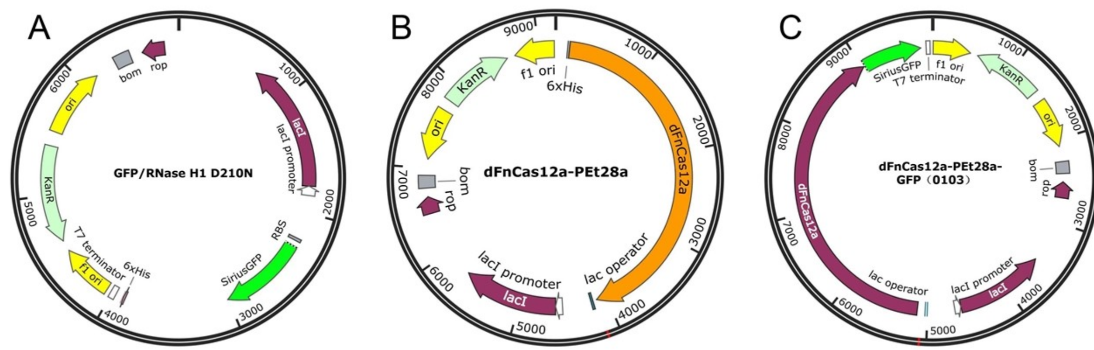

Figure S1. The plasmid maps of RNase H1 D210N (A), dFnCas12a-PET28a (B) and dFnCas12a-PET28a-GFP (C).

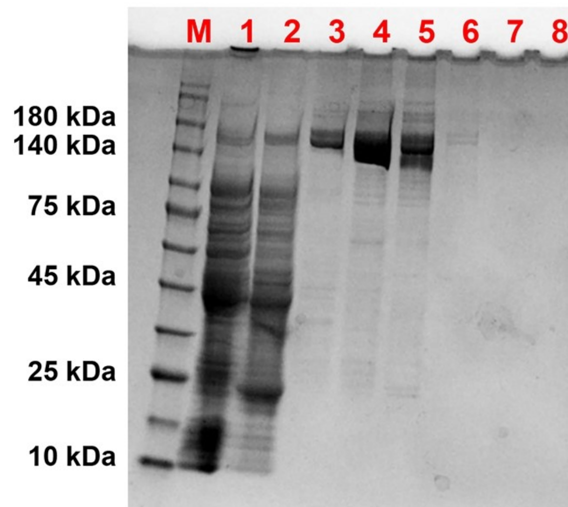

Figure S2. The image of dFnCas12a-GFP fusion protein extraction. Lane 1-8 represents different concentrations of imidazole elution (Flowing fluid, 10 mM, 25 mM, 50 mM, 100 mM, 150 mM, 200 mM, and 500 mM of imidazole buffer). Imidazole buffer was prepared by different concentrations of imidazole and lysis buffe.

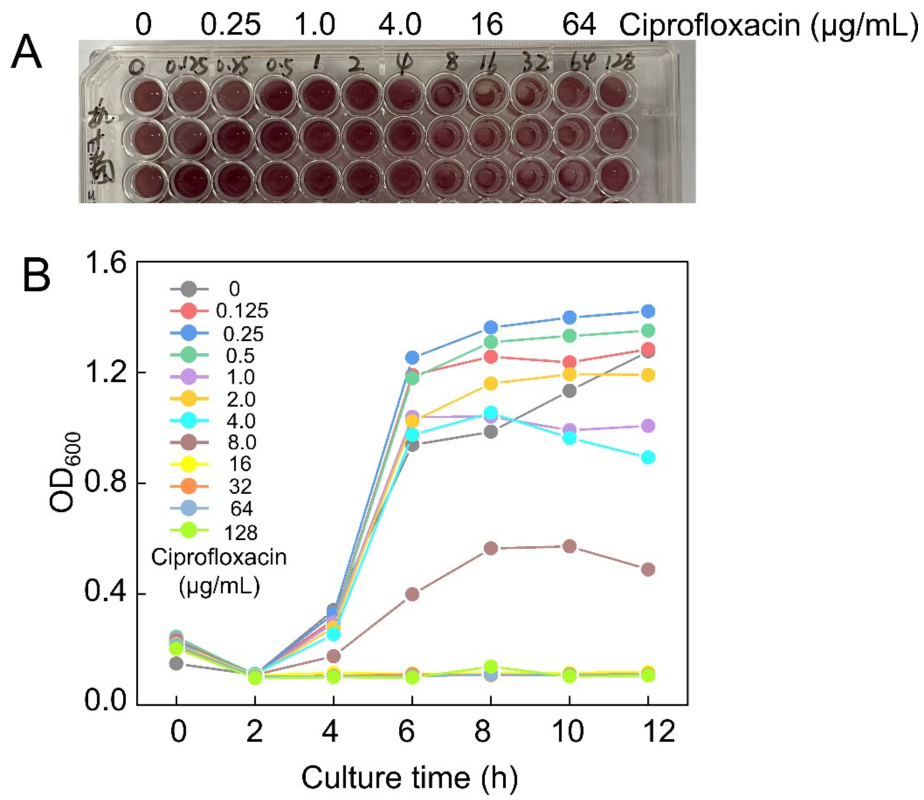

Figure S3 (A) The minimum inhibitory concentration (MIC) test of ciprofloxacin for *oqxB*-positive *S. enterica*. (B) The growth curves of *oqxB*-positive *S. enterica* under different concentrations of ciprofloxacin.

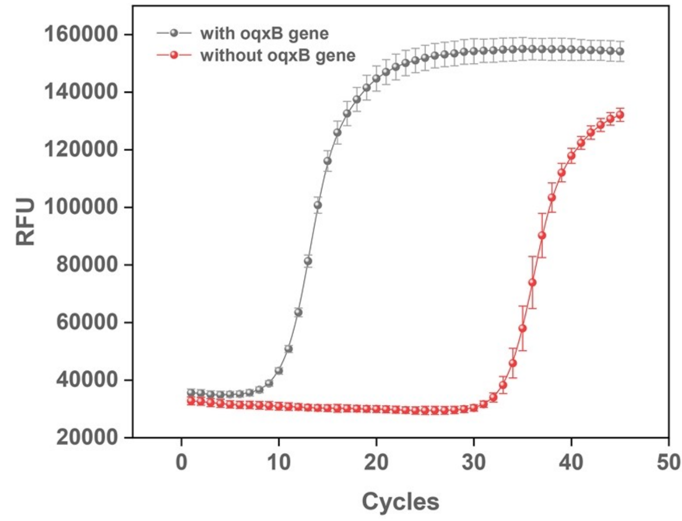

Figure S4 The qPCR curves of the quinolone-resistant and quinolone-sensitive *S. enterica* strain. Tests were carried in three replicates.

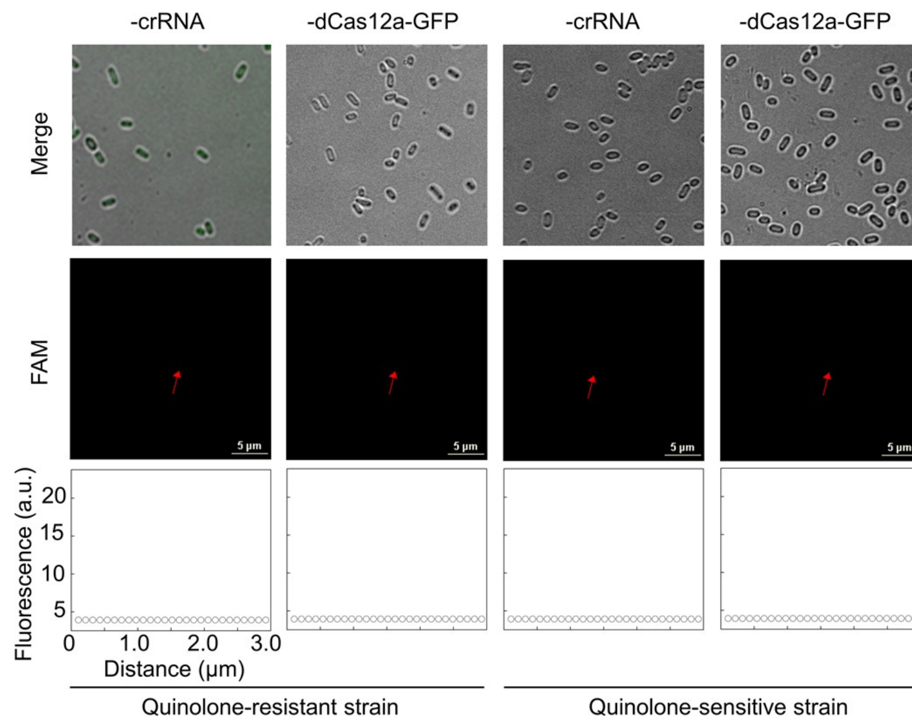

Figure S5 Cellular imaging and fluorescence intensity profile of control groups lacking crRNA or dCas12a-GFP.

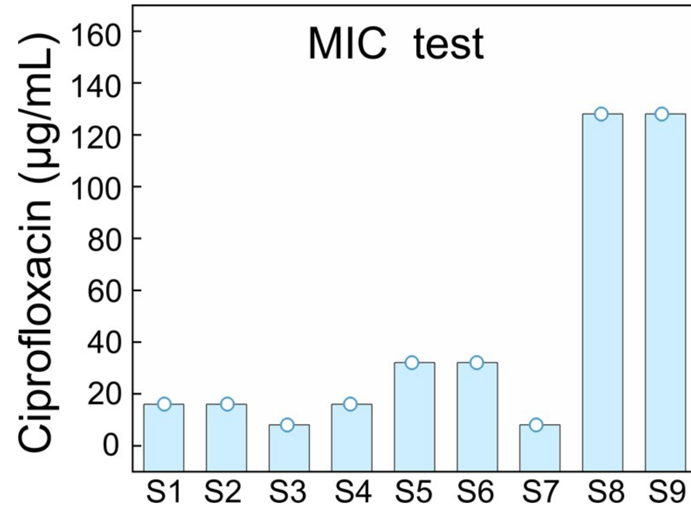

Figure S6 The minimum inhibitory concentration (MIC) test of ciprofloxacin for 9 quinolone-resistant *S. enterica* strains isolated from the poultry farm.