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Supplementary material

Title: Eliminated colistin-resistance dissemination by carbon nanotube - mediated CRISPR/Cas9 system- a combined effect of curing plasmid and conjugation blocking

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List of Supporting Information

Text S1 Measurement of bacteria respiration rate.

Fig. S1. The curing efficiency of sensitization treatment with CRISPR/Cas9 systems containing different sgRNA.

Fig. S2. SWCNTs internalization inside *E. coli* cell.

Fig. S3. Zeta potential of *E. coli* cells exposed to SWCNTs.

Fig. S4. Bacterial conjugation between *E. coli* exposed to PEI-SWCNTs at different concentrations.

Fig. S5. Effects of PEI-SWCNTs on the conjugative transfer frequency of *mcr-1* from *E. coli* in the conjugative transfer system at 2h treatment.

Fig. S6. EPS content of *E. coli* exposed to PEI-SWCNTs.

Fig. S7. Effects of PEI-SWCNTs on the conjugative transfer frequency of IncX4 plasmid carrying *mcr-1*.

Table S1. Bacterial strains and plasmids used in this study.

Table S2. Primers used in this study.

Text S1 Measurement of bacteria respiration rate.

The bacterial suspension (OD_{600}=0.4) with or without PEI-SWCNTs was transferred to respiration chambers. Oxygen levels were measured every 20 seconds for 30 minutes using a chart recorder. The slope of the oxygen concentration decrease over time was used to determine the rate of oxygen consumption. The rate of bacterial respiration was calculated as the reduction of oxygen concentration per unit time.

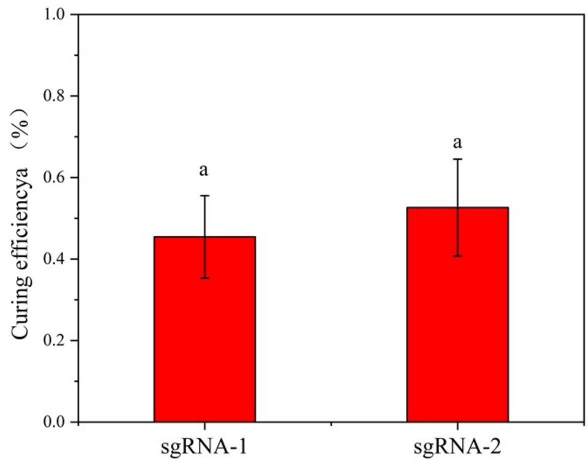

Fig. S1. The curing efficiency of sensitization treatment with CRISPR/Cas9 systems containing different sgRNA.

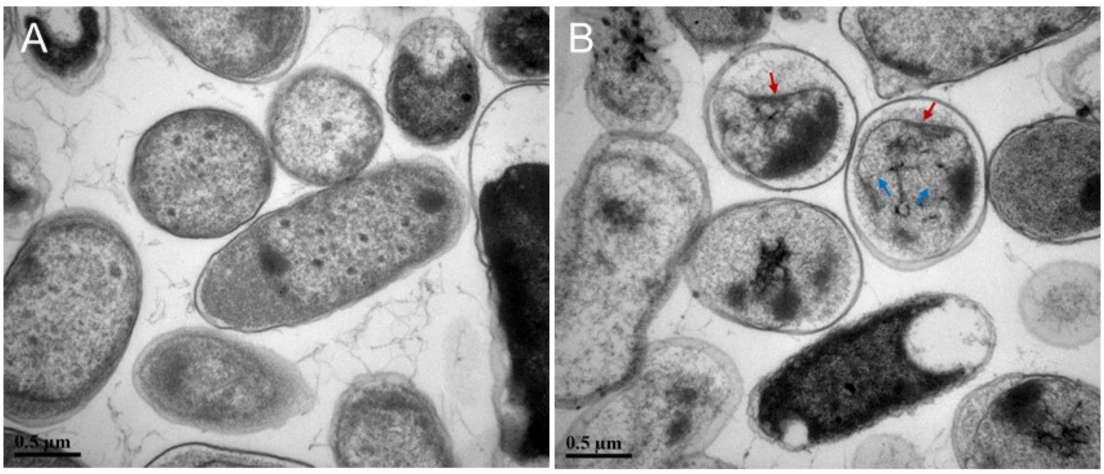

Fig. S2. SWCNTs internalization inside *E. coli* cell. Annotated TEM images of non-treated (A) and PEI-SWCNTs treated (B). Blue arrows indicate SWCNTs, the red arrow indicates the cell membrane.

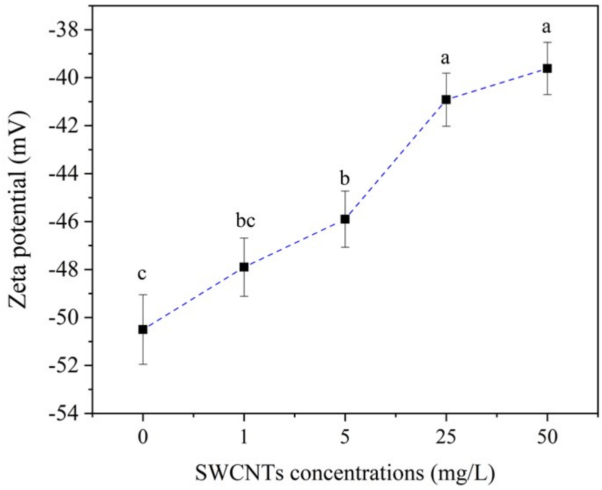

Fig. S3. Zeta potential of *E. coli* cells exposed to SWCNTs.

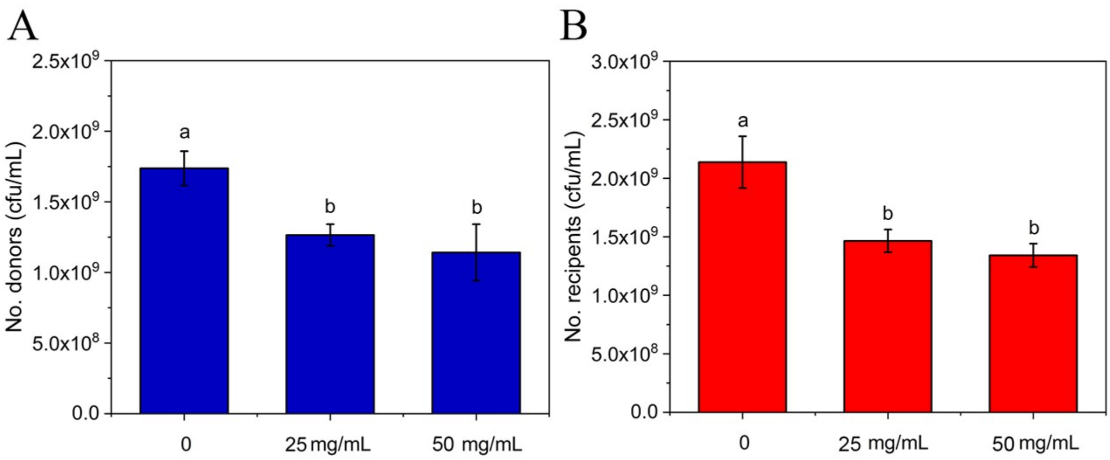

Fig. S4. Bacterial conjugation between *E. coli* exposed to PEI-SWCNTs at different concentrations. The number of donors (A) and recipients (B).

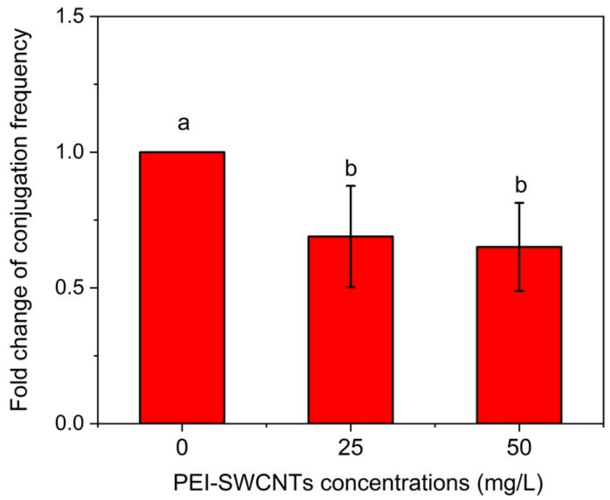

Fig. S5. Effects of PEI-SWCNTs on the conjugative transfer frequency of *mcr-1* from *E. coli* in the conjugative transfer system at 2h treatment.

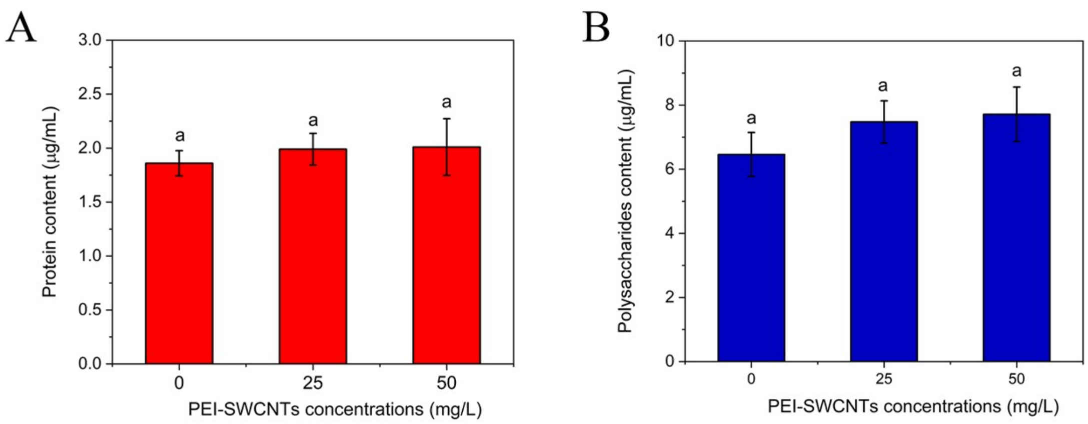

Fig. S6. EPS content of *E. coli* exposed to PEI-SWCNTs. Protein content (A), polysaccharides content (B).

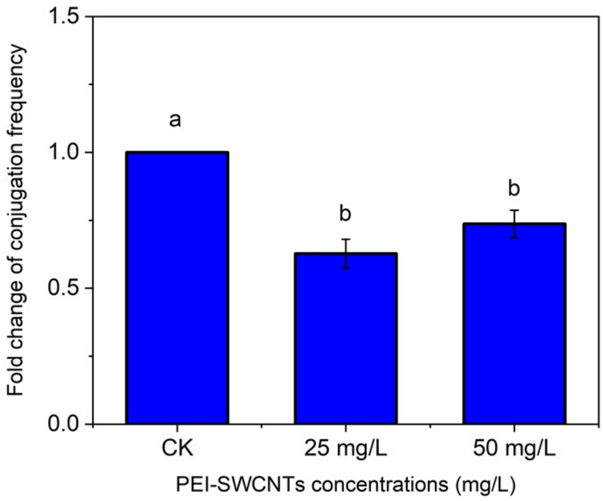

Fig. S7. Effects of PEI-SWCNTs on the conjugative transfer frequency of IncX4 plasmid carrying *mcr-1*.

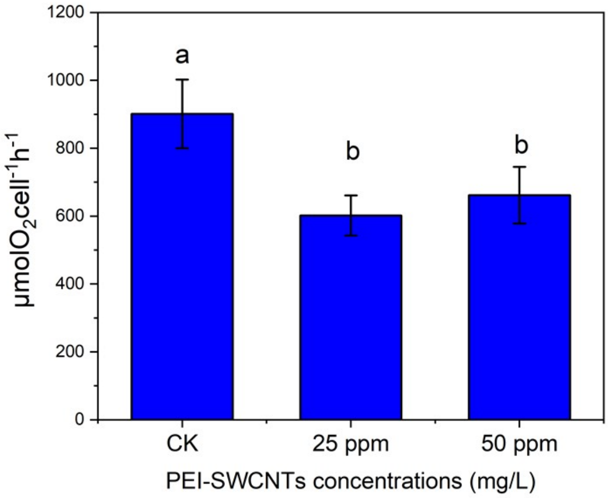

Fig. S8. Effects of PEI-SWCNTs on the respiratory rate in bacteria.

Table S1 Bacterial strains and plasmids used in this study.

Strain or plasmid	Antibiotic resistance	Source or reference
E. coli DH5α	None	Laboratory stock
E. coli SHP45	Colistin	[1]
pHNSHP45	Colistin	[1]
pwtCas9-bacteria	Ampicillin	addgene

Table S2 Primers used in this study.

Gene	Primer	Sequence of primer (5′-3′)
sgRNA-1	sgRNA-1-R	ACGCTCGGTCAGTCCGTTTG
	sgRNA-1-F	CAAACGGACTGACCGAGCGT
sgRNA-2	sgRNA-2-R	TTTGCTATTAATCATGGGCG
	sgRNA-2-F	CGCCCATGATTAATAGCAAA
BBa_J23119		TTGACAGCTAGCTCAGTCC
		TAGGTATAATGCTAGC
nuoA	*nuoA*-F	TGTTGCCTGATGCTGGTAGG
nuoA	*nuoA*-R	ACTGTTCGTTTCCGGGTTCA
nuoB	*nuoB*-F	GCGTTTTGGCGCAGAAGTAT
nuoB	*nuoB*-R	TTTATCGACGCCCTGCACAA
nuoC	*nuoC*-F	CACGTACCGACCTTCACCAA
nuoC	*nuoC*-R	CCTGTTTGGCTTTGGTCAGC
nuoE	*nuoE*-F	AGCACGAGATGCACCACTAC
nuoE	*nuoE*-R	ACAGACCACGCTGTCACAAT
nuoF	*nuoF*-F	AATGGAGCCTGATGCCGAAA
nuoF	*nuoF*-R	CGCAGATTAACTGCCGCTTC
nuoG	*nuoG*-F	TAGGTTTTGCCATCGCCCAT
nuoG	*nuoG*-R	GCGTTCGTCCCGGAGATAAT
nuoH	*nuoH*-F	TGTGGAACGTTATCCCGCAA
nuoH	*nuoH*-R	CCCACGAAGAACAGACCGAA
nuoI	*nuoI*-F	GCGATTCAGTTAACCCCGGA
nuoI	*nuoI*-R	ACGTCGATAGGCTTGGCTTC
nuoJ	*nuoJ*-F	CTTCTCACTGGGCGCTTACT
nuoJ	*nuoJ*-R	TAGCACTGATTGGCGTACCG
nuoK	*nuoK*-F	GGTCTGGTTATCCGTCGCAA
nuoK	*nuoK*-R	AAGGCCGATACTCGCTTCTG

nuoL	*nuoL*-F	CACTGCGGGCTTCTTCAGTA
nuoL	*nuoL*-R	TGAGTTACCCCTTTCACGGC
nuoM	*nuoM*-F	CGTCTGATCGCCTACACCTC
nuoM	*nuoM*-R	GTCGCGGGTATGGATACGTT
nuoN	*nuoN*-F	GCTGGTTTCGCTCTGGTTTG
nuoN	*nuoN*-R	ACAGAGACGCCAGATGGTTG
sdhC	*sdhC*-F	GCAAGCTTCCGCGATTATGG
sdhC	*sdhC-R*	ATACGAGGACTCCTGCGAGA
sdhD	*sdhD*-F	CTGGATCGGTTTCTTCGCCT
sdhD	*sdhD-R*	CCAGTGCAACGACAATCACC
sdhA	*sdhA*-F	TACTGGTCACCGAAGGTTGC
sdhA	*sdhA-R*	GTCGGAATACCGCCCATCAT
sdhB	*sdhB*-F	GCTCCGCGTATGCAGGATTA
sdhB	*sdhB-R*	GCTGGTTGAGTGCCGAAATC
frdA	*frdA-F*	CTTCCATATGCTGCACACGC
frdA	*frdA-R*	CCATACCCATACCGTCACCG
frdB	*frdB-F*	CCGCGGATCAGGGTACTAAC
frdB	*frdB-R*	GAGCAGTAGCCCACGAAAGT
frdC	*frdC-F*	TGATTTTCGGGCTGTTTGCC
frdC	*frdC-R*	TGATATTGGCCGCTTTCGGT
frdD	*frdD-F*	GGTATTCTGGGGCCTCTTCG
frdD	*frdD-R*	AGGAACAGGAATACGCGACC
cyoC	*cyoC-F*	TGCCGGATTTATCGGGATGG
cyoC	*cyoC-R*	GGGTACGGTTAGTGCTGGTC
cyoA	*cyoA-F*	GCATCTGATCGCCAACGAAC
cyoA	*cyoA-R*	CAGTTTTTCGAACGCAGCCA
cyoB	*cyoB-F*	GGCAACCTTCTCGCGTAAAC
cyoB	*cyoB-R*	AAAACCGATGGTCCACAGCA
cyoD	*cyoD-F*	TGGCAGTGGTACAGGTTCTG

cyoD	*cyoD-R*	AGCCTACAACCAGGATAGCG
atpA	*atpA*-F	ACTGTCCGGTGGTATCCGTA
atpA	*atpA-R*	CCAGGTAACCACGTTCTGCT
atpD	*atpD*-F	TGAACGTACTGGGTGAACCG
atpD	*atpD-R*	TTTACCTACACCCGCACCAC
atpG	*atpG*-F	TGGAAGACCGCGACGTTAAA
atpG	*atpG-R*	CCCACGGAGTTGAAGAACGA

References

1. Y. Y. Liu, Y. Wang, T. R. Walsh, L. X. Yi, R. Zhang and J. Spencer, Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study, *Lancet Infect. Dis.*, 2016, **16**, 161-168.