Supplementary materials

Phage-delivered sensitisation with subsequent antibiotic treatment reveals sustained effect against antimicrobial resistant bacteria

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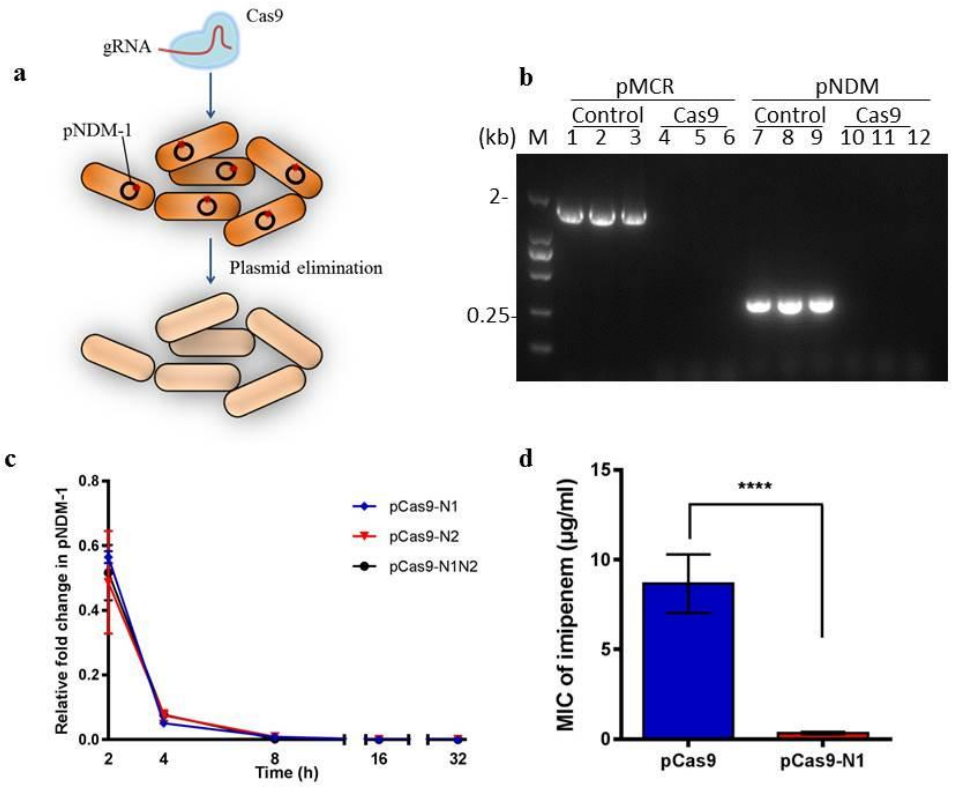
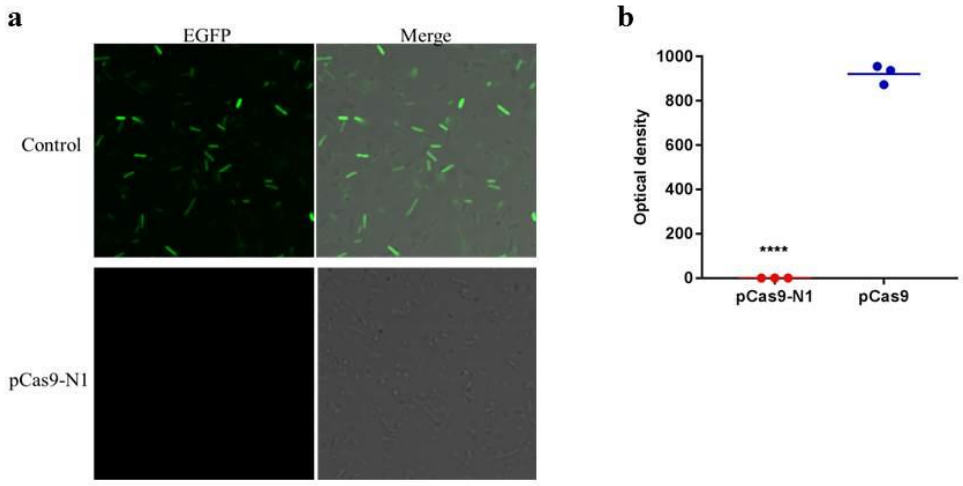
Figure S1. Eradication of *bla*$_{NDM-1}$ possessing plasmids using pCas9-N1



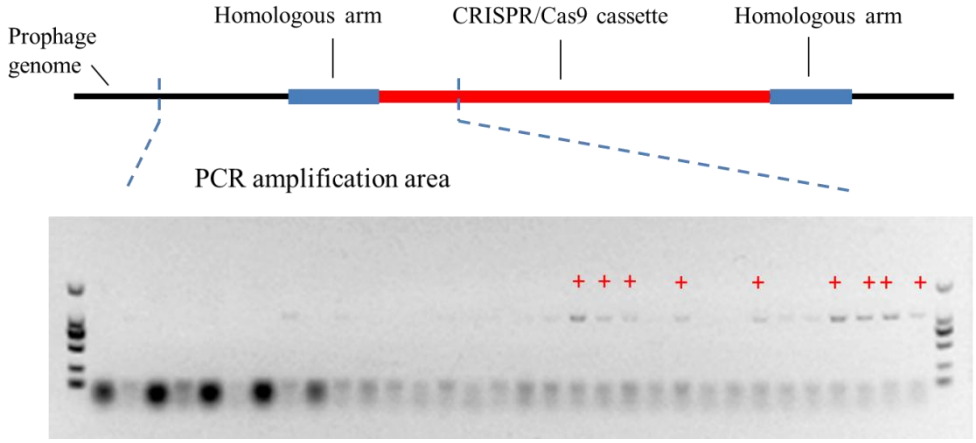
Sensitizing effect to antimicrobial resistant bacteria by CRISPR/Cas9 plasmid was determined *in vitro*. (a) The specific CRISPR/Cas9 plasmid was transformed into resistant *E.coli* J53 cells. (b) The specific CRISPR/Cas9 plasmids were delivered into J53 cells to target pNDM and pMCR. PCR detection of transformed colonies indicated that guided Cas9 enzyme could digest these resistant plasmids separately, controlled by pCas9 blank vector. (c) J53 pNDM-1 was sensitized using CRISPR/Cas9 plasmids targeting one site in *bla*$_{NDM-1}$ (pCas9-N1, pCas9-N2) or two sites (pCas9-N1N2). The relative fold change of pNDM-1 copies were determined at various time points by qPCR (n = 3, mean ± SD). Results revealed that targeted resistant plasmid was eliminated by over 99.9% after 8h. (d) Antimicrobial susceptibility test showed that MIC of imipenem of J53 pNDM-1 cells was obviously decreased after transformation of pCas9-N1 (unpaired t-test, n=3, $p<0.001$).

Figure S2. Eliminate high-copy NDM-1 plasmid using pCas9-N1



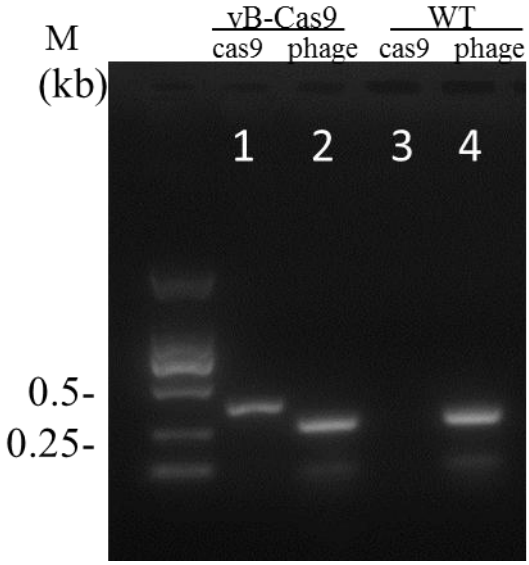
High-copy plasmids pEGFP-ndm co-expressed $bla_{\text{NDM-1}}$ and *EGFP*. (a) The *E. coli* DH5α pEGFP-ndm were transformed with pCas9-N1 and pCas9 control separately. EGFP signals were scanned by fluorescence confocal microscopy. EGFP signals were significantly quenched in pCas9-N1 group compared to those of the control. (b) The integrated optical fluorescent density decreased from 921.5 ± 24.99 to 0 (unpaired *t*-test, $P < 0.0001$, $n = 3$), representing a decrease in $bla_{\text{NDM-1}}$ of greater than 99.9%.

Figure S3. PCR screening of dsDNA transformant colonies



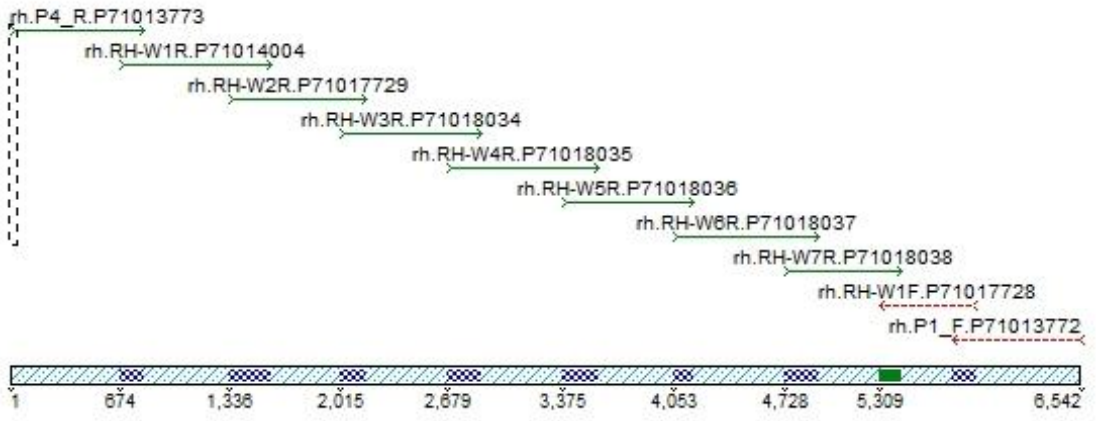
Colonies transformed with dsDNA were screened by PCR. The amplification flanked the upstream homologous arm. Using this screening strategy, colonies did not complete the recombination would show negative results in PCR screening, and transformants recombined with CRISPR/Cas9 cassette in prophage genome could be identified. Nine out of thirty-two colonies were identified as positive recombinant transformants by PCR detection. Positive rate was 28.1%.

Figure S4. PCR examination to engineered genome of modified phage



Genome-engineered phage was examined by PCR, compared with control. The vB_Cas9 phage group (lane1, 2) showed positive for both cas9 gene and phage genome, while wild-type control (lane3, 4) was negative for cas9 sequence.

Figure S5. Verification the integration of CRISPR/Cas9 in phage genome by PCR sequencing



PCR sequencing of a 6.542 kb fragment in modified phage genome containing full length of CRISPR/Cas9 cassette (5.081 kb), also flanking full sequence of dsDNA donor for phage recombination. This whole fragment was assembled by results of ten sequencing reactions, and its sequence was displayed as below.

Assembled sequencing results:

ATTATATTTAGCCCTACCAGTGACAATAGCGTAACGGAAAACCTCGCCGCAT
CTTCTGCGGGCTTTGTTGGCTCGCTCCATTGCACCGCGATCTTCAAATCTGC
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GATGATAGGTAAAATGTCGTCATCAAACATTTTTGCAAGTTCAGTTGCATAG
CCTACTGACCAGACTTGCTTCTTGTGCTCGTACCATTCCTTGTAAATCGCAC
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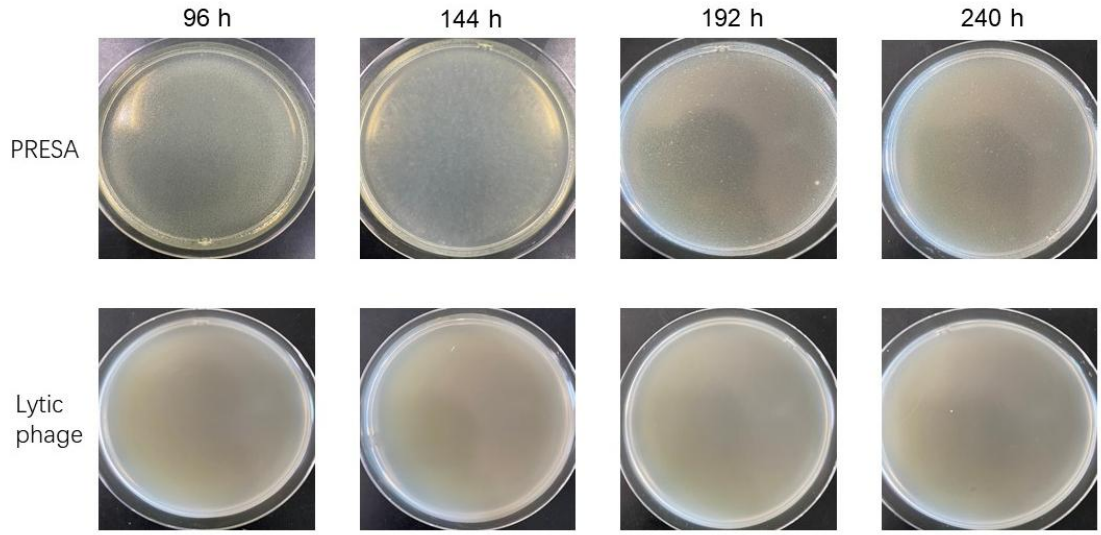
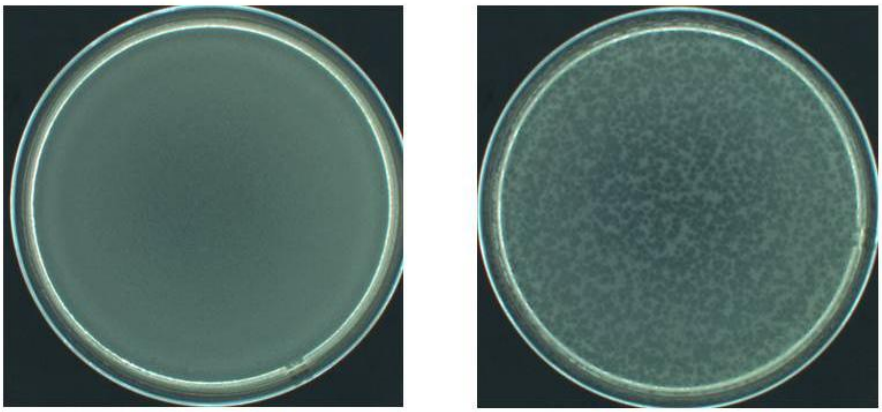
Figure S6. Phage plaque test of MG1655 cells separated from phage treatments during 240 h observation



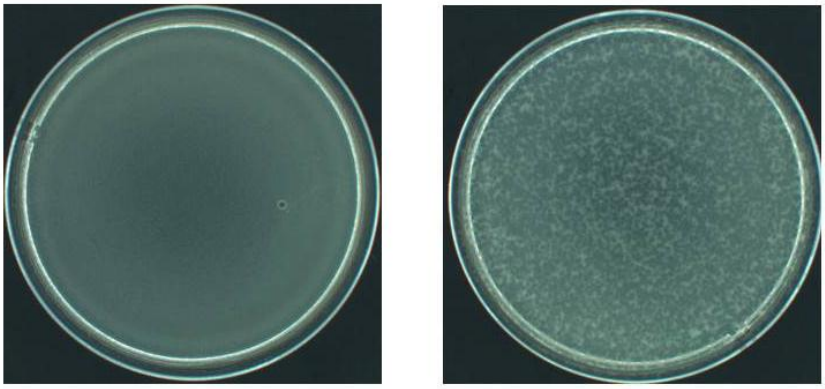
Host strain of phage plaque test were arranged using bacterial cells separating from the 240-hour in-vitro surveillance in different time points, to demonstrate the development of resistance of corresponding phages. Photos above represents the result of vB_253 plaque, which host strain were from the lytic phage treatment strategy. No forming plaque were found in this test. Photos below represents the result of SSC induced vB_Cas9 plaques, which host strain were from the vB_Cas9&Kan group. Even the rarely remaining cells in 192-hour treatment were also sensitive to phage infection.

Figure S7. Phage plaque test of MG1655 cells separated from phage treatments in mice skin.



Phage plaque test were arranged using bacterial cells separating from the mice skin model in day 6, to demonstrate the development of resistance of corresponding phages in vivo. Left photo represent the result of vB_253 plaque, with host strain from the lytic phage strategy treatment. No forming plaque were found in this test, indicating the developed resistance to lytic phage of separated bacteria. Right photo represent the result of SSC induced vB_Cas9 plaques, which host strain were from the vB_Cas9&Kan group. The remaining bacteria were also sensitive to phage infection.

Figure S8. Phage plaque test of MG1655 cells separated from phage treatments in mice intestine.



Phage plaque test were arranged using bacterial cells separating from the mice intestine in day 7, to demonstrate the development of resistance of corresponding phages in vivo. Left photo represent the result of vB_253 plaque, whose host strain were from the lytic phage strategy treatment. No forming plaque were observed, indicating the developed resistance to lytic phage of separated bacteria. Right photo represent the result of SSC induced vB_Cas9 plaques, which host strain were from the vB_Cas9&Kan group. The remaining bacteria showed no phage resistance.

Table S1. Primers in this research

Primers	Sequence (5'-3')	Use
ndm300-F	GGCGGAATGGCTCATCACGA	detection of *bla*NDM-1
ndm300-R	CGCAACACAGCCTGACTTTC	detection of *bla*NDM-1
NDM1001-F	CGGTCGACCACCTCATGTTTG AATTCGC	detection of *bla*NDM-1
NDM1001-R	CCCTCTAGACTCTGTCACATC GAAATCGC	detection of *bla*NDM-1
16s-F	AGAGTTTGATCCTGGCTCAG	determination of reference gene
16s-R	CTGCTGCCTCCCGTAGGAGT	determination of reference gene
spacer-F	AACACGCATTGATTTGAG	detection of CRISPR loci
spacer-R	ATAGGAAGGTATCCGACT	detection of CRISPR loci
N1Kana-F	GTCGACACCGCATTAGCCGC TGCATTGATGCTGAGCAGGG CGCAAGGGCTGCTAAAG	amplification of KanR gene with target and PAM sequence
N1Kana-R	GGATCCTCAGAAGAACTCGT CAAGAAGGC	amplification of KanR gene with target and PAM sequence
Kana-F	AGCGTCGACTCAGGGCGCAA GGGCTGCTAAAG	amplification of KanR gene
Kana-R	CGCGGATCCTCAGAAGAACT CGTCAAGAAGGC	amplification of KanR gene
kana200-F	GCCCTCTGGTAAGGTTGG	determination of KanR gene
kana200-R	GCCGATTGTCTGTTGTGCC	determination of KanR gene
phageup-F	GCCTTGAACTGAAATGCCCG	amplification of upstream homologous arm
phageup-R	ATGATTTCGTAGGGCCCTCAT CGCCATTGCTCCCCAA	amplification of upstream homologous arm
spCas9-F	ATGGCGATGAGGGCCCTACG AAATCATCCTGTGGA	amplification of CRISPR/Cas9 cassette
spCas9-R	AACCATTACGAACTGTGAAC ACACTACTCTTCTTTTGC	amplification of CRISPR/Cas9 cassette
phagedown-F	AGAGTAGTGTGTTCACAGTT CGTAATGGTT	amplification of downstream homologous arm
phagedown-R	TTTCCTCGCGGTCCATGC	amplification of downstream homologous arm
phicas-F	CTGCGTTACCGAGTGGAT	confirm the recombination

		of CRISPR/Cas9 within the temperate phage
phicas-R	AAGGCTAGTCCGTTATCAA	confirm the recombination of CRISPR/Cas9 within the temperate phage
Cas350-F	GCCGTCGTTGGAACTGCTTT	GCCGTCGTTGGAACTGCTTT
Cas350-R	TCCTTGGAGAATCCGCCTGT	TCCTTGGAGAATCCGCCTGT
Phage200-F	ggaccagcatacgatctgccgt	detection of the temperate phage
Phage200-R	caggcaaagtccgtggctgat	detection of the temperate phage

Table S2. Comparison of techniques for construction of phage-delivered CRISPR/Cas9 system

Study	This study	Previous study(*13*)	Previous study(*12*)	Previous study(*15*)
Phage name	vB_EcoM_IME365	M13	ΦNM1	λ
Method for integration of CRISPR/Cas9	Homologous recombination	Phagemid construction	Phagemid construction	Homologous recombination
Method for Recombinant screening	Suicide gene based counter-selection, avoid resistant marker	Resistant marker based selection	Resistant marker based selection	Resistant marker based selection
Resistance residue	none	Remained in phage particle	Remained in phage particle	Deleted by another Flippase recombination

Text S1. Whole genome sequence of vB_365

>Seq1 [organism=Escherichia virus Lambda-like phage vB_EcoM_IME365], chromosomeTAAGCATCAGTGAAACCTCGCCGCTTGCTCTGCTGGTTCAATG
GAAATAACCAGGTCTTTGTCGCCTAACTGCCAGATAATTCCCTGTTCTTCTT
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