Title: Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria

Short Title: Novel antimicrobials for AMR bacteria

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Abstract: Emergence of antimicrobial-resistant bacteria is an increasingly serious threat to global health, necessitating the development of innovative antimicrobials. We established a series of CRISPR-Cas13a-based antimicrobials, termed PhagoCas13a(s), capable of sequence-specific killing of carbapenem-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* through promiscuous RNA cleavage after recognizing corresponding antimicrobial resistance genes. PhagoCas13a constructs were generated by packaging CRISPR-Cas13a into a bacteriophage to target antimicrobial resistance genes. Contrary to Cas9-based antimicrobials that lack bacterial killing capacity when the target genes are located on a plasmid, the PhagoCas13a(s) exhibited strong bacterial killing activities upon recognizing target genes regardless of their location. The antimicrobials' treatment efficacy was confirmed using a *Galleria mellonella* larvae model. Further, we demonstrated that the PhagoCas13a(s) can assist in bacterial gene detection without employing nucleic acid amplification and optical devices.

One Sentence Summary: Novel gene-specific antimicrobials capable of killing drug-resistant bacteria and applicable to detect bacterial genes were developed.

Main Text: The emergence and spread of antimicrobial resistance among pathogenic bacteria has been a growing global public health concern for several decades (*1*). A recent nationwide survey of infectious disease specialists, conducted by the Emerging Infections Network of The Infectious Diseases Society of America, found that more than 60% of the participants attended to a patient with pan-resistant, untreatable bacterial infection within the previous year (*2*). It is also predicted that antimicrobial-resistant (AMR) pathogens will cause 10 million fatalities per year by the year 2050 if new antimicrobial strategies are not developed (*3*). In fact, the emergence of AMR bacteria has led to the present post-antibiotic era, in which many currently available antimicrobials are no

longer effective. This is due in part to the decline in antibiotic innovation, as no new class of antibiotics has been developed against Gram-negative bacteria in more than 45 years, and only a limited number of antibiotics was in either phase II or III clinical trial as of 2016 [https://www.edisongroup.com/edison-explains/antibiotics/]. Therefore, there is an urgent need for new strategies to develop alternative therapeutic approaches to prevent infections of AMR bacteria.

To this end, various nucleic acid-based antibacterials, novel peptides, bacteriophage therapies, antibodies, bacteriocins, and anti-virulence compounds have been recently developed (*4*). Among these, CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9-encoding phages provide a means to combat such threats by selectively killing AMR bacteria. The CRISPR-Cas9 genome-editing construct, which is designed to target AMR genes, was packaged into phages to generate gene-specific antimicrobials. The resulting phage-mediated CRISPR-Cas9-based antimicrobials can be employed to selectively kill bacteria carrying targeted AMR genes (*5*). However, even though the adaptability of CRISPR-Cas9 allows for the building of large libraries of CRISPR-Cas9-guided RNAs to act against rapidly evolving AMR bacteria, this strategy is ineffective against AMR bacteria carrying plasmid-encoded AMR genes, unless a lethal system, such as a toxin-antitoxin system, is also encoded by the plasmid because sole DNA cleavage does not simply result in bacterial death (*6*).

CRISPR-Cas13a is the most recently identified CRISPR-Cas type VI class 2 system and is characterized by RNA-guided single-stranded RNA (ssRNA) cleavage activity (*7*, *8*). A 2016 study by Abudayyeh *et al*. demonstrated that CRISPR-Cas13a has promiscuous ssRNA cleavage activities and slows down the host bacteria growth due to degradation of host RNAs (*8*). Our group recently reported the whole genome sequencing of 11 strains of six *Leptotrichia* species and analysis of the associated CRISPR-Cas13a systems showed that *Leptotrichia shahii* Cas13a (LshCas13a) had relatively high growth inhibition effect on host cells (*9*), consistent with the observation in a previous study (*8*). In order to understand whether this growth inhibition was due to cell death by Cas13a-mediated cytotoxicity, we constructed two phasmid systems (pKLC21 and pKLC26, see later) carrying CRIPSR-Cas13a and target gene, respectively, and co-introduced them into *E. coli* to test their effect on the host cells. We found that LshCas13a clearly showed target sequence-specific cell killing activity if the spacer was appropriately designed (Fig. S1). It opened a possibility that the CRISPR-Cas13a system can be applied for the development of new types of antimicrobials to cleave the mRNA of target genes regardless if the gene is encoded by the chromosome or plasmid, which might overcome the limitations of CRISPR-Cas9-based antimicrobials (*6, 10–11*). This possibility prompted us to explore the development of CRISPR-Cas13a-based antimicrobials, designated PhagoCas13a(s), which are expected to selectively kill bacteria that encode target genes on either the bacterial chromosome or plasmid. In order to determine if LshCas13a cleavage of ssRNA is sufficient to kill target bacteria, Lsh*Cas13a* and a minimal CRISPR array containing one spacer were inserted into the plasmid pC003 to generate the plasmid pKLC21, which thus was programed to target the carbapenem resistance gene $bla_{\text{IMP-1}}$. The resulting pKLC21_$bla_{\text{IMP-1}}$ construct was transformed into *E. coli* NEB5α F´I^{q} cells with or without $bla_{\text{IMP-1}}$ expression using pKLC26. Growth of the $bla_{\text{IMP-1}}$-expressing transformant was significantly inhibited, as compared to those without $bla_{\text{IMP-1}}$ expression (Fig. 1A). Similar growth arrest was also observed in other *E. coli* cells transformed with a series of pKLC21 plasmids programed to target various carbapenem and colistin resistance genes, as well as other genes of interest (Fig. S1). We interpreted these results as demonstrating CRISPR-Cas13a-mediated

sequence-specific killing of antimicrobial-resistant *E. coli*. Following verification of the bactericidal activity of the CRISPR-Cas13a_bla_{IMP-1} construct against bla_{IMP-1}-positive bacteria, AMR bacteria-specific PhagoCas13a constructs were developed by loading the CRISPR-Cas13a system onto a phage. As an initial attempt, CRISPR-Cas13a_bla_{IMP-1} was loaded onto *E. coli* phage M13, which can infect F pili-positive *E. coli*, to generate EC-PhagoCas13a_bla_{IMP-1} (Fig. 1B), which demonstrated sequence-specific killing activity against bacteria carrying the bla_{IMP-1} gene in an EC-PhagoCas13a_bla_{IMP-1} concentration-dependent manner (Fig. 1C). Subsequently, the same sequence-specific killing activities were confirmed with a series of PhagoCas13a constructs programmed to target different AMR genes, including those conferring resistance to carbapenem (bla_{IMP-1}, bla_{OXA-48}, bla_{VIM-2}, bla_{NDM-1}, bla_{KPC-2}) and colistin (*mcr*-1, *mcr*-2), all of which are the representatives of currently problematic genetic factors in the clinical setting (Fig. 1D). Conversely, non-targeting PhagoCas13a killed no bacteria under any circumstance, indicating that this construct has ideal potential for gene-directed antimicrobial therapy against AMR bacterial infections.

A Cas9-based EC-PhagoCas9_bla_{NDM-1} construct was also generated with the same protocol, but in this instance, CRISPR-Cas13a_bla_{NDM-1} was replaced with CRISPR-Cas9_bla_{NDM-1} and its bacteria-killing manner was compared with that of EC-PhagoCas13a_bla_{NDM-1}. As expected, EC-PhagoCas9_bla_{NDM-1} killed only the bacteria with a chromosomal target gene, while EC-PhagoCas13a_bla_{NDM-1} killed the bacteria that carried the target gene on either the chromosome or plasmid (Fig. 1E). Since the majority of clinically important AMR genes are encoded on plasmids (*12*), the killing efficiency of PhagoCas13a was expected to be superior to that of PhagoCas9 in light of the current difficulty with the threat of Carbapenem-resistant Enterobacteriaceae (CRE) carrying resistant genes on the plasmids. Besides, previous studies have suggested that CRISPR-Cas9 is prone to unexpected genetic mutations due to its DNA-cleavage activity (*13–15*). On the contrary, instead of DNA, PhagoCas13a targets RNA; therefore, unintended evolution of the bacteria is unlikely.

In order to determine whether the PhagoCas13a(s) can selectively kill target bacteria among a mixed population of AMR bacteria, an artificial mixture of *E. coli* NEB5α F'I^q expressing the carbapenem resistance gene bla_{IMP-1}, the colistin resistance gene *mcr-2*, or with no resistance gene, as a control, was treated with each gene-specific EC-PhagoCas13a construct (i.e., EC-PhagoCas13a_bla_{IMP-1} and EC-PhagoCas13a_*mcr-2*, respectively). Subsequently, the abundance of each bacterial strain was determined. As shown in Fig. 2, the percentage of cell numbers of NEB5α F'I^q (bla_{IMP-1}) and NEB5α F'I^q (*mcr-2*) were significantly decreased from 30.5% and 35% to 3.5% and 1.9%, respectively, as a result of eradicating the target cells, when the cell mixtures were treated with EC-PhagoCas13a_bla_{IMP-1} and EC-PhagoCas13a_*mcr-2*, respectively, while there was no change in the abundance of cell populations after treated with non-targeting EC-PhagoCas13a_null control. These highly selective killing activities of PhagoCas13a(s) demonstrate their potential as microbial control agents that can modify or manipulate the bacterial flora without affecting undesired bacterial populations by eliminating those with specific genomic contents. In addition to demonstration of the versatility of PhagoCas13a, we investigated its *in vivo* therapeutic efficacy using a *G. mellonella* larvae infection model. Administration of EC-PhagoCas13a_bla_{IMP-1} to *G. mellonella* larvae infected with *E. coli* R10-61 carrying bla_{IMP-1} showed significantly improved survival over no treatment (p = 0.0016) or a non-targeting PhagoCas13a_null, as a control (p = 0.044) (Fig. 3). This outcome further strengthened the therapeutic prospects of the PhagoCas13a constructs for treatment of infections with AMR bacteria.

Owing to the fact that the prevalence of stealth bacteria (carrying AMR genes but not identifiable by existing susceptibility tests) has been increasing (*16*), the establishment of a simple and easy-to-use detection method for such strains at the genetic level is an important task in the clinical setting. Our results demonstrated that the PhagoCas13a(s) could be applied for the detection and identification of AMR genes. To this end, the PhagoCas13a constructs were modified for the detection of bacterial AMR genes. First, the spacer sequence of EC-PhagoCas13a_$bla_{\text{IMP-1}}$ targeting the $bla_{\text{IMP-1}}$ gene was optimized in order to improve the killing efficiency, since the bactericidal activity of CRISPR-Cas13a differed greatly depending on the spacer sequences (Fig. S1). The optimization started with the construction of a CRISPR-Cas13a expression plasmid library, in which 121 different spacer sequences targeting $bla_{\text{IMP-1}}$ were inserted into the CRISPR array. Then, all plasmids were transformed into $bla_{\text{IMP-1}}$-expressing *E. coli* cells and the resulting transformants were analyzed to identify the most effective spacer sequence (Fig. S2A). The calculation of the number of spacer reads after deep sequencing of the total plasmid DNAs extracted from the transformants found that all of the tested spacer sequences mediated target-specific bacterial killing, at least to some extent, when judged by the depletion efficiency of the plasmid DNAs (Fig. S2B and Supplementary Table 4). Among these, 13 spacer sequences had depletion efficiencies of >100 and the $bla_{\text{IMP-1}}$–563 spacer sequence GACTTTGGCCAAGCTTCTATATTTGCGT, which had the highest depletion efficiency of 364.1 was chosen as the best spacer sequence for use in subsequent experiments (Fig. S2C). Then, the carrier phage M13 was replaced with the lysogenic phage Φ80 with the use of the PICI (phage-inducible chromosomal island) packaging system (*17, 18*) (Fig. 4A and Fig. S3). The M13 phage described in the previous experiment was less than ideal for clinical applications since it can only infect bacteria carrying F pili. Finally, the kanamycin resistance gene KanR was inserted as a selection marker to generate the PICI-based EC-PhagoCas13a::KanR_$bla_{\text{IMP-1}}$ construct, which was expected to have improved detection sensitivity. A simulation to test the detection efficiency for $bla_{\text{IMP-1}}$ was conducted by spotting 25 μL of 10-fold serial dilutions of EC-PhagoCas13a::KanR_$bla_{\text{IMP-1}}$ onto fresh top agar lawns of the test strains in LB agar with or without supplementation of kanamycin. To judge the results, we opted to determine the bacterial killing effect against kanamycin-resistant cells on kanamycin plates (Fig. 4B), rather than assessing the bacterial killing effect against original cells on kanamycin-free plates (Fig. 4C). When the EC-PhagoCas13a::KanR_$bla_{\text{IMP-1}}$ carrying the kanamycin-resistance gene was applied on the soft agar bacterial lawn grown on bottom agar containing kanamycin, the cells infected by this phage acquired kanamycin resistance and, hence, could grow in the presence of kanamycin. Nevertheless, with the bacterial cells carrying the target AMR gene $bla_{\text{IMP-1}}$, there was no observable growth due to the bactericidal effect of the CRISPR-Cas13a construct (Fig. 4B). This method was shown to be about 1,000 times more sensitive than direct observation of bacterial growth inhibition, as with the previous method (Figs. 1C, 1D, and 4C). The efficiency of this system was also confirmed with the use of the M13 phage-based EC-PhagoCas13a::KanR_$bla_{\text{IMP-1}}$ construct (Figs. S4A, B, and C). A subsequent experiment showed that the target AMR genes of interest located on either the plasmid or chromosome could be precisely detected, demonstrating that the AMR bacteria could be identified by this method (Figs. 4D, 4E, S4D, and S4E). This finding was further confirmed by two other EC-PhagoCas13a::KanR constructs targeting $bla_{\text{OXA-48}}$ and $bla_{\text{VIM-2}}$ (Figs. 4D, 4E, S4D, and S4E). Although the sensitivity was slightly lower, it was even possible to detect target genes by directly spotting the PhagoCas13a(s) onto the bacteria swabbed on an agar plate instead of using the soft agar overlay method (Fig. S5). The PhagoCas13a(s) could also simultaneously detect the toxin-encoding genes *stx*1 and *stx*2 (Fig. 4F), indicating that this method is applicable for the detection of any bacterial gene.

Next, the applicability of the PhagoCas13a constructs for identification of carbapenem-resistant clinical isolates of *E. coli* carrying bla_{IMP-1} or bla_{NDM-1} was tested. As these strains were not susceptible to kanamycin, the KanR of EC-PhagoCas13a::KanR was replaced with the hygromycin resistance gene, HygroR, to generate the constructs EC-PhagoCas13a::HygroR_bla_{IMP-1} and EC-PhagoCas13a::HygroR_bla_{NDM-1}. The test results with these two PhagoCas13a(s) showed that the *E. coli* isolates carrying bla_{IMP-1} and bla_{NDM-1} were clearly detected, which was consistent with the results of polymerase chain reaction (PCR) analysis (Fig. 4G). So far, there have been amazing developments of novel methods to detect bacterial genes, such as *in situ* hybridization, allele-specific PCR, loop-mediated isothermal amplification, endonuclease cleavage, direct DNA sequencing, primer extension, and oligonucleotide ligation. All these methods have unique merits in terms of sensitivity, accuracy, and robustness, allowing the wide use of each, but all have serious drawbacks that have often reduced general use. An intrinsic limitation to these molecular methods is that all are based on nucleic acid (DNA or RNA) amplification technology, which requires the use of expensive reagents as well as substantial operating costs. Reagents, disposable materials (e.g., PCR reaction tubes and pipette tips), thermal cyclers, and the necessary gel electrophoresis equipment are fairly expensive. In contrast, the proposed detection system using the PhagoCas13a constructs can be performed without amplification of DNA or RNA, electrophoresis equipment, or optical devices, as only bacterial culture plates are required. In addition, more than 10^{10} transducing forming units (TFU) of PhagoCas13a constructs can be harvested per liter of host bacterial culture, although only 2-3 μL of 10^5 constructs per mL of solution is required for a single spot test to accurately determine the presence or absence of target genes. Together, these features highlight the elegant potentials of PhagoCas13a(s) for bacterial gene detection.

Lastly, in addition to demonstrating the bactericidal activity of CRISPR-Cas13a against Gram-negative bacteria, we also attempted to confirm the collateral activity of CRISPR-Cas13a against Gram-positive bacteria using *Staphylococcus aureus*. At first, a set of *E. coli-S. aureus* shuttle vectors, namely pKCL4(s), were generated carrying the CRISPR-Cas13a construct with or without a spacer sequence targeting the *S. aureus rpsE* genes. Transformation of the vector into *S. aureus* strain RN4220 showed that the bactericidal activity of CRISPR-Cas13a with an appropriate spacer sequence was similar to that in *E. coli* (Fig. S6A). Then, a SA-PhagoCas13a_*mecA* construct was produced to target methicillin-resistant gene *mecA* of methicillin-resistant *S. aureus* (MRSA), one of the most prevalent AMR pathogens worldwide (*19*). Optimization of the spacer sequences (Figs. S6B, C, D and E) and the CRISPR-Cas13a_*mecA* carrying vector (pKCL-SP_*mecA*) construction were carried out in the same way as above for CRISPR-Cas13a_bla_{IMP-1}, and packing of CRISPR-Cas13a_*mecA* into *S. aureus* phage Φ80α was performed in accordance with the method established by Carles Ubeda *et al*. using the *S. aureus* pathogenicity island (SaPI) system (*20, 21*) (Fig. S7). This packing system simultaneously imparted tetracycline resistance to the resulting SA-PhagoCas13a::TetR_*mecA* construct, since the SaPI carried the tetracycline resistance gene, which made it possible to be tested for both bacterial killing ability against MRSA and capability of MRSA detection by targeting *mecA*. As expected, when the methicillin-susceptible *S. aureus* (MSSA) strain RN4220 and MRSA USA300 or *mecA* knockout USA300 (USA300-Δ*mecA*) was infected with SA-PhagoCas13a_*mecA*, the growth of only the USA300 strain carrying *mecA* was significantly inhibited (Fig. 4H and Fig. S8), clearly demonstrating the sequence-specific bacterial killing ability of CRISPR-Cas13a against the Gram-positive bacteria *S. aureus*.

In this study, the promiscuous RNA cleavage ability of CRISPR-Cas13a via recognition of target RNA by CRISPR-RNA (crRNA), which resulted in host cell death, was exploited to generate a

new type of sequence-specific bacterial antimicrobials. Although there are still many questions to be answered concerning the host range of the carrier phages, catalytic mode of Cas13a (*22*), the efficiency of phage packaging, and ethical issues regarding genetic recombinants, etc., our strategy demonstrated that CRISPR-Cas13a-based antimicrobials are likely to be developed for at least three application categories: (1) promising antibacterial therapeutic agents targeting any bacterial gene, including AMR genes, or selective killing of targeted toxin-producing bacteria; (2) a simple and inexpensive bacterial gene detection system for bacterial identification and efficient molecular epidemiological investigations without the need for the amplification of nucleic acids or optic devices; and (3) tools to manipulate the bacterial flora by targeting and eliminating a specific bacterial population without disruption to other irrelevant bacterial populations. In conclusion, the proposed CRISPR-Cas13a-based antimicrobials are expected to have a great impact in the field of antimicrobial resistance, infection control as well as bacterial flora control.

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Acknowledgments: We thank Dr. Feng Zhang of the Massachusetts Institute of Technology, Dr. Luciano A Marraffini of The Rockefeller University, Dr. David Bikard of The Institut Pasteur and Dr. Timothy K. Lu of the Massachusetts Institute of Technology for kindly gifting the plasmids. We thank Hibiki Research Group for Clinical Microbiology for kindly providing Carbapenem-resistance *E. coli* strains R10-61 and R10-79. We thank Dr. Keiichi Hiramatsu for kindly providing USA300 strain.; **Funding:** This work was supported by JSPS KAKENHI (Grant No. 18K15149 to KK, 15H05654 and 19K08960 to SW, 17K15691 to YS, 19K15740 to MK and 17K19570 to LC), Mochida Memorial Foundation for Medical and Pharmaceutical Research (KK), the Takeda Science Foundation (SW, LC), and the Japan Agency for Medical Research and Development J-PRIDE (grant no. JP17fm0208028, JP18fm0208028, and JP19fm0208028 to LC). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript; **Author contributions:** KK, LC designed the study, analyzed the data, and wrote the manuscript. XT, VC contributed to acquisition, analysis and interpretation of data, and assisted the preparation of the manuscript. JP contributed to design of study, interpretation of data and assisted the preparation of the manuscript. All other authors contributed to data collection and interpretation, and critically revised the manuscript. All authors approved the final version of the manuscript and agreed for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **Competing interests:** The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Materials:

Materials and Methods

Figures S1-S8

Tables S1-S4

Legends

Fig. 1. *In vitro* sequence-specific bactericidal activity of CRISPR-Cas13a from *Leptotrichia shahii* (LshCas13a). (A) LshCas13a targeting the carbapenem resistance gene bla_{IMP-1} demonstrated strong killing activity against *E. coli* harboring bla_{IMP-1}. *E. coli* DH5α cells were co-transformed with a CRISPR-Cas13a expression vector targeting bla_{IMP-1} and tetracycline-inducible bla_{IMP-1} expression vector with anhydrotetracycline-induced bla_{IMP-1} expression. Activation of the CRISPR-Cas13a system upon recognition of bla_{IMP-1} hindered *E. coli* growth, as shown by cell growth at an optical density at 650 nm as well as measurement of cell culture turbidity. The absence of bla_{IMP-1}-spacer and/or the target gene bla_{IMP-1} did not kill the host cell, as expected, indicating that both elements are

crucial for the functioning of the CRISPR-Cas13a system. (B) Graphical concept model depicting the mode of action of PhagoCas13a. CRISPR-Cas13a_bla_{IMP-1} packaged onto *E. coli* M13 phage (EC-PhagoCas13a_bla_{IMP-1}) was delivered into the host bacterial cells. In the presence of the target gene (bla_{IMP-1}-expressing *E. coli*), CRISPR-Cas13a was activated and the subsequent non-sequence-specific RNase activity resulted in host cell death. On the other hand, *E. coli* cells without the bla_{IMP-1} gene were unaffected. (C) EC-PhagoCas13a-bla_{IMP-1} triggered cell death or growth inhibition only when the target cells expressed bla_{IMP-1} (L-arabinose induction). (D) PhagoCas13a(s) programmed to target specific carbapenem resistance genes (bla_{IMP-1}, bla_{OXA-48}, bla_{VIM-2}, bla_{NDM-1}, and bla_{KPC-2}) and colistin resistance genes (*mcr-1* and *mcr-2*) selectively killed only the bacteria harboring the corresponding resistance gene(s), as demonstrated by plaque formation. (E) Unlike the CRISPR-Cas9 system, the CRISPR-Cas13a system selectively killed bacterial cells carrying target genes on the chromosome as well as the plasmid. Conversely, the activity of the CRISPR-Cas9 system was restricted only to the target gene located on the chromosome.

Fig. 2. PhagoCas13a(s) selectively eradicated the target cells from the mixed cell population. A mixed cell population was prepared by mixing *E. coli* NEB5α F'I^q with equal numbers of NEB5α F'I^q carrying bla_{IMP-1} and NEB5α F'I^q carrying *mcr-2*. The cells mixtures were divided into three tubes and each was treated with individual PhagoCas13a(s) targeting bla_{IMP-1}, *mcr-2*, or non-targeting PhagoCas13a. Note that the PhagoCas13a(s) dramatically reduced the numbers of bacterial cells carrying the target genes, while the growth of non-targeted bacteria remained unchanged. Similarly, PhagoCas13a(s) with non-targeting spacer had no effect on the proportions of cells in the mixture.

Fig. 3. Time course of *G. mellonella* cell death. The therapeutic efficacies of the PhagoCas13a(s) were evaluated using a *G. mellonella* infection model. As shown by the Kaplan–Meier survival curve, administration of PhagoCas13a_bla_{IMP-1} targeting bla_{IMP-1} to the *G. mellonella* larvae infected with carbapenem-resistant clinical isolates of *E. coli* carrying bla_{IMP-1} significantly improved survival of the *G. mellonella* larvae, when compared to those treated with PhagoCas13a_null (control) ($p < 0.05$) or phosphate-buffered saline ($p < 0.01$) (n = 20 per group). The results are presented as the average values of three independent experiments.

Fig. 4. PhagoCas13a as a tool for bacterial gene detection. (A) A schematic illustration of the generation of PICI-based EC-PhagoCas13a::KarnR_bla_{IMP-1} for detecting bacteria carrying bla_{IMP-1}. (B) In the assay for detecting bla_{IMP-1} gene of carbapenem-resistant *E. coli* isolates, the bacteria were mixed with soft agar and poured onto LB bottom agar plates containing kanamycin, followed by spotting of 10-fold serially diluted EC-PhagoCas13a::KanR_bla_{IMP-1} with the kanamycin resistance gene KanR onto the surface of the plates. The sensitivity of the test was enhanced by about 1,000 times by inserting the KanR gene into the EC-PhagoCas13a_bla_{IMP-1} to generate EC-PhagoCas13a::KanR_bla_{IMP-1} compared with the results of directly using EC-PhagoCas13a_bla_{IMP-1} (C). The applicability of EC-PhagoCas13a::KanR(s) for detecting other carbapenem resistance genes (bla_{OXA-48}, bla_{VIM-2}, and bla_{IMP-1}) located on both the plasmid (D) and chromosome (E) was tested. Resistance genes on either the plasmid or chromosome can be clearly detected and identified using this method. (F) Furthermore, EC-PhagoCas13a::KanR(s) were also effectively detecting toxin-encoding genes. (G) The carbapenem-resistant *E. coli* clinical isolate carrying bla_{IMP-1} was detected, where KanR was replaced with the hygromycin resistance gene HyrogR and the bottom agar contained hygromycin (200 μg/mL), as these clinical isolates were kanamycin resistant. (H) SA-PhagoCas13a::TetR_*mecA* generated by packaging CRISPR-Cas13a targeting *mecA* of MRSA

into the *S. aureus* phage exhibited *mecA*-specific bacterial killing activity against MRSA but not *S. aureus* strains deficient in *mecA*.

Fig. 1

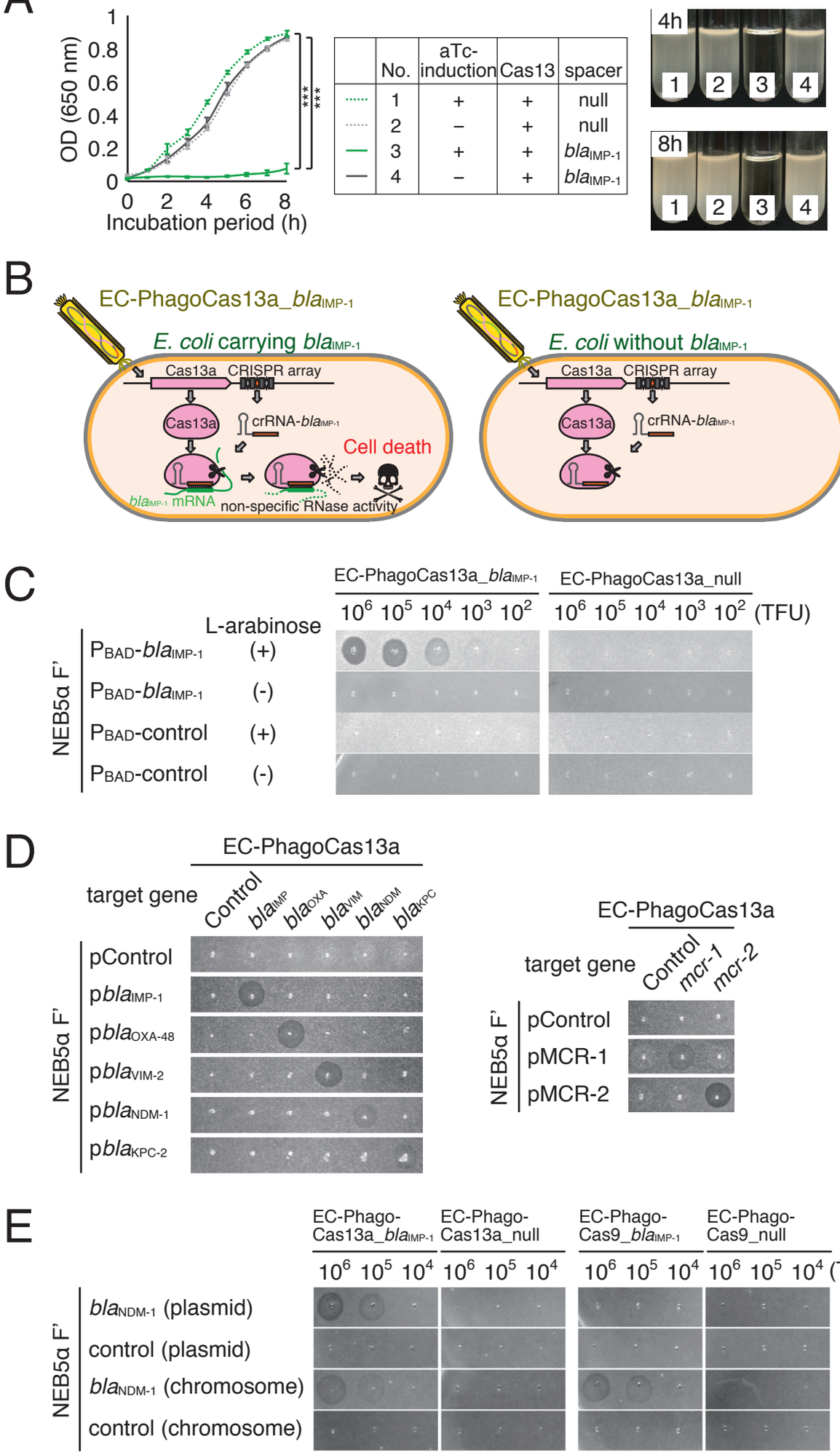

A
OD (650 nm)
Incubation period (h)
***
***
No. | aTc-induction | Cas13 | spacer
1 | + | + | null
2 | – | + | null
3 | + | + | blaIMP-1
4 | – | + | blaIMP-1
4h
8h
B
EC-PhagoCas13a_blaIMP-1
E. coli carrying blaIMP-1
Cas13a
CRISPR array
crRNA-blaIMP-1
Cell death
blaIMP-1 mRNA
non-specific RNase activity
EC-PhagoCas13a_blaIMP-1
E. coli without blaIMP-1
C
EC-PhagoCas13a_blaIMP-1
EC-PhagoCas13a_null
10^6 10^5 10^4 10^3 10^2 (TFU)
L-arabinose
NEB5α F'
PBAD-blaIMP-1 (+)
PBAD-blaIMP-1 (-)
PBAD-control (+)
PBAD-control (-)
D
EC-PhagoCas13a
target gene
Control
blaIMP
blaOXA
blaVIM
blaNDM
blaKPC
NEB5α F'
pControl
pblaIMP-1
pblaOXA-48
pblaVIM-2
pblaNDM-1
pblaKPC-2
EC-PhagoCas13a
target gene
Control
mcr-1
mcr-2
NEB5α F'
pControl
pMCR-1
pMCR-2
E
EC-Phago-Cas13a_blaIMP-1
EC-Phago-Cas13a_null
EC-Phago-Cas9_blaIMP-1
EC-Phago-Cas9_null
10^6 10^5 10^4 10^6 10^5 10^4 10^6 10^5 10^4 10^6 10^5 10^4 (TFU)
NEB5α F'
blaNDM-1 (plasmid)
control (plasmid)
blaNDM-1 (chromosome)
control (chromosome)

Fig. 2

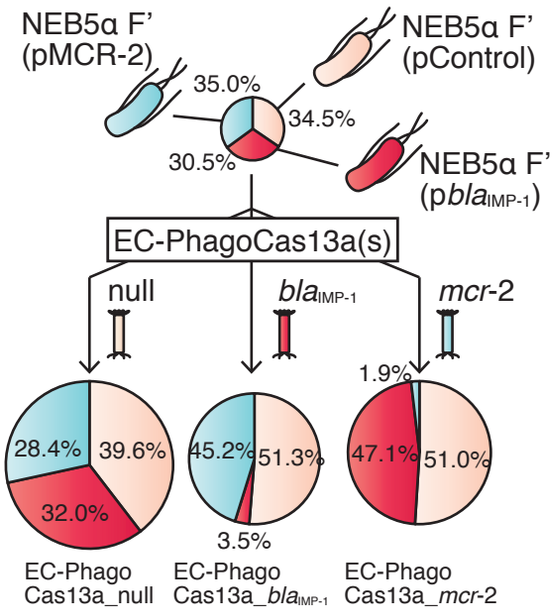

NEB5α F'
(pMCR-2)
NEB5α F'
(pControl)
NEB5α F'
(p*bla*IMP-1)
35.0%
34.5%
30.5%
EC-PhagoCas13a(s)
null
*bla*IMP-1
mcr-2
28.4%
39.6%
32.0%
45.2%
51.3%
3.5%
1.9%
47.1%
51.0%
EC-Phago
Cas13a_null
EC-Phago
Cas13a_*bla*IMP-1
EC-Phago
Cas13a_*mcr*-2

Fig. 3

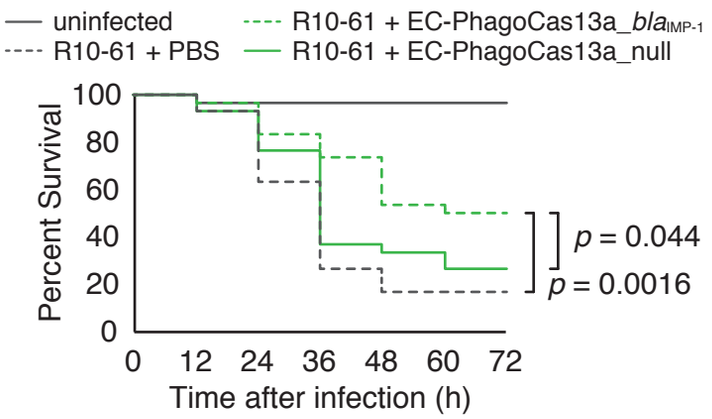
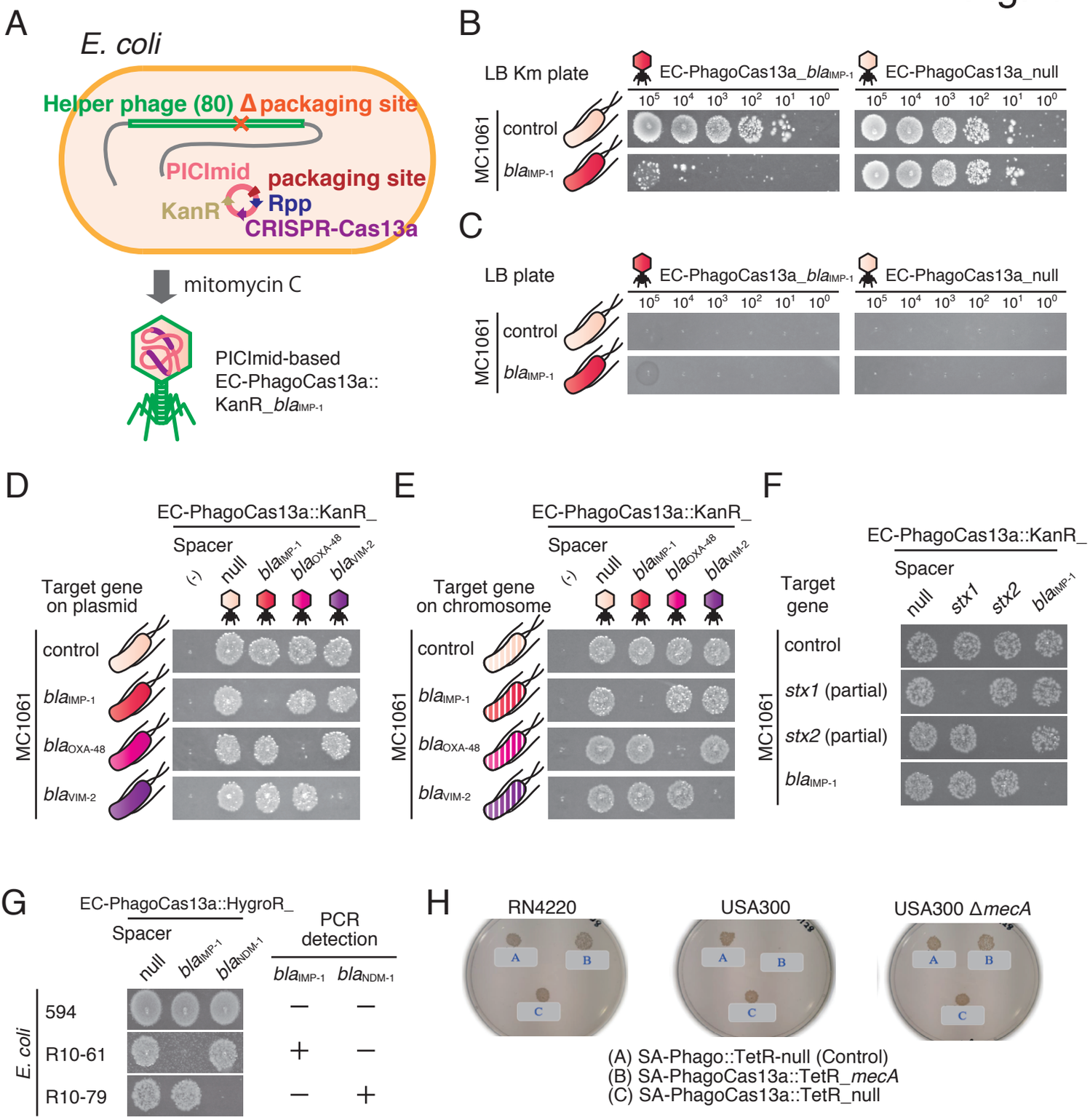

uninfected
R10-61 + PBS
R10-61 + EC-PhagoCas13a_*bla*IMP-1
R10-61 + EC-PhagoCas13a_null
Percent Survival
100
80
60
40
20
0
0
12
24
36
48
60
72
Time after infection (h)
p = 0.044
p = 0.0016

Fig. 4


A
E. coli
Helper phage (80) Δpackaging site
PICImid
packaging site
KanR
Rpp
CRISPR-Cas13a
mitomycin C
PICImid-based
EC-PhagoCas13a::
KanR_blaIMP-1
B
LB Km plate
EC-PhagoCas13a_blaIMP-1
EC-PhagoCas13a_null
10^5 10^4 10^3 10^2 10^1 10^0
MC1061
control
blaIMP-1
C
LB plate
EC-PhagoCas13a_blaIMP-1
EC-PhagoCas13a_null
10^5 10^4 10^3 10^2 10^1 10^0
MC1061
control
blaIMP-1
D
EC-PhagoCas13a::KanR_
Spacer
(-) null blaIMP-1 blaOXA-48 blaVIM-2
Target gene on plasmid
MC1061
control
blaIMP-1
blaOXA-48
blaVIM-2
E
EC-PhagoCas13a::KanR_
Spacer
(-) null blaIMP-1 blaOXA-48 blaVIM-2
Target gene on chromosome
MC1061
control
blaIMP-1
blaOXA-48
blaVIM-2
F
EC-PhagoCas13a::KanR_
Spacer
null stx1 stx2 blaIMP-1
Target gene
MC1061
control
stx1 (partial)
stx2 (partial)
blaIMP-1
G
EC-PhagoCas13a::HygroR_
Spacer
null blaIMP-1 blaNDM-1
PCR detection
blaIMP-1 blaNDM-1
E. coli
594 — —
R10-61 + —
R10-79 — +
H
RN4220
USA300
USA300 ΔmecA
A B C
(A) SA-Phago::TetR-null (Control)
(B) SA-PhagoCas13a::TetR_mecA
(C) SA-PhagoCas13a::TetR_null