





# Bacteriophage-Mediated Modulation of Bacterial Competition during Selective Enrichment of *Campylobacter*

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**ABSTRACT** Selective media using antimicrobial supplements generate unique microbial ecology to facilitate bacterial isolation. However, antibiotic-resistant bacteria indigenous to samples can interfere with the isolation process using selective media. Recent studies showed that extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* is highly prevalent on retail raw chicken and compromises the efficacy of *Campylobacter* isolation because ESBL-producing *E. coli* are resistant to antimicrobial supplements in *Campylobacter*-selective media and outgrows *Campylobacter*. The objective of this study was to improve *Campylobacter* isolation by inhibiting the growth of ESBL-producing *E. coli* using bacteriophages (phages). The supplementation of *Campylobacter*-selective media with *E. coli* phages reduced the level of ESBL-producing *E. coli* during the enrichment step. When *E. coli* phages were combined with the antimicrobial supplements of *Campylobacter*-selective media, antimicrobial synergy was observed, particularly with rifampicin, an antibiotic used in Preston medium. Although the same materials (i.e., phages and selective media) were used, the sequence of combining the materials markedly influenced the inhibition of ESBL-producing *E. coli* and the isolation of *Campylobacter*. These findings indicated that the modulation of microbial competition at the enrichment step was critical to the successful isolation of fastidious bacteria and that phages can be utilized to facilitate the selective enrichment of target bacteria by inhibiting their competitive bacteria.

**IMPORTANCE** Phages are promising antimicrobial alternatives. In this study, we first demonstrated that phages can be used to facilitate selective isolation of fastidious bacteria that are prone to be outgrown by bacterial competitors during isolation. The effectiveness of a phage-based isolation method was primarily dependent on the antimicrobial synergy between phages and antibiotics used in selective media. The same approach could be applied to the development of isolation methods for other fastidious bacteria.

**KEYWORDS** *Campylobacter* isolation, bacteriophage, antimicrobial synergy, ESBL-producing *Escherichia coli*

Bacterial isolation involves a series of procedures that selectively enrich and culture only target bacteria by inhibiting the growth of bacterial competitors that are indigenous to samples. *Campylobacter* is a bacterial pathogen that contaminates food, particularly retail poultry because *Campylobacter* heavily inhabits the gastrointestinal tract of avian species (1, 2). However, despite the abundance of *Campylobacter* in poultry products, it is difficult to isolate *Campylobacter* mainly because *Campylobacter* needs fastidious growth requirements. For instance, *Campylobacter* is unable to use glucose as a nutrient due to a defect in glycolysis and requires microaerobic conditions and elevated temperatures (i.e., 42°C) for optimal growth (3, 4).

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*Campylobacter* isolation is conducted using selective media supplemented with antibiotics, to which *Campylobacter* is intrinsically resistant (5, 6). Antibiotics in selective supplements are designed to promote the growth of *Campylobacter* while inhibiting contaminating bacteria. However, the efficacy of *Campylobacter* isolation has been significantly compromised by bacteria that are resistant to antimicrobial supplements in *Campylobacter*-selective media. Particularly, extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* are resistant to cefoperazone, a 3rd generation cephalosporin drug used in *Campylobacter*-selective media, such as Bolton selective medium and modified charcoal-cefoperazone-deoxycholate (mCCD) agar. ESBL-producing *E. coli* are highly prevalent in chicken and interrupts *Campylobacter* isolation using current protocols (7, 8), leading to a revision of the international standard for the detection and enumeration of *Campylobacter* (9, 10). Our previous analysis of microbiome changes during the enrichment of *Campylobacter* in selective media demonstrated that ESBL-producing *E. coli* are more abundantly enriched than *Campylobacter* (11, 12). These studies suggested that inhibiting ESBL-producing *E. coli* at the enrichment step is the key to the success in *Campylobacter* isolation.

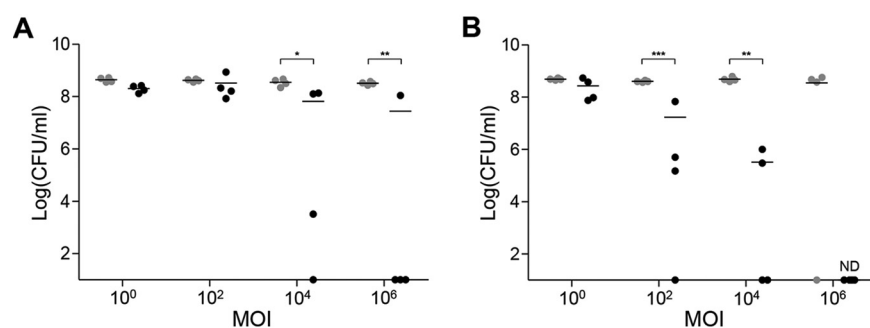
Because bacteriophages (phages) specifically infect only the host bacteria without affecting other bacteria (13), phages are considered promising antimicrobial alternatives to control antibiotic resistance (14, 15). Unlike antibiotics, phages have unique features, such as strict host specificity, low toxicity, and the capability to self-replicate (16). However, the use of a single phage is generally ineffective because the host range of phage infection is too narrow, and bacteria resistant to phage infection emerge easily (16). To overcome the limitations, phages are normally used as a cocktail consisting of multiple phages that infect different hosts or recognize different receptors (17). In our recent study, we discovered that *E. coli* phages have different infection efficacy depending on the phylogenetic group of *E. coli* (18). Because phylogroups A and B1 are predominant in *E. coli* isolates from chicken (19–23), a cocktail consisting of phages that infect *E. coli* in phylogroups A and B1 effectively inhibited antibiotic-resistant *E. coli* on chicken carcasses (18).

We hypothesized that *E. coli* phages could be used as an antimicrobial alternative to inhibit ESBL-producing *E. coli* during the isolation of *Campylobacter*. Using the *E. coli* phage cocktail, we demonstrated that phages could be used to facilitate *Campylobacter* isolation from chicken carcasses by modulating the growth competition between *Campylobacter* and ESBL-producing *E. coli*.

## RESULTS

### Phage inhibition of ESBL-producing *E. coli* in *Campylobacter*-selective media.

Five phages (JEP1, 4, 6, 7, and 8), which effectively inhibit ESBL-producing *E. coli* isolates from chicken carcasses, were identified and characterized in our previous study (18). Because *Campylobacter*-selective Bolton broth (BB) and Preston broth (PB) are the culture mediums adopted by the International Organization for Standardization (ISO) in the protocol for *Campylobacter* isolation (10), BB and PB were used in this study. Although phylogroups A and B1 dominate in *E. coli* isolates from chicken, *E. coli* in phylogroups B2 and D have also been isolated from chicken carcasses and can cause extraintestinal infections in humans (19–23). Thus, we prepared a mixed culture consisting of ESBL-producing *E. coli* strains (E20, E41, E55, and E59), which belong to the phylogroups A, B1, B2, and D, respectively, as reported in our previous study (23). The levels of ESBL-producing *E. coli* were measured after infecting the mixed culture of *E. coli* strains with the phage cocktail in BB and PB. Interestingly, the efficacy of *E. coli* inhibition by the phage cocktail was different depending on the *Campylobacter*-selective medium (Fig. 1). Whereas the phage cocktail reduced ESBL-producing *E. coli* marginally (0.7–1.1 log colony forming units per milliliter [CFU/mL] in mean values) in BB even after treatment with a high multiplicity of infections (MOIs;  $10^4$  and  $10^6$ ) (Fig. 1A), the phage cocktail markedly reduced ESBL-producing *E. coli* in PB in proportion to the MOI (Fig. 1B). At the highest MOI ( $10^6$ ), ESBL-producing *E. coli* were undetectable ( $<10$  CFU/mL) in PB (Fig. 1B). When combined with the



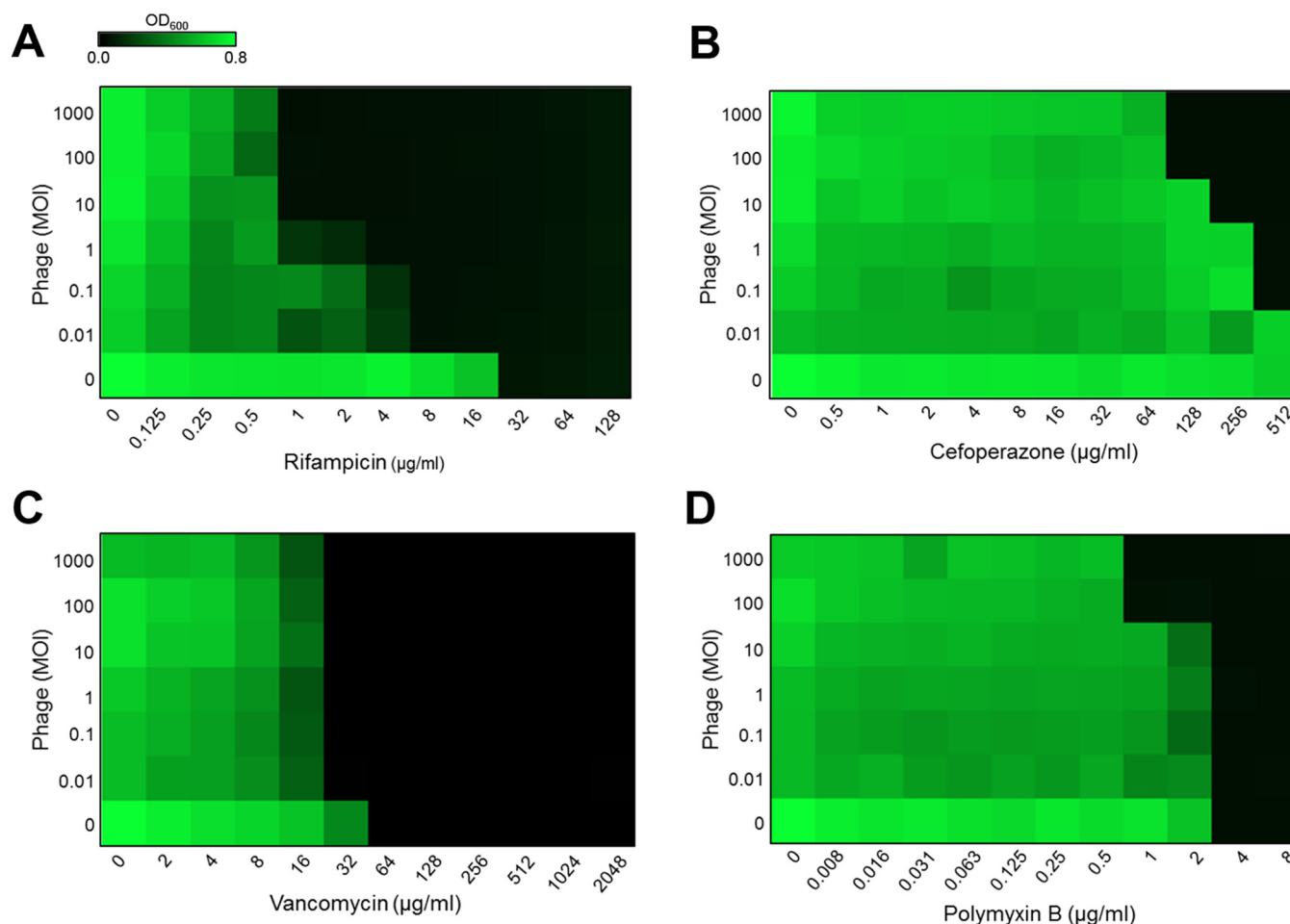
**FIG 1** Phage inhibition of ESBL-producing *E. coli* in *Campylobacter*-selective media. The inhibition of ESBL-producing *E. coli* by the phage cocktail in *Campylobacter*-selective Bolton broth (A) and *Campylobacter*-selective Preston broth (B). Each dot represents the mean of the results of quadruplicate samples in a single experiment. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; ND, not detected (detection limit: 10 CFU/mL); MOI, multiplicity of infection.

phage cocktail, PB generated more significant antimicrobial activity against ESBL-producing *E. coli* than BB.

**Antimicrobial synergy between phages and antibiotics.** Because the phages inhibited ESBL-producing *E. coli* differentially depending on the selective medium, we hypothesized that the level of antimicrobial synergy between phages and *Campylobacter*-selective medium was dependent on the antibiotic used in the selective supplements of BB and PB. The hypothesis was examined by conducting checkerboard titration assays in the presence of five antibiotics (cefoperazone, vancomycin, polymyxin B, rifampicin, and trimethoprim) that are used as antimicrobial supplements in BB and PB. The most substantial synergy was observed when the phage cocktail was combined with rifampicin, an antibiotic supplement in PB (Fig. 2A). Although the MIC of cefoperazone was high ( $>512 \mu\text{g/mL}$ ) due to cephalosporin resistance that was conferred by ESBL, the susceptibility of ESBL-producing *E. coli* to cefoperazone was increased when the phages were used at high MOIs (Fig. 2B). The cotreatment with the phage cocktail reduced the MICs of vancomycin and polymyxin B by 2-fold and 4-fold, respectively (Fig. 2C and D). Trimethoprim is commonly used as an antibiotic supplement in both BB and PB. Despite cotreatment with the phage cocktail, trimethoprim did not reduce the level of ESBL-producing *E. coli* (Fig. S1). These results indicated that phages could enhance the antimicrobial activity of antibiotics when used together, and the efficacy of antimicrobial synergy was affected by the antibiotic combined with phages. Based on these findings, the substantial inhibition of ESBL-producing *E. coli* by the phage cocktail in PB (Fig. 1B) could be ascribed to the antimicrobial synergy between the phages and rifampicin (Fig. 2A).

#### Inhibition of ESBL-producing *E. coli* by phages during selective enrichment.

The levels of ESBL-producing *E. coli* were measured under eight different enrichment conditions that were made by combining two kinds of selective medium (BB and PB), two temperatures (37°C and 42°C), and with/without the phage cocktail (Fig. S2). After enrichment of chicken samples under the eight enrichment conditions, the levels of *E. coli* were quantified by qPCR (Fig. 3A). *E. coli* was detected in all samples, and the phages significantly reduced *E. coli* under all the conditions except on BB at 37°C (Fig. 3A). Moreover, phage treatment substantially reduced the level of ESBL-producing *E. coli* based on the enumeration of colonies on MacConkey agars supplemented with cefotaxime, a 3rd generation cephalosporin drug (Fig. 3B). *E. coli* was significantly reduced when the phages were used in PB at 42°C (Fig. 3). In contrast, the level of *Campylobacter* was increased by phage treatment under the enrichment conditions, but the differences were not statistically significant (Fig. S3), suggesting unidentified factors may still affect the enrichment of *Campylobacter*. Nevertheless, these results

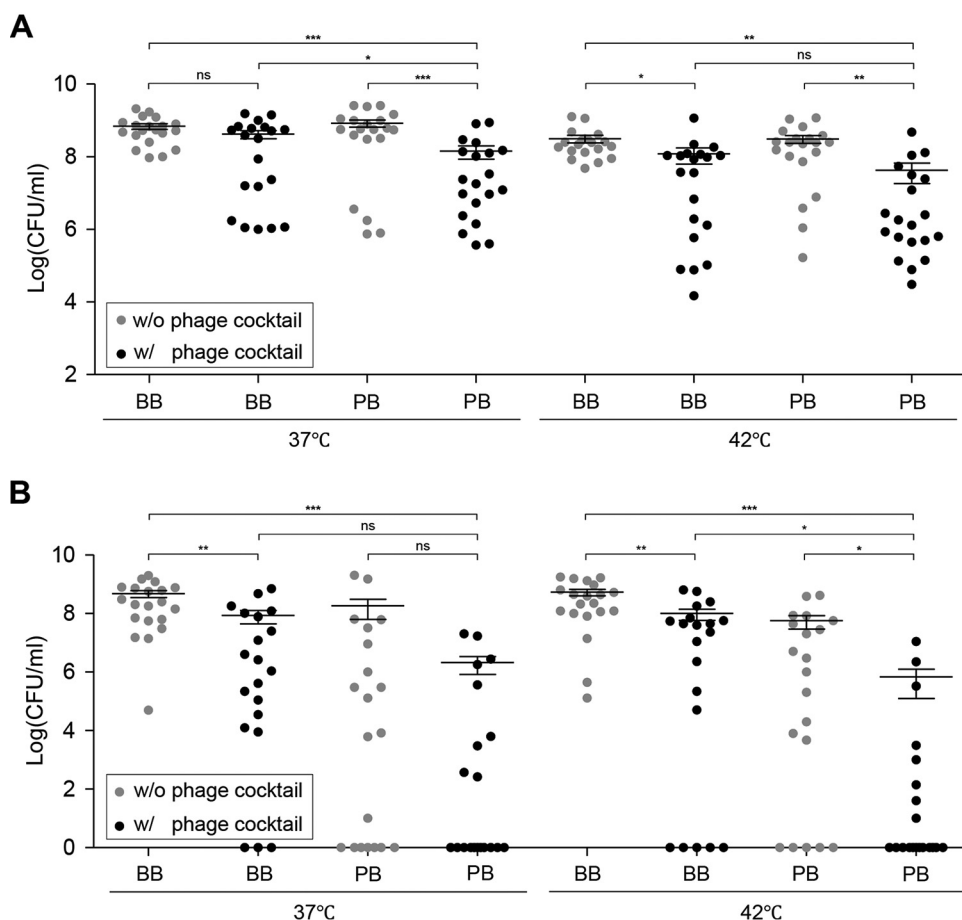


**FIG 2** Antimicrobial synergy between phages and antibiotics. Checkerboard titration assays were conducted using the phage cocktail and the antibiotics of *Campylobacter*-selective Bolton and Preston media, including rifampicin (A), cefoperazone (B), vancomycin (C), and polymyxin B (D). The experiments were repeated at least three times and generated similar results. MOI, multiplicity of infection.

suggested that the phages successfully reduced the level of ESBL-producing *E. coli*, the major bacterial competitor affecting the growth of *Campylobacter*.

**Improvement of *Campylobacter* isolation by modulating bacterial competition using phages.** To evaluate how phage inhibition of *E. coli* affects *Campylobacter* isolation, the frequency of *Campylobacter* isolation was measured in 16 different culture conditions where we combined two of the 1st enrichment mediums (BB and PB), two of the 2nd selective solid medium (Bolton agar [BA] and Preston agar [PA]), two enrichment temperatures (37°C and 42°C), and with/without the phage cocktail (Fig. S2). Depending on the condition, the isolation frequencies varied from 0% to 45% (Fig. 4 and Table S2). *Campylobacter* was not isolated in 8 out of 20 chicken samples under the 16 conditions (Table S2). The use of the phage cocktail at the enrichment step markedly increased the isolation frequency, especially when the samples were enriched at 42°C in PB followed by plating on BA (Fig. 4).

Moreover, when the phage cocktail was added to the enrichment medium, the number of *E. coli* colonies was substantially reduced, whereas *Campylobacter* colonies were easily recognized (Fig. 5). Without using the phage cocktail, *Campylobacter*-selective agar plates were heavily covered with colonies of ESBL-producing *E. coli* (Fig. 5A). However, the use of the phage cocktail markedly prevented the formation of *E. coli* colonies and made *Campylobacter* colonies easily recognizable (Fig. 5B), indicating that the phages successfully inhibited ESBL-producing *E. coli* and facilitated the growth of *Campylobacter*.



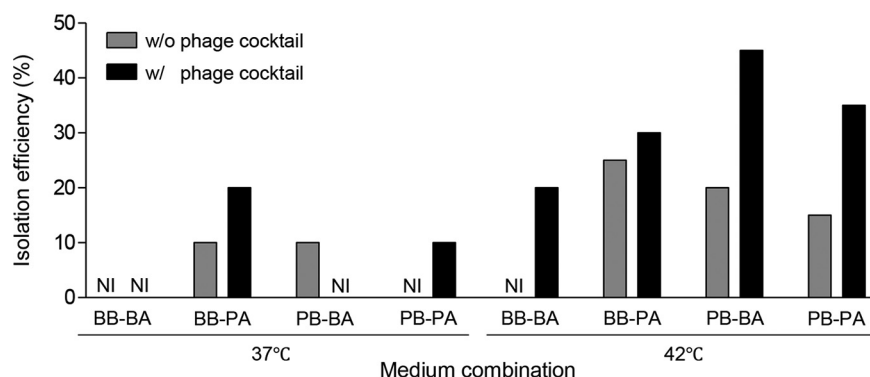
**FIG 3** Inhibition of EBL-producing *E. coli* by the phage cocktail in *Campylobacter*-selective enrichment broth. The level of EBL-producing *E. coli* was determined in 20 chicken carcasses in *Campylobacter*-selective enrichment media using qPCR amplifying *uidA* (A) and CFU counting on MacConkey agars supplemented with 1  $\mu$ g/mL cefotaxime (B). SM buffer was added to nontreated controls. BB, Bolton broth; PB, Preston broth. The bars show the mean and the standard deviations. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; ns, not significant.

## DISCUSSION

Successful bacterial isolation relies on how effectively selective culture media can eliminate bacterial competitors while facilitating the growth of the target bacteria. Our previous studies demonstrated that each *Campylobacter*-selective medium makes substantial changes in the microbiome of enrichment cultures because of the selective pressure generated by antimicrobial supplements (11, 12). Proteobacteria and Firmicutes are the major phyla after the enrichment of chicken carcasses with BB, whereas Proteobacteria and Fusobacteria were dominant after the enrichment with PB (11). Commonly, antibiotic-resistant *E. coli*, such as EBL-producing *E. coli*, is predominantly enriched in both BB and PB (11). Consistently, it has been reported that EBL-producing *E. coli* prevalent on chicken carcasses significantly compromises the efficacy of *Campylobacter* isolation (7, 11), suggesting the need for improving the current isolation methods. However, EBL genes in *E. coli* are generally encoded on plasmids harboring a cluster of antibiotic resistance genes (24, 25). Thus, the identification of additional antimicrobial supplements, which inhibit EBL-producing *E. coli* and do not affect the growth of *Campylobacter*, is extremely challenging.

To address this issue, we used phages as an antimicrobial supplement to inhibit EBL-producing *E. coli*, the primary bacterial competitor interrupting *Campylobacter* isolation. The isolation of fastidious bacteria, such as *Campylobacter*, is a technical challenge to many microbiology laboratories because *Campylobacter* is normally

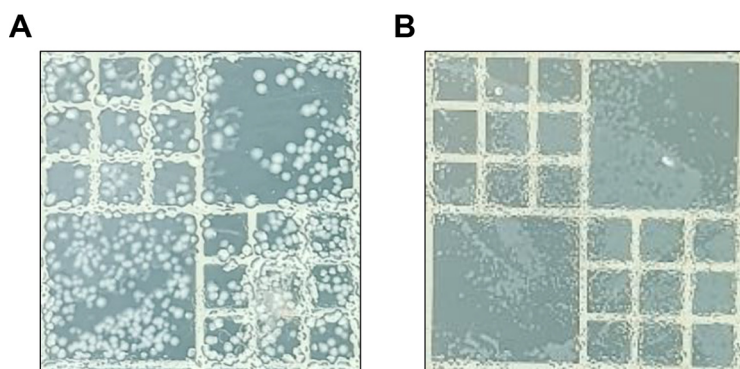




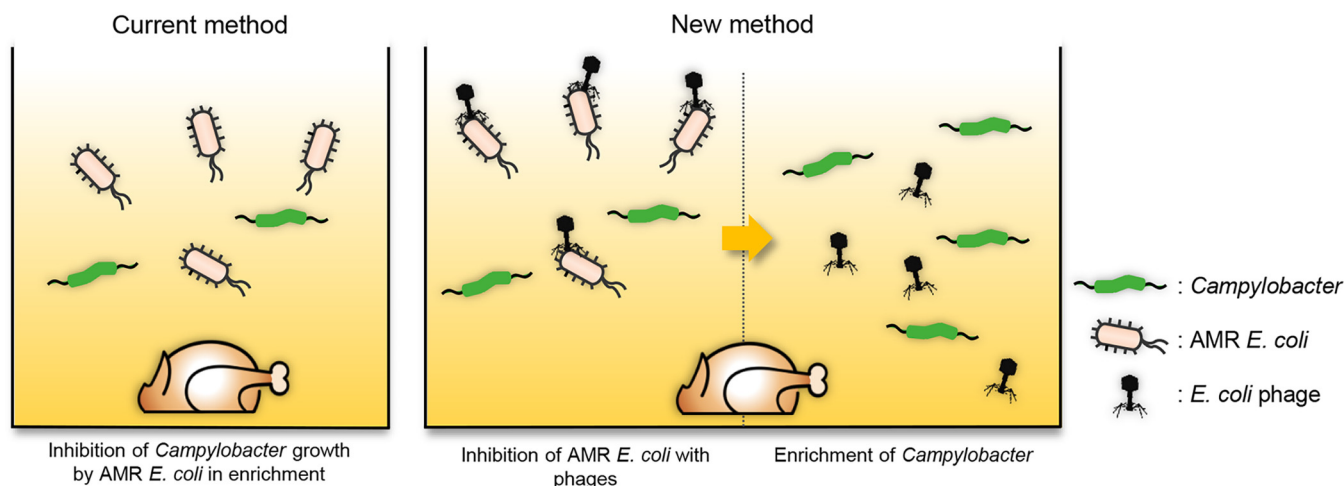
**FIG 4** Effects of phage cocktail treatment on *Campylobacter* isolation. NI, not isolated; BB, Bolton broth; BA, Bolton agar; PB, Preston broth; PA, Preston agar.

outcompeted by other fast-growing bacteria that are indigenous to samples. For this, selective media were supplemented with antibiotics to enhance the isolation of *Campylobacter* by suppressing competing bacteria (11, 26). This study first demonstrated that phages can be utilized to improve the efficacy of *Campylobacter* isolation by controlling microbial competition between *Campylobacter* and ESBL-producing *E. coli* (Fig. 6). When current methods are used to isolate *Campylobacter* from chicken, ESBL-producing *E. coli* could outgrow *Campylobacter* during enrichment (Fig. 6). However, phages could inhibit ESBL-producing *E. coli* and improved *Campylobacter* isolation (Fig. 6).

The antimicrobial synergy between phages and antibiotics enhanced the inhibition of ESBL-producing *E. coli*, indicating that phages could be used complementarily with antibiotics to inhibit antibiotic-resistant bacteria. Importantly, the level of antimicrobial synergy between phages and antibiotics was affected by the antibiotic combined with phages. The most significant antimicrobial synergy was observed when the phages were mixed with rifampicin, a broad-spectrum antibiotic used in PB. The phage cocktail reduced the MIC of rifampicin by 32-fold even at low MOIs, such as 10 (Fig. 2A). The synergy between phages and rifampicin may account for the substantial reduction in ESBL-producing *E. coli* by the phage cocktail in PB (Fig. 1B). In addition, the level of antimicrobial susceptibility is another factor that determines antimicrobial synergy. The phage treatment reduced the MIC only by 2-fold in combination with vancomycin regardless of MOIs, because vancomycin is not effective against Gram-negative bacteria. A recent study also reported that phages generate synergistic antimicrobial effects with antibiotics depending on the mode of action of an antibiotic possibly because the impairment of critical cellular functions in bacteria, such as DNA replication and pro-



**FIG 5** Reduced formation of ESBL-producing *E. coli* colonies by phage treatment. The photos show representative Bolton agars inoculated with an aliquot of *Campylobacter*-selective Preston enrichment broth without (A) and with (B) the phage cocktail at 42°C. Whitish colonies are ESBL-producing *E. coli* and transparent colonies are *Campylobacter*.



**FIG 6** Modulation of microbial competition with phages at the step of *Campylobacter* enrichment. AMR, antibiotic-resistant.

tein synthesis, by antibiotics can also affect the life cycle of phages within bacterial hosts (27).

It is worth noting that the sequence of combining selective mediums made substantial differences in the inhibition of ESBL-producing *E. coli* and, consequently, the efficacy of *Campylobacter* isolation. Although the same selective medium and phages were used in the experiments, the frequency of *Campylobacter* isolation varied widely depending on how the materials were used in combination (Fig. 4). The highest isolation efficiency was achieved when chicken samples were enriched in PB in the presence of the phage cocktail at 42°C followed by plating on BA (Fig. 4). However, the enrichment of chicken samples in BB with the phage cocktail followed by plating on PA exhibited only 20% of isolation efficiency at 37°C (Fig. 4). Moreover, enrichment temperatures were another important factor determining the efficacy of *Campylobacter* isolation using phages. Overall, the frequency of *Campylobacter* isolation was higher at 42°C than 37°C (Fig. 4), presumably because *Campylobacter* optimally grows at 42°C. Although the supplementation of selective media with phages provides additional selective pressure to improve *Campylobacter* isolation, environmental factors should also be optimized to facilitate the growth of target bacteria.

Here, we demonstrated that phages can be used to facilitate the isolation of *Campylobacter* from retail raw chicken by inhibiting ESBL-producing *E. coli*, the primary bacterial competitor interrupting *Campylobacter* isolation. The findings in this study provide new insights into how phages can be incorporated into the procedures to isolate fastidious bacteria.

## MATERIALS AND METHODS

**Bacterial strains, phages, and growth conditions.** Five phages (JEP1, 4, 6, 7, and 8) were reported in our previous study to inhibit ESBL-producing *E. coli* isolates from chicken carcasses (18). All cesium chloride (CsCl)-purified phages ( $\geq 10^{10}$  plaque forming units per milliliter [PFU/mL]) were used in this experiment. ESBL-producing *E. coli* strains E20, E41, E55, and E59 belonging to phylogroups A, B1, B2, and D, respectively, were isolated from retail raw chicken in our previous study (18) and routinely cultured at 37°C in Luria Bertani (LB) medium (Difco, NJ, USA). *Campylobacter* was cultured at 42°C in Mueller-Hinton (MH) medium (Oxoid, Hampshire, UK) under microaerobic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>). All bacterial strains were stored at -80°C in LB or MH broth with 15% glycerol. The sodium chloride-magnesium sulfate (SM) buffer (100 mM NaCl, 8 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, and 50 mM Tris-HCl, pH 7.5) was used as the phage buffer.

**Inhibition assay in Bolton and Preston *Campylobacter*-selective mediums.** Overnight cultures of ESBL-producing *E. coli* strains E20, E41, E55, and E59 in LB broth were diluted in fresh LB broth by 100-fold. After the cultivation of the ESBL-producing *E. coli* strains (E20, E41, E55, and E59) to an optical density at 600 nm (OD<sub>600</sub>) of 0.5, an equal volume of the cultures was mixed in a single tube. The mixed culture was diluted and added to 3 mL of *Campylobacter*-selective Bolton medium (Oxoid, UK) and Preston medium (Thermo-Fisher Scientific, OH, USA) at 10<sup>1</sup>, 10<sup>3</sup>, 10<sup>5</sup>, or 10<sup>7</sup> CFU/mL followed by the addition of

the phage cocktail (40  $\mu$ L;  $10^7$  PFU/mL) at a range of MOI values ( $10^5$  to  $10^6$ ). After incubation at 42°C for 24 h, a 10-fold serially diluted culture was plated onto LB agar plates to enumerate *E. coli*.

**Checkerboard titration assay.** The checkerboard titration assay was performed as described previously (28) to evaluate whether phages could inhibit ESBL-producing *E. coli* synergistically with the antibiotics in Bolton and Preston mediums, including cefoperazone (0 to 512  $\mu$ g/mL; MilliporeSigma, MO, USA), vancomycin (0 to 2048  $\mu$ g/mL; MilliporeSigma), and trimethoprim (0 to 512  $\mu$ g/mL; MilliporeSigma) in Bolton medium, and polymyxin B (0 to 8  $\mu$ g/mL; MilliporeSigma), rifampicin (0 to 128  $\mu$ g/mL; MilliporeSigma), and trimethoprim (0 to 512  $\mu$ g/mL; MilliporeSigma) in Preston medium. Five antibiotics (cefoperazone, vancomycin, polymyxin B, rifampicin, and trimethoprim) were selected for the assay. The antibiotics were serially diluted 2-fold on each column in a 96-well plate, and the phage cocktail was diluted 10-fold on each row. The mixed culture of *E. coli* strains (E20, E41, E55, and E59) was prepared as described above and added to the 96-well plate ( $10^5$  CFU per well). The plate was incubated at 42°C for 24 h. The OD<sub>600</sub> was measured with SpectraMax i3 Platform (Molecular Devices, CA, USA).

**Sample collection and selective enrichment.** Twenty whole chicken carcasses (20 different brands of 16 different companies) were purchased from retail stores. The thigh meat was cut with a sterilized knife. Approximately 10 g of chicken thigh meat was enriched with 90 mL of Bolton or Preston broth with phage cocktail ( $10^8$  PFU/mL) or SM buffer at different temperatures (37°C or 42°C) for 24 h under microaerobic conditions.

**Quantitative PCR (qPCR) of *E. coli* in enrichment broth.** To determine the level of *E. coli* at the enrichment step, qPCR was performed as described previously (29). A standard curve was prepared using the genomic DNA (gDNA) of *E. coli* K-12 W3110. The gDNA was extracted from *E. coli* K-12 W3110 grown to an OD<sub>600</sub> of 1 using the G-spin<sup>TM</sup> Genomic DNA Extraction kit (Intronbio, Seongnam, Republic of Korea) according to the manufacturer's instructions. The purified gDNA was serially diluted to create a standard curve. A standard curve correlating gDNA copy number with CFU was generated using *uidA* (29). The enrichment cultures (20 mL) were concentrated by centrifugation at  $4,000 \times g$ , 4°C for 7 min, and pellets were resuspended with 2 mL of PBS. The gDNA was extracted from 1 mL resuspension and analyzed by qPCR. The qPCR mixture contained 10  $\mu$ L  $2 \times$  iQ SYBR green Supermix (Bio-Rad, CA, USA) and 0.3  $\mu$ M each primer in a reaction volume of 20  $\mu$ L, *uidA*-qPCR-F, and *uidA*-qPCR-R (Table S1). All qPCRs were performed using the CFX Connect<sup>TM</sup> real-time PCR detection system (Bio-Rad, USA), and the cycling parameters were as follows: 95°C for 5 min; 39 cycles at 95°C for 15 s, 55°C for 15 s, 72°C for 30 s; 72°C for 7 min.

**Quantification of ESBL-producing *E. coli* in enrichment broth.** Enrichment cultures (20 mL) were centrifuged at  $4,000 \times g$ , 4°C for 7 min and resuspended with 2 mL of PBS. ESBL-producing *E. coli* was counted by 10-fold serial dilution and plating on MacConkey agars (Oxoid, UK) supplemented with 1  $\mu$ g/mL cefotaxime.

**Isolation of *Campylobacter* under 16 different combinations.** Enrichment cultures (100  $\mu$ L) prepared as described above were spread onto Bolton and Preston agar plates supplemented with *Campylobacter*-selective supplements. The plates were incubated at 42°C for 48 h under microaerobic conditions. For each sample, 15 colonies were analyzed and isolated to indicate *Campylobacter* isolation efficiency. Based on colony morphology, 15 presumptive *Campylobacter* colonies were randomly selected and tested with multiplex PCR using the primer sets specific for *Campylobacter* 16S rRNA and three *Campylobacter* species, including *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* (Table S1).

**Statistics analysis.** Statistical significance was evaluated with Student's *t* test using GraphPad Prism version 5.01 (GraphPad Software, Inc., CA, USA).

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.4 MB.

## ACKNOWLEDGMENTS

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J.K., S.R., and B.J. designed the study. J.K. and J.I.H. performed the experiments. J.K. and B.J. analyzed data and wrote the paper. S.R. and B.J. supervised the study.

We declare no conflicts of interest.

## REFERENCES

1. Jeon B, Muraoka WT, Zhang Q. 2010. Advances in *Campylobacter* biology and implications for biotechnological applications. *Microb Biotechnol* 3: 242–258. <https://doi.org/10.1111/j.1751-7915.2009.00118.x>.
2. Skarp CP, Hanninen ML, Rautelin HI. 2016. *Campylobacteriosis*: the role of poultry meat. *Clin Microbiol Infect* 22:103–109. <https://doi.org/10.1016/j.cmi.2015.11.019>.



3. Davis L, DiRita V. 2008. Growth and laboratory maintenance of *Campylobacter jejuni*. Curr Protoc Microbiol Chapter 8:Unit 8A.1.1–8A.1.7. <https://doi.org/10.1002/9780471729259.mc08a01s10>.
4. Burnham PM, Hendrixson DR. 2018. *Campylobacter jejuni*: collective components promoting a successful enteric lifestyle. Nat Rev Microbiol 16: 551–565. <https://doi.org/10.1038/s41579-018-0037-9>.
5. Taylor DE, Courvalin P. 1988. Mechanisms of antibiotic-resistance in *Campylobacter* species. Antimicrob Agents Chemother 32:1107–1112. <https://doi.org/10.1128/AAC.32.8.1107>.
6. Corry JEL, Post DE, Colin P, Laisney MJ. 1995. Culture media for the isolation of campylobacters. Int J Food Microbiol 26:43–76. [https://doi.org/10.1016/0168-1605\(95\)00044-K](https://doi.org/10.1016/0168-1605(95)00044-K).
7. Hazeleger WC, Jacobs-Reitsma WF, den Besten HM. 2016. Quantification of growth of *Campylobacter* and extended spectrum  $\beta$ -lactamase producing bacteria sheds light on black box of enrichment procedures. Front Microbiol 7:1430. <https://doi.org/10.3389/fmicb.2016.01430>.
8. Chon JW, Kim YJ, Kim YJ, Jung JY, Bae D, Khan S, Seo KH, Sung K. 2017. Addition of rifampicin to Bolton broth to inhibit extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* for the detection of *Campylobacter*. J Food Sci 82:1688–1692. <https://doi.org/10.1111/1750-3841.13761>.
9. International Organization for Standardization. 2006. ISO 10272-1. 2006. Microbiology of food and animal feeding stuffs—Horizontal method for detection and enumeration of *Campylobacter* spp.—part 1: detection method. Geneva, Switzerland.
10. International Organization for Standardization. 2017. ISO 10272-1. 2017. Microbiology of the food chain—horizontal method for detection and enumeration of *Campylobacter* spp.—part 1: detection method. Geneva, Switzerland.
11. Kim J, Shin H, Park H, Jung H, Kim J, Cho S, Ryu S, Jeon B. 2019. Microbiota analysis for the optimization of *Campylobacter* isolation from chicken carcasses using selective media. Front Microbiol 10:1381. <https://doi.org/10.3389/fmicb.2019.01381>.
12. Kim J, Guk J-H, Mun S-H, An J-U, Song H, Kim J, Ryu S, Jeon B, Cho S. 2019. Metagenomic analysis of isolation methods of a targeted microbe, *Campylobacter jejuni*, from chicken feces with high microbial contamination. Microbiome 7:1–16.
13. Sulakvelidze A, Alavidze Z, Morris JG. 2001. Bacteriophage therapy. Antimicrob Agents Chemother 45:649–659. <https://doi.org/10.1128/AAC.45.3.649-659.2001>.
14. Skurnik M, Strauch E. 2006. Phage therapy: facts and fiction. Int J Med Microbiol 296:5–14. <https://doi.org/10.1016/j.ijmm.2005.09.002>.
15. Endersen L, O'Mahony J, Hill C, Ross RP, McAuliffe O, Coffey A. 2014. Phage therapy in the food industry. Annu Rev Food Sci Technol 5: 327–349. <https://doi.org/10.1146/annurev-food-030713-092415>.
16. Drulis-Kawa Z, Majkowska-Skrobek G, Maciejewska B, Delattre A-S, Lavigne R. 2012. Learning from bacteriophages—advantages and limitations of phage and phage-encoded protein applications. Curr Protein Pept Sci 13:699–722. <https://doi.org/10.2174/138920312804871193>.
17. Nilsson AS. 2014. Phage therapy—constraints and possibilities. Ups J Med Sci 119:192–198. <https://doi.org/10.3109/03009734.2014.902878>.
18. Kim J, Park H, Ryu S, Jeon B. 2021. Inhibition of antimicrobial-resistant *Escherichia coli* using a broad host range phage cocktail targeting various bacterial phylogenetic groups. Front Microbiol 12:699630. <https://doi.org/10.3389/fmicb.2021.699630>.
19. Coura FM, Diniz SA, Silva MX, Mussi JMS, Barbosa SM, Lage AP, Heinemann MB. 2015. Phylogenetic group determination of *Escherichia coli* isolated from animal samples. Sci World J 2015:1–258424. <https://doi.org/10.1155/2015/258424>.
20. Unno T, Han D, Jang J, Lee S-N, Ko G, Choi HY, Kim JH, Sadowsky MJ, Hur H-G. 2009. Absence of *Escherichia coli* phylogenetic group B2 strains in humans and domesticated animals from Jeonnam Province, Republic of Korea. Appl Environ Microbiol 75:5659–5666. <https://doi.org/10.1128/AEM.00443-09>.
21. Xu X, Cui S, Zhang F, Luo Y, Gu Y, Yang B, Li F, Chen Q, Zhou G, Wang Y, Pang L, Lin L. 2014. Prevalence and characterization of cefotaxime and ciprofloxacin co-resistant *Escherichia coli* isolates in retail chicken carcasses and ground pork, China. Microb Drug Resist 20:73–81. <https://doi.org/10.1089/mdr.2012.0224>.
22. Kluytmans JAJW, Overvest ITMA, Willemsen I, Kluytmans-van den Bergh MFQ, van der Zwaluw K, Heck M, Rijnsburger M, Vandenbroucke-Grauls CMJE, Savelkoul PHM, Johnston BD, Gordon D, Johnson JR. 2013. Extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. Clin Infect Dis 56:478–487. <https://doi.org/10.1093/cid/cis929>.
23. Park H, Kim J, Ryu S, Jeon B. 2019. Predominance of bla<sub>CTX-M-65</sub> and bla<sub>CTX-M-55</sub> in extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* from raw retail chicken in South Korea. J Glob Antimicrob Resist 17:216–220. <https://doi.org/10.1016/j.jgar.2019.01.005>.
24. Kim J, Hwang BK, Choi H, Wang Y, Choi SH, Ryu S, Jeon B. 2019. Characterization of *mcr-1*-harboring plasmids from pan drug-resistant *Escherichia coli* strains isolated from retail raw chicken in South Korea. Microorganisms 7:344. <https://doi.org/10.3390/microorganisms7090344>.
25. Li Q, Chang W, Zhang H, Hu D, Wang X. 2019. The role of plasmids in the multiple antibiotic resistance transfer in ESBLs-producing *Escherichia coli* isolated from wastewater treatment plants. Front Microbiol 10:633. <https://doi.org/10.3389/fmicb.2019.00633>.
26. Ehrmann E, Jolivet-Gougeon A, Bonnaure-Mallet M, Fosse T. 2013. Antibiotic content of selective culture media for isolation of *Capnocytophaga* species from oral polymicrobial samples. Lett Appl Microbiol 57:303–309. <https://doi.org/10.1111/lam.12112>.
27. Liu CG, Green SI, Min L, Clark JR, Salazar KC, Terwilliger AL, Kaplan HB, Trautner BW, Ramig RF, Maresco AW. 2020. Phage-antibiotic synergy is driven by a unique combination of antibacterial mechanism of action and stoichiometry. mBio 11:e01462-20. <https://doi.org/10.1128/mBio.01462-20>.
28. Hsieh MH, Yu CM, Yu VL, Chow JW. 1993. Synergy assessed by checkerboard a critical analysis. Diagn Microbiol Infect Dis 16:343–349. [https://doi.org/10.1016/0732-8893\(93\)90087-N](https://doi.org/10.1016/0732-8893(93)90087-N).
29. Taskin B, Gozen AG, Duran M. 2011. Selective quantification of viable *Escherichia coli* bacteria in biosolids by quantitative PCR with propidium monoazide modification. Appl Environ Microbiol 77:4329–4335. <https://doi.org/10.1128/AEM.02895-10>.