Isolation and characterization of *Escherichia coli* phage and enhance its bactericidal activity that collaborative with kanamycin sulfate

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Abstract

**Background:** *Escherichia coli* is the most important and widespread bacteria in worldwide, which mainly found in contaminated food, human and animal faeces. Unfortunately, Some of *E. coli* strains are multidrug-resistant (MDR) pathogen leading significant public health concern globally. Biofilm is a multicellular community of microorganisms. Phages and their derivatives are ideal candidates for replacing or compensating for antibiotic problems in the future.

**Method:** Here, we aimed to isolation and characterization of *Escherichia coli* phage and research its bactericidal activity that individually or collaborative with kanamycin sulfate

**Results:** In this study, three virulent phages Flora, T4 and WJ were isolated from the laboratory and drug sample in Wuxi, China. It’s belonged to the *Myoviridae* family and optimum temperature is 42 °C, optimum pH= 7, optimum MOI is 0.0001 and the genome size of Flora, T4 and WJ were 168, 909, 168903 and 168, 900 bp respectively. Flora has two exonuclease, whereas T4 and WJ have only one. Antibiotics have better bactericidal activity than phages in a low concentration medium of bacteria, nonetheless, phages have better bactericidal activity than antibiotics in a high concentration of bacteria, and that, collaboration of phages and antibiotics have better bactericidal activity effect than alone of phages or antibiotics in a low concentration medium of bacteria.

**Conclusion:** The excellent performance of phage Flora for its therapeutic potential on clinic. The data of this study provided the strong evidence that the application of phage could reduce the growth and biofilm of *E. coli* that are important to maintain public health.

**Keywords:** *Escherichia coli*, phage, lytic spectrum, biofilm, antibiotic

Introduction

*Escherichia coli* is widespread in the digestive tract of humans, and some of these strains cause intestinal disease[1 – 3]. What is more, the human pathogen of *E. coli* O157:H7 is an important pathogen that is associated with abdominal pain, gastroenteritis, haemorrhagic colitis, diarrhoea to potentially fatal haemolytic-uraemic syndrome, hemolytic-uremic syndrome (HUS), hemorrhagic colitis and death[4 – 6]. With the normal use and abuse of antibiotics, some strains were capable of
resistant to 14 different antibiotic, which was existed in food that sold to consumers[7 – 9]. Furthermore, the emergence of emergence of multi-drug resistant bacterial strains urgent need alternative treatments to antibiotics[10,11].

E. coli can adhere to and be internalized in epithelial cells. The persistence of this pathogen in cattle mammary glands causes an infection called mastitis, whose recurrence may be related to biofilm formation[12]. The antibiotic resistance and mastitis were relevanted with the formation of E. coli biofilm.

Biofilm is a multicellular community of microorganisms where microorganisms are embedded self-produced extracellular matrix and attached to highly hydrated extracellular matrix on, non-biological and biological surfaces[13 – 15]. The extracellular polymeric substances matrix of biofilm acts as a barrier that reduces the penetration of antimicrobial agents and microbiotic into the interior of the biofilm[16,17]. Biofilm of microorganism are highly resistant to desiccation, heat, antibiotics and acidic condition[18]. Bacteria in the biofilm are approximately 10 to 1000 times less sensitive to antimicrobial agents than planktonic bacteria, on account of extracellular polymeric substances of the biofilm that prevent contact with antimicrobial agents[19,20]. This makes totally eliminate of biofilm in clinic, food industry and husbandry are scarcely possible[21].

Penicillin has been around since 1943, since then antibiotics play an important role in controlling bacterial infections and protecting human health. Nevertheless, with the aggravation of antibiotic abuse, widespread drug resistance problems, adverse consequences and serious harm have been caused. As early as 2003, statistics from the ministry of health, PRC showed that the annual death toll caused by the abuse of antibiotics in China was as high as 80,000[22].

Phages and their derivatives are ideal candidates for replacing or compensating for antibiotic problems in the future[23]. Phages are viruses that with bacterial lysis activity[24,25]. Due to the ability of kill bacteria, which appear to be a good alternative to antimicrobials and disinfectants[26]. Above all, phages infect only bacteria and not be harmful to humans, making them safe for apply to clinic and food products[27]. Recent study found that that bacteriophage has high efficiency in reducing and control bacterial biofilms on various surfaces formed by E. coli, Salmonella, Listeria
monocytogenes and Pseudomonas aeruginosa[28 – 32].

Narrow spectrum of lytic has been restricting the application of phages,

The aim of this study was to isolate and characterize the genomic characteristics of endonuclease, exonuclease and lysozyme among three different lytic spectrum phages strains which possess activity of potential biocontrol agent for biofilm of multidrug-resistant strains of E. coli. Afterwards, we have studied the E. coli phage Flora to reduce biofilm formation or control mature biofilms of E. coli. The significant differences of lysozyme and exonuclease were found among Flora, T4 and WJ strains. Our aim was to provide direction and theoretical basis for development and modification of broad-spectrum phages.

**Materials And Methods**

**Bacterial strains and growth conditions**

*Coli* was isolated from the patient in Yunnan first people's hospital in China, was used as host for isolation of phages. The host strain and phage host range determination strains were grown aerobically on LB plates or in LB broth (Difco, Detroit, MI, USA) and stirring incubated at 37 °C for 18 h. Soft top agar containing LB broth was prepared with 0.5% agar for phage plaque confirmation and LB agar plates were prepared with broth supplemented with 1.8% agar. All strains of *E. coli* stock cultures were stored at −80 °C in the LB broth (Difco, Detroit, MI, USA) containing 20% (v/v) glycerol.

**Bacteriophage isolation and purification**

*coli* targeting phages were isolated from laboratory and drug. The phages isolate method was modified as follows[33]. Briefly, 10 g of each sample was mixed with 20 mL sterile normal saline (0.9% NaCl) buffered in sterile 50 mL centrifuge tube and then shock for 2 h using incubator with 200 rpm at room temperature. Then, samples were centrifuged at 5000×g for 15 min and filtered using 0.22 um filter membrane. 10 mL of each filtering medium was added to 30 mL of LB broth containing the 1% of overnight culture of the host strain and then incubated for 48 h. After that, Cultures were centrifuged at 8 000×g for 15 min and the supernatant was filtered using 0.22 um filter membrane. The filtrate was diluted 10 times in series and mixing in 5 mL of molten 0.5% LB soft agar containing
E. coli (2×10^8 cfu/mL), and immediately add to LB plate that containing 1.8 agar. Overnight culture and plaque formation was observed. Single phage plaque was selected for phage purification and repeat three times.

The thermotolerance, optimum pH, optimum MOI, growth curve and transmission electron microscopy (TEM) of isolated phages

Thermotolerance

Take 1 mL phage pure cultures of titer for 1 × 10^7 pfu/mL, respectively set in 4 °C, 25 °C, 37 °C, 42 °C, 50 °C, 60 °C and 90 °C for 1 h. Following determination of phage titer at different temperature.

The experiment was repeated three times.

Optimum pH

Take 0.99 mL buffer liquid with pH of 3, 4, 5, 6, 7, 8, 9, 10 and 11 (Citrate buffer 50 mmol/L, pH 3, pH 4, pH 5; phosphate buffer 50 mmol/L, pH 6, pH 7, pH 8; Tris-HCl buffer 50 mmol/L, pH 9; Sodium carbonate buffer 50 mmol/L, pH 10, pH 11) in 1.5 mL sterile centrifuge tube, add phage pure cultures 0.01 mL of titer for 1 × 10^8 pfu/mL to each tube. Place at room temperature for 1 hour then determination of phage titer at different pH. The experiment was repeated three times.

Optimum MOI

Multiplicity of infection (MOI) was the ratio of the number of phages added to the number of host bacteria at the time of initial infection. According to the MOI of 0.0001, 0.001, 0.01, 0.1 1, 10 and 100 add phage pure culture solution and host bacteria suspension, then transfer to LB medium and shock culture at 37 °C for 8 hours. The cultures were centrifuged at 10 000×g for 15min at 4 °C, then the supernatant was filtered by 0.22 um filter to obtain the phage increment solution, finally the titer of phage increment solution was determined through double plate method. The experiment was repeated three times.
**Growth curve**

Added phage (1 × 10^7 pfu/mL) to LB culture that containing 1/250 seed fluid of host bacteria according the optimum MOI and string culture at 37 °C, intermittent sampling was used to determine the titer of the phage.

**Transmission electron microscopy**

The morphology of the phages particles was observed by transmission electron microscopy (TEM).

Briefly, each phage stock dilution (approximately 2×10^8 to 2×10^9 pfu/mL) was deposited on copper grids with carbon-coated Formvar films, stained with 2% uranyl-acetate (pH 4.0). Phage samples were imaged using a Philips EM 300 electron microscope, operated at 80 kV at the Jiangnan university (Wuxi, China). Phages were classified and identified referring to the International Committee on Taxonomy of Viruses[34].

**Bacteriophage genomic DNA extraction, sequencing and bioinformatics analysis**

**Bacteriophage genomic extraction and restriction enzyme digestion**

Firstly, phage was purified from concentrated to a high titer stock using 10 kd filter (about 10^9 to 10^10). Purified phages were treated with RNase (1U) and DNase (1U) at 37 °C for 1 h. Then, Takara minibest viral RNA/DNA Extraction kit (Cat#9766) was carried out to obtain purified phage genomic DNA. Restriction endonuclease Ecorl, Hind III, Not I and Xhol I were used for phage genome digestion, respectively.

**Sequencing and bioinformatics analysis**

Extracted phage genomic DNA was sequenced using a Illumina Hiseq (Sangon Biotech, China). The original sequencing data were evaluated by FastQC and assembled with SPAdes assembler software. The NCBI Blast compare with multiple databases of CDD, KOG, COG, NR, NT, PFAM, Swissprot and
TrEMBL were used for function annotation information of gene protein sequence.

**Host-range determination and characteristic of host**

**Host-range determination**

The host range of the phages Flora, T4 and WJ were determined by the spot test method[35]. The reference strains (ten strains of *E. coli* from clinical patients, *E. coli* DH5α and *E. coli* BL21) were tested for susceptibility of phage Flora, T4 and WJ. Generally, each of 200 uL reference strains (10^9 cfu/mL) was added to 5 mL of liquefied LB soft agar (LB broth with 0.5% agar), and poured over the LB 1.8% agar plate. Three minutes later, single drops of phage suspension were added and incubated at 37 ºC for 24 h.

**Antimicrobial susceptibility testing**

**Antibiotic sensitivity testing**

Antibiotic sensitivity of the Ten strains of *E. coli* from clinical patients, *E. coli* DH5α and *E. coli* BL21 were tested against seventeen antibiotics by minimal inhibitory concentration (MIC) method. The antimicrobials tested were Penicillin, Streptomycin, kanamycin sulfate, cefoxitin, ampicillin, ceftriaxone, gentamicin, ertapenem, aztreonam, amoxicillin, Ciprofloxacin, imipenem, levofloxacin, cefepime, macrodantin, amikacin.

**The different effects of phages and antibiotics on biofilms**

**Scanning electron microscopy**

Make first-phase preparations, A 48 - well cell slide was placed into a 24 - well plate. Seed solution was inoculated into 100 mL LB culture solution at the rate of 4‰. Inoculate 1 mL of bacterial solution into 24-well plate. One group, added phages, antibiotics, and mixtures of antibiotics and phages, respectively, nothing added as a control (the addition amount of phage was MOI=1, The final concentration of kanamycin sulfate was 10 ug/ml), Incubation (37 ºC, 24 h). The other group, firstly culture for 12 hours, after that, added phages, antibiotics, and mixtures of antibiotics and phages,
respectively, nothing added as a control (The addition amount of phage was MOI=1, the final concentration of kanamycin sulfate was 10 ug/ml), Incubation (37 °C, 12 h). The cfu of each sample was measured by plate counting method. Following the recovered culture washed twice with PBS buffer; and fixed with 2.5% pre-cooling glutaraldehyde at room temperature for 3 h in dark place. Wash twice with PBS buffer, then dehydrated in an increasing ethanolic gradient (15%, 30%, 50%, 70%, 100% v/v), for 10 min at each step. Afterward, dry overnight and gilt, the results obtained through scanning electron microscope with an accelerating voltage 20 kV.

**Microplate reader detected the ability of biofilm formation**

coli seed solution was inoculated in LB for the proportion 4‰ and overnight culture. Then 200 times dilution with LB and added to 96-well plate (200 ul/hole), each sample has three multiple holes. One group, added phages, antibiotics (kanamycin sulfate (10 ug/mL) ), and mixtures of antibiotics and phages, respectively, nothing added as a control (The addition amount of phage was MOI= 0.1, the final concentration of kanamycin sulfate was 10 um/ml), incubation (37 °C, 24 h). The other group, firstly culture for 12 hours, after that, added phages, antibiotics, and mixtures of antibiotics and phages, respectively, nothing added as a control (The addition amount of phage was MOI= 0.1, the final concentration of kanamycin sulfate was 10 ug/ml), incubation (37 °C, 12 h). The bacterial population density (OD\textsubscript{600} nm) was measured using a ELIASA (Thermo Scientific, EUA) and discarded bacteria solution. The wells washed twice with PBS to remove unattached cells, repeated three times, added 99% methanol and fix for 15 min, then discard methanol and dry at room temperature, following added 2% crystal violet and stain for 8 min. Rinse the culture plate with running water until the water is colorless. After drying, measured the absorption light at 570 nm wavelength with a microplate reader. The experiment was repeated three times.

Results

**3.1 Characteristics and morphology of isolated phages**

A total of three virulent *Escherichia coli* phages T4, WJ and MYP were isolated from the laboratory and drug sample. The plaqu of phages T4, WJ and MYP were appeared 4 mm in diameter after overnight
incubation at 37°C (Fig. 1).

Negatively stained of purified *E. coli* phages T4, Flora and WJ were observed with an electron microscope. Transmission electron microscopy (TEM) revealed phages T4, Flora and WJ virions with an icosahedral head 60 ± 2 nm in diameter, and a contractile tail 150 ± 5 nm long (Fig. 2). The morphology of phage T4, WJ and Flora indicated they belonged to the *Myoviridae* family.

A growth curve of the phages T4, Flora and WJ were obtained by inoculation on *E. coli* BL21 according to MOI of 0.1 at 37°C (Fig. 3). The latent period for the phages T4, Flora and WJ were 55 min. The titers of phages phages T4, Flora and WJ were reached peaks very quickly in 20 hours, then slowly decrease. The amplification factor of phages T4, Flora and WJ were approximately 2000 times.

### 3.2 Optimum temperature, pH and MOI of isolated phages

Phages T4, Flora and WJ have the highest activity after treatment for 1 h at 42°C, then there was a noticeable decline at 60 °C and complete inactivation until 90 °C (Fig. 4). The result show that phages T4, Flora and WJ have low temperature adaptability and which consistent with the optimum survival temperature of it’s host.

Phages T4, Flora and WJ have the most plaque at pH=7, furthermore, T4, Flora and WJ still have high activity at pH=3 and pH=11, (Fig. 4). These results indicated that the phages T4, Flora and WJ have good tolerance to alkali and acids.

Multiplicity of infection (MOI) refers the ratio of the number of phages to cells. The optimum MOI of phages T4, Flora and WJ were 0.0001, among them, the plaque of T4, Flora and WJ were decreased significantly after 0.0001 and reach minimum at MOI=100(Fig. 4).

### 3.3 DNA extractionrestriction endonuclease digestion and genome analysis
The genome size of phages T4, Flora and WJ were 161,903, 161,909 and 161, 900 bp respectively. We identified 263 protein-coding genes (open reading frames (ORFs) for Flora and 153 protein-coding genes for WJ. T4 DNA was digested by Hind III and Xho I, WJ DNA was digested by EcoRI, nevertheless, Flora DNA was can't be digested by EcoRI, Hind III, NotI and Xho I. This is consistent with the results of phage genomic analysis of that phage Flora has more and significant difference endonuclease and exonuclease sites. Genome analysis revealed that phages T4, Flora and WJ are virulent phages (Fig. 5,6).

3.4 Phages bacteriocidal spectrum, host resistance and phylogenetic analysis the genes of 16S, host biofilm and crispr

Antimicrobial susceptibility

The E. coli were isolated from clinical patients in the laboratory of first people's hospital of Yunnan province. Unfortunately, they have a broad spectrum of resistance (Table 1), but fortunately, most of them can be removed by the phages that we have isolated (Table 2). They are all possess resistant to Penicillin, Streptomycin, kanamycin sulfate, ertapenem, amoxicillin, imipenem, cefepime, macrodantin and amikacin, but sensitive to ampicillin and ceftriaxone.

| Antibiotic             | A | B | C | D | E | F | G | H | I | J | DH5α | BL21 |
|------------------------|---|---|---|---|---|---|---|---|---|---|------|------|
| Penicillin             | S | S | S | S | S | S | S | S | S | S | S    | S    |
| Streptomycin           | S | S | S | S | S | S | S | S | S | S | S    | S    |
| kanamycin sulfate      | S | S | S | S | S | S | S | S | S | S | S    | S    |
| cefoxitin              | S | S | S | S | S | S | S | S | S | S | S    | S    |
| ampicillin             | R | R | R | R | R | R | R | R | R | R | R    | R    |
| gentamicin             | R | S | R | R | S | S | S | S | S | S | R    | R    |
| aztreonam              | R | S | R | R | S | S | S | S | S | S | R    | R    |
| Ciprofloxacin          | R | R | R | R | S | S | S | S | S | S | R    | R    |
| levofloxacin           | R | R | R | R | S | S | S | S | S | S | R    | R    |
| macrodantin            | S | S | S | S | S | S | S | S | S | S | S    | S    |
| amikacin               | S | S | S | S | S | S | S | S | S | S | S    | S    |
| cefepime               | S | S | R | R | S | S | S | S | S | S | S    | S    |
| imipenem               | S | S | R | R | S | S | S | S | S | S | S    | S    |
| amoxicillin            | R | S | S | S | S | S | S | S | S | S | S    | S    |
| ertapenem              | S | S | S | S | S | S | S | S | S | S | S    | S    |
| ceftriaxone            | R | S | R | R | R | R | R | R | R | R | R    | R    |

Note: S, sensitive
R, resistive
The lytic phages of *E. coli* Flora was able to infect three or more *E. coli* strains which were isolated from Clinical patients at The First People Hospital of Yunnan Province, China (Table 2). The *E. coli* exhibited sensitivity to T4, Flora and WJ. This analysis underlined the wide host range of the isolated phage Flora.

**Table 2. Host range analysis of phages T4, Flora and WJ.**

| Strain                  | T4 | Flora | WJ |
|-------------------------|----|-------|----|
| *Escherichia coli*-A    | +  | +     | +  |
| *Escherichia coli*-B    | -  | +     | -  |
| *Escherichia coli*-C    | -  | -     | -  |
| *Escherichia coli*-D    | -  | -     | -  |
| *Escherichia coli*-E    | -  | +     | -  |
| *Escherichia coli*-F    | -  | +     | -  |
| *Escherichia coli*-G    | -  | -     | -  |
| *Escherichia coli*-H    | -  | -     | -  |
| *Escherichia coli*-I    | -  | -     | -  |
| *Escherichia coli*-DH5α | +  | -     | -  |
| *Escherichia coli*-BL21 | +  | +     | +  |

### 3.5 Compare the effects of phages and antibiotics on host biofilms

SEM was used to assess *E.coli* biofilm formation on round coverslip that was affected by Flora (MOI=0.1) and kanamycin sulfate (10 ug/mL). Under the condition of *E.coli* inoculation at a rate of 4‰, added Flora (MOI=0.1) and kanamycin sulfate (10 ug/mL) in immediately and culture for 24 hours, antibiotics have better sterilization effect than phages whether in the case of scanning electron micrograph, OD$_{600}$ of bacterial culture solution, or microplate reader OD$_{570}$ of bacterial biofilm (Fig. 7, 8, 9). Nevertheless, under the condition of *E.coli* inoculation at a rate of 4‰ and culture for 12 hours, then Flora (MOI=0.1) and kanamycin sulfate (10 ug/mL) were added and cultured for 12 hours, phages have better sterilization effect than antibiotics whether in the case of scanning electron micrograph, OD$_{600}$ of bacterial culture solution, or microplate reader OD$_{570}$ of bacterial biofilm (Fig. 7, 8, 9). In addition, under the condition of *E.coli* inoculation at a rate of 4‰ and culture for 12 hours, then Flora (MOI=0.1) and kanamycin sulfate (10 ug/mL) were added and cultured for 12 hours, collaboration of phages and antibiotics have better sterilization effect than alone of bacteriophages or antibiotics whether in the case of scanning electron micrograph, OD$_{600}$ of bacterial culture solution, or microplate reader OD$_{570}$ of bacterial biofilm (Fig. 7, 8, 9).
The result of host colony-forming unit indicate that phage and antibiotics have their own ways of killing bacteria (Fig. 9). Meanwhile, under the condition of *E. coli* inoculation at a rate of 4‰ and culture for 12 hours, then Flora (MOI=0.1) and kanamycin sulfate (10 ug/mL) were added and cultured for 12 hours, bacteriophages have better sterilization effect than antibiotics (Fig. 9).

**Discussion**

*E. coli* is the most important and widespread bacteria in worldwide, which mainly found in contaminated food, human and animal faeces. Unfortunately, Some of *E. coli* strains are multidrug-resistant (MDR) pathogen leading significant public health concern globally[36-39]. The *E. coli* strains used in this study were isolated from clinical patients in the first people's hospital of Yunnan province and all of them were belonged to multidrug-resistant strains (table 2). With the rise of antibiotic abuse, multiple resistant bacteria and superbacteria hence a public health hazard. Alternatives to antibiotics are urgently needed, phage, are dawn of this increasing drug resistance. The isolated *E. coli* phage Flora was ideal substitute for antibiotics for they strong lyase performance. What's more, phage have better sterilization effect than antibiotics in a high concentration of bacteria, and that, collaboration of phages and antibiotics have better sterilization effect than alone of phages or antibiotics in a low concentration medium of bacteria (fig. 7,8)[40].

The isolated *E. coli* phages Flora and WJ belongs to *Myoviridae* family and the genome sizes was 168, 909 and 168900 bp. Corresponding, the genome sizes of *E. coli* phage phiLLS was 107, 263 bp and phage vB_EcoM-Ro111lw, vB_EcoS-Ro145lw and vB_EcoM-Ro157lw were 42kb to 149kb , the consistent part was they all belong to the *Myoviridae* family[37,41]. The genomic characteristics of *E. coli* phages Flora, T4 and WJ genome reveal that Flora has a special endonucleas and possess one more unique exonuclease. The extra part of endonuclease and exonuclease maybe the reason for its wider lytic spectrum of Flora. Another reason for wider lytic spectrum of Flora could be the greater ability of resistance to restriction enzymes than T4 and WJ.

The ability of microorganism to form biofilms on different food surfaces increases the risk of cross-contamination, particularly in poultry products, which was a serious problem for food industries, clinic
and public health[42-44]. Although the significant problems in pathogen control caused by biofilms, exploiting effective eliminate of biofilms is still challenging[45]. Until now, there is no ideal technology of biofilm control, hence, the new control strategies for biofilm are constantly recommended[46]. In this study, we demonstrated that the phage Flora has better properties than antibiotics to reduced biofilm formation of \textit{E. coli} (fig. 7,8).

The result shown that phage Flora and kanamycin sulfate can infect \textit{E. coli} biofilm and has the potential to reduce tested \textit{E. coli} strains. Antibiotics have better anti-biofilm effect than phages in a low concentration medium of bacteria (Fig. 7,8). Nonetheless, phages have better anti-biofilm effect than antibiotics in a high concentration of bacteria (fig. 7,8). The data of this study provided the strong evidence that the application of phage could reduce the growth and biofilm of \textit{E. coli} that are important to maintain public health.

Conclusion
In conclusion, firstly, we have isolated and characterized of lytic \textit{E. coli} phages and acquired its biological properties, then, we have found that combination use of phages and antibiotics possess significantly better anti-biofilm and bactericidal properties on host bacteria than either antibiotics or phages alone. The data of this study provided the strong evidence that the application of phage could reduce the growth and biofilm of \textit{E. coli} that are important to maintain public health.

Declarations

\textbf{Abbreviation}

S: sensitive; R: resistive; cfu: colony-forming unit; pfu: plaque forming unit; MOI: Multiplicity of Infection

\textbf{Acknowledgements}

Not applicable

\textbf{Authors’ contributions}
Conceived and designed the experiments: LMJ. Performed the experiments: LMJ. Analyzed the data: LMJ. Contributed reagents/materials/analysis tools: RZ LMJ. Wrote the paper: LMJ. Both authors read and approved the final manuscript.

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**Ethics Statement**

Ethical approval was obtained from the Institutional Ethics Committee (The First People’s Hospital of Yunnan Province, Kunming, Yunnan, China). The study protocol was in accordance with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000). Written informed consent was received from each patient before sample collection.

**Availability of data and material**

Please contact author for data requests.

**Consent for publication**

Not applicable.

**Conflicts of Interest**

The authors declare no conflict of interest.
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Figures
Figure 1

Plaques formed by phages E. coli phages T4, WJ and Flora, respectively, the host strains of E. coli BL21 after an overnight incubation at 37°C.

Figure 2

Morphological features of Escherichia coli phages T4, Flora and WJ by transmission electron microscopy (TEM).
Population dynamics of phages T4, Flora and WJ inoculate in Escherichia coli BL21.

Optimum temperature, pH and MOI of isolated phages T4, Flora and WJ.
Restriction enzyme digests of phages T4, Flora and WJ. T4 DNA was digested by Hind III and Xhol I (lane 7 and 9), WJ DNA was digested by EcoRI (lane 16), Flora DNA was can't be digested by EcoRI, Hind III, NotI and Xhol I (lane 11, 12, 13 and 14).

Line map of the phage Flora genome. In the Flora track, genes colored red instructions for lysozyme, genes colored purple instructions for endonuclease, genes colored yellow instructions for tail structure gene. The arrows represent the ORFs and point the direction of transcription.
Figure 7

Scanning electron micrograph (SEM) of E.coli colonization before and after phage Flora (MOI=0.1) and kanamycin sulfate (10 ug/mL) application to biofilms formed on round coverslip. (A) E.coli inoculation at a rate of 4‰ and culture for 24 hours, (B) A added phage Flora (MOI=0.1), (C) A added kanamycin sulfate (10 ug/mL). (D) E.coli inoculation at a rate of 4‰ and culture for 12 hours, then phage Flora (MOI=0.1) was added and cultured for 12 hours, (E) E.coli inoculation at a rate of 4‰ and culture for 12 hours, then kanamycin sulfate (10 ug/mL) was added and cultured for 12 hours, (F) E.coli inoculation at a rate of 4‰ and culture for 12 hours, then phage Flora (MOI=0.1) and kanamycin sulfate (10 ug/mL) were added and cultured for 12 hours. (5,000× magnification)
Figure 8

Effects of phages and kanamycin sulfate (10 ug/mL) on biofilms. (A,B) Effects of phage SHI and kanamycin sulfate (10 ug/mL) on E. coli (inoculation at a rate of 4‰) growth that culture for 12 h and 24 h (OD600). (E, F) Effects of phage Flora and kanamycin sulfate (10 ug/mL) on E. coli (inoculation at a rate of 4‰) biofilm that culture for 12 h and 24 h (OD570). (C, D) Effects of phage Flora and kanamycin sulfate (10 ug/mL) on E. coli (inoculation at a rate of 4‰) growth, first culture for 12 hours, then phage Flora and kanamycin sulfate (10 ug/mL) were added and cultured for 12 h and 24 h (OD600). (G,H) Effects of phage Flora and kanamycin sulfate (10 ug/mL) on E. coli (inoculation at a rate of 4‰) biofilm, first culture for 12 hours, then phage Flora and kanamycin sulfate (10 ug/mL) were added and cultured for 12 h and 24 h (OD570).
Effects of phage and kanamycin sulfate (10 ug/mL) on colony-forming unit of E. coli. (A) Effects of phage Flora and kanamycin sulfate (10 ug/mL) on E. coli (inoculation at a rate of 4‰) that culture for 24 h. (B) Effects of phage Flora and kanamycin sulfate (10 ug/mL) on E. coli (inoculation at a rate of 4‰), first culture for 12 hours, then phages Flora and kanamycin sulfate (10 ug/mL) were added and cultured for 12 h.