Isolation and characterization of *Salmonella* paratyphi phage and its lytic spectrum that correlation with pili

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Abstract

Background

Prevention and control of *Salmonella* biofilm have great importance for economic point and medical. Phages and their derivatives are ideal candidates for replacing or compensating of antibiotic problems in the future.

Results

In this study, the phage KM16 was isolated from slaughterhouse sump samples. It belonged to the *Myoviridae* family and optimal growth temperature was 42 °C, the pH of optimal preservation buffer was 6 ~ 7, optimal multiplicity of infection (MOI) was 0.0001 and the genome size was 170,126 bp. The phage KM16 has the ability to lytic most clinical strains of *Salmonella paratyphi* A and *Salmonella paratyphi* B. Phylogenetic analysis found that the 16S rRNA, *crispr 1* and *fimA* genes of *Salmonella paratyphi* have a high similarity and correlation with lytic spectrum of phage KM16, but not correlated with the genes of *invA*, *isrK* and *luxS*. Above all, the lytic spectrum of phage KM16 correlation with tertiary structure of *Salmonella* pili, the pili of *Salmonella* was the recognition site for phage adsorption. Collaboration of phage KM16 and antibiotics have better anti-biofilm effect than alone of phage or antibiotics in low concentration of bacteria culture and phage have better anti-biofilm effect than antibiotics in a high concentration of bacteria culture.

Conclusions

The data of this study provided a new perspective to understand the relationship between phage lytic spectrum and difference of host strains.

Background

Salmonella as the most serious foodborne pathogens among worldwide and it was distributing widely over natural environment, in addition, it was widely existed on all kinds of food and food raw materials [1]. *Salmonella* mainly lodges in common domestic animals, and humans are also one of its main hosts. It's a serious zoonosis original microbes, which not only cause disease in livestock, poultry animals, rats and mice, but also cause of food poisoning in human [2]. Majowicz and Deng have reported there are 94 million gastroenteritis cases and 155, 000 people deaths caused by *Salmonella* in the world every year [3, 4].

Biofilm was a multicellular community of microorganisms where microorganisms are embedded self-produced extracellular matrix and attached to highly hydrated extracellular matrixon, non-biological and biological surfaces [5–7]. 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 [8, 9]. Biofilm of microorganism are highly resistant to desiccation, heat, antibiotics and acidic condition [10]. 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 [11, 12]. This makes totally eliminate of biofilm in clinic, food industry and husbandry are scarcely possible [13].

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. The food industry and clinic faces a serious *Salmonella* contamination problem, what is more exacerbated by the overuse of antibiotics, resulting in an increasing number of antibiotic-resistant foodborne and clinic *Salmonella*. Efforts to develop new ways to control *Salmonella* contamination in food and its processing environment are important. The applications of antibiotics and disinfectants at stationary phases to eradicate *Salmonella* biofilms could be have adverse effects on human health [14].

Phages and their derivatives can be used as a novel, feasible, and safe biological product for the prevention, treatment, and elimination of *Salmonella* in food, clinic and food processing environments. Phages and their derivatives are ideal candidates for replacing or compensating for antibiotic problems of the future [15]. Phages are viruses that with bacterial lysis activity [16, 17]. Due to the ability of kill bacteria, which appear to be a good alternative to antimicrobials and disinfectants [18]. Above all, phages infect only bacteria and not be harmful to humans, making them safe for apply to clinic and food products [19]. Recent study found that that phage has high efficiency in reducing and control bacterial biofilms on various surfaces formed by *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* [20–24].

Prevention and control of contamination caused by multidrug-resistance (MDR) bacteria of *Salmonella* has great importance for an economic point of view and medical [25]. The main challenges for such a phage therapy of the relatively narrow lytic range against bacterial strain [26]. Here we report a new isolated lytic phages named KM16 have better anti-biofilm effect than antibiotics in high concentration of bacteria. In addition, phylogenetic analysis found that the lytic spectrum of phage KM16 correlation with tertiary structure of *Salmonella* pili, the pili of *Salmonella* was the recognition site for phage adsorption.

Results

Characteristics and morphology of isolated phages

Virulent phage KM16 was isolated from the samples of slaughterhouse sump that according to the host of *Salmonella paratyphi* NA3. The plaque of phage KM16 was appeared 1 mm in diameter after overnight incubation at 37°C (**Figure 1**).

Negatively stained of purified phage KM16 was observed with an electron microscope. Transmission electron microscopy (TEM) revealed KM16 virions with an icosahedral head 110 ± 5 nm in diameter, and a non contractile tail 110 ± 5 nm long (**Figure 2**). The morphology of phage KM16 indicated they belonged to the *Myoviridae* family.

After standing for 5 min at 15 °C, nearly 85% of phage particles were adsorbed to the host bacteria, after incubation for 20 min, almost all phages were adsorbed to host bacteria (**Figure 3**). A growth curve of the phage KM16 was obtained by inoculation on *Salmonella paratyphi* A NA3 according to MOI of 0.1 at 37°C (Fig. 3). The latent period of phage KM16 was 60 min. The titer of phage KM16 were explosive growth before 170 min and reached peak at 9 h, and then it's appear going down at 15 h later. The amplification factor of phage KM16 was approximately 200 times.

Optimum temperature, pH and MOI of isolated phages

The phage titer of KM16 was measured by the double plate method at different temperature after 1 h of processed. KM16 have the highest activity after treatment for 1 h at 42°C, then there was a noticeable decline at 50 °C and complete inactivation until 90 °C (**Figure 4**). The result show that phage KM16 have low temperature adaptability and which consistent with the optimum survival temperature of it's host.

Phage KM16 have the most plaque at pH=6~7, further, plaque at pH=10 ~11 were significantly higher than that at pH3~4 (**Figure 4**). These results indicated that the phage KM16 have good tolerance to alkali but extremely intolerant to acids.

Multiplicity of infection (MOI) refers the ratio of the number of phages to cells. The optimum MOI of phage KM16 were 0.0001, among them, the plaque of KM16 were decreased significantly after 0.001 and reach minimum at MOI=100 (Fig. 4).

DNA extraction—restriction endonuclease digestion and genome analysis

The genome size of phage KM16 is 170,126 bp. We have identified 278 protein-coding genes for KM16 genome (Table S1). KM16 genome DNA was digested by *EcoRI*, *Hind III*, *NotI* and *XhoI*. This is consistent with the results of phage genome analysis of that phage KM16 has more endonuclease sites. Genome analysis revealed that phage KM16 was virulent phages (**Figure 5,6**).

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

Antimicrobial susceptibility

The *Salmonella paratyphi* A and *Salmonella paratyphi* B 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 phage of KM16 (Table 2). They

are all possess resistant to penicillin, streptomycin, cefoxitin, gentamicin, ciprofloxacin and tobramycin, but sensitive to kanamycin sulfate and macrodantin.

Table 1. Antibiotic resistance of *Salmonella* isolates used in this study

Antibiotic	<i>Salmonella paratyphi</i> A								<i>Salmonella paratyphi</i> B			
	A	B	C	D	E	F	JL1	JJ9	NA5	NA3	G	H
Penicillin	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	R	R	R	R	R	R	R	R	R	R	R	R
kanamycin sulfate	S	S	S	S	S	S	S	S	S	S	S	S
cefoxitin	R	R	R	R	R	R	R	R	R	R	R	R
ampicillin	S	S	S	S	S	R	S	S	S	S	S	R
gentamicin	R	R	R	R	R	R	R	R	R	R	R	R
aztreonam	S	S	S	S	S	S	S	S	S	S	S	R
ceftriaxone	S	S	S	S	S	S	S	S	S	S	S	R
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	R	R
levofloxacin	S	S	S	S	S	S	S	S	S	S	S	S
tobramycin	R	R	R	R	R	R	R	R	R	R	R	R
macrodantin	S	S	S	S	S	S	S	S	S	S	S	S

The lytic phage of *Salmonella paratyph* KM16 was able to infect most strains of *Salmonella paratyphi* which were isolated from The First People Hospital of Yunnan Province, China (Table 2). The *Salmonella paratyphi* A JJ9 and *Salmonella paratyphi* A NA3 exhibited sensitivity to KM16. This analysis underlined the wide host range of the isolated phage KM16.

Table 2. Host range analysis of phage KM16.

Strain	KM16
<i>Salmonella paratyphi</i> A-A	+
<i>Salmonella paratyphi</i> A-B	+
<i>Salmonella paratyphi</i> A-C	+
<i>Salmonella paratyphi</i> A-D	+
<i>Salmonella paratyphi</i> A-E	+
<i>Salmonella paratyphi</i> A-F	+
<i>Salmonella paratyphi</i> A-JL1	+
<i>Salmonella paratyphi</i> A-JJ9	+
<i>Salmonella paratyphi</i> A-NA5	+
<i>Salmonella paratyphi</i> A-NA3	+
<i>Salmonella paratyphi</i> B-G	-
<i>Salmonella paratyphi</i> B-H	-

Phylogenetic analysis.

The genes of 16S rRNA (bacterial systematics), *crispr* 1 (Clustered regularly interspaced short palindromic repeats, an immune weapon produced by bacteria and phages fighting each othe), *fimA* (*Salmonella* pili), *invA* (Virulence genes), *isrK* (Quorum sensing gene) and *luxS* (Quorum sensing gene) of *Salmonella paratyphi* were aligned for phylogenetic analysis. Phylogenetic tree analysis indicated that genes of 16S, *crispr* 1 and *fimA* of *Salmonella paratyphi* have a high similarity and correlation with lytic spectrum of phage KM16, but not correlated with genes of *invA*, *isrK* and *luxS*. The results indicate that the relatedness, immune system and pili of host were corresponded with the lytic spectrum of phage,

nevertheless, the virulence, and quorum sensing of host were not corresponded with the lytic spectrum of phage (Figure 7).

Structure of *Salmonella* fimA (pili) protein

The pili of *Salmonella* was the recognition site for phage adsorption. Comparison of *Salmonella* fimA among *Salmonella paratyphi* A-A, *Salmonella paratyphi* A-NA3 and *Salmonella paratyphi* B-H using SWISS-MODEL (<https://swissmodel.expasy.org/>) for tertiary structure prediction. SWISS-MODEL searches with other *Salmonella* fimA with experimentally solved tertiary structure revealed a significant match (<https://www.rcsb.org/structure/6erj>) (Figure 8). There were an significant differences tertiary structure of *Salmonella pili* among phage KM16 sensitive and resistant strains (Figure 8).

Compare the effects of phages and antibiotics on host biofilms

The effects of KM16 (MOI=0.1) and kanamycin sulfate (10 µg/mL) on round coverslip of *Salmonella paratyphi* A NA3 biofilm were assessed through Scanning electron micrograph (SEM). In this case of *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250, added KM16 (MOI=0.1) and kanamycin sulfate (10 µg/mL) in immediately and culture for 24 h, antibiotics have better sterilization effect than phages whether in the results of scanning electron micrograph, OD₆₀₀ of bacterial culture solution, or microplate reader OD₅₇₀ of bacterial biofilm (Fig. 9, 10).

Nevertheless, In another case of *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250 and culture for 12 h later, then KM16 (MOI=0.1) and kanamycin sulfate (10 µg/mL) were added and cultured for 12 h, phages have better sterilization effect than antibiotics whether in the results of scanning electron micrograph, OD₆₀₀ of bacterial culture solution, or microplate reader OD₅₇₀ of bacterial biofilm (Fig. 9, 10). In addition, in case of of *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250 and culture for 12 h later, then KM16 (MOI=0.1) and kanamycin sulfate (10 µg/mL) were added and cultured for 12 h, collaboration of phage and antibiotics have better sterilization effect than alone of phages or antibiotics whether in the results of scanning electron micrograph, OD₆₀₀ of bacterial culture solution, or microplate reader OD₅₇₀ of bacterial biofilm (Figure 9,10).

The result of host colony-forming unit indicate collaboration of phage and antibiotics have better sterilization effect than alone of phage or antibiotics (Figure 11). Meanwhile, under the case of *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250 and culture for 12 h later, then KM16 (MOI=0.1) and kanamycin sulfate (10 µg/mL) were added and cultured for 12 h, phages have better sterilization effect than antibiotics (Figure 11).

Discussion

Salmonella is a common foodborne pathogen, which mainly found in contaminated food and cause severe medical problems and foodborne diseases [27, 28]. The *Salmonella paratyphi* A NA3 used in this study was isolated from a clinical patient in the first people's hospital of Yunnan province, China who

accidentally ate *Salmonella* contaminated bread. It's regrettable of that overuse of antibiotics has increased the severity of *salmonella* [29]. The main reasons of drug resistance was that antibiotic resistance (AR) and multidrug-resistance (MDR) genes were in the genome of almost all *Salmonella* strains [30].

The isolated *Salmonella* phage KM16 belongs to *Myoviridae* and the genome sizes was 170, 126 bp. Corresponding, the genome sizes of *Salmonella* phage Φ Stp1 was 112,149 bp and *Salmonella* phages SPFM was 233 to 242 Kb, the consistent part was they all belong to the *Myoviridae* family [31, 32].

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 *Salmonella* phages KM16 were ideal substitute for antibiotics for they can lysin multidrug-resistant bacteria. What's more, phages 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. 9,10,11).

Phylogenetically of *Salmonella paratyphi fimA* reveal that has a high similarity and correlation with lytic spectrum of phage KM16. Similar results were found that tail fiber adhesion features was a rare polyglycine rich domain for host recognition of *Salmonella* phage S16 [33]. Furthermore, the deficient outer core of lipopolysaccharides was proposed for *Escherichia coli* O157:H7 KIT03 recognise and infect host bacteria [34].

The ability of *Salmonella* 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 and public health [35–37]. Although the significant problems in pathogen control caused by biofilms, exploiting effective eliminate of biofilms is still challenging [38]. Until now, there is no ideal technology of biofilm control, hence, the new control strategies for biofilm are constantly recommended [39]. In this study, we demonstrated that the phage KM16 and kanamycin sulfate reduced biofilm formation of *Salmonella*.

The phage cleavage spectrum was the bottleneck of phage wide application. At present, some studies have reported that the phage cleavage spectrum is related to the phage itself and the host. For phage, Wu., et al. have found that the endolysin of *Acinetobacter baumannii* PD-6A3 with activity of extended lytic [40], Sergueev., et al. have reported that a distinct allele in hypervariable complex repeat structure in the Sb-1 genome correlated with phage lytic spectrum [41], Olszak., et al. have found that the jumbo phage in the bacterial population correlates with reduced *Pseudomonas aeruginosa* virulence [42]. For the host, Benešík., et al. have found that the binding spectrum of SH3b domain of Kayvirus endolysin LysF1 was a promising feature for creating new chimeolysins through combining it with more effective catalytic domains [43], Hoai., et al. have reported that the phage cleavage spectrum of *Lactococcus garvieae* affected by bacterial capsule and phage [44].

The result shown that phage KM16 and kanamycin sulfate can infect *Salmonella* biofilm and has the potential to reduce tested *Salmonella paratyphi* A NA3 strains. Antibiotics have better anti-biofilm effect than phages in a low concentration medium of bacteria (Fig. 9,10,11). Nonetheless, phages have better anti-biofilm effect than antibiotics in a high concentration of bacteria, and that, collaboration of phages and antibiotics have better anti-biofilm effect than alone of phages or antibiotics in a low concentration medium of bacteria (Fig. 9,10,11). The data of this study provided the strong evidence that the application of phage could reduce the growth and biofilm of *Salmonella* that are important to maintain public health.

Conclusions

In this work, we have found the host specific lytic effect of *Salmonella paratyphi* phage correlation with tertiary structure of *Salmonella* pili, which this research provided a new perspective to understand the relationship between phage lytic spectrum and difference of host strains, this is important in field of phage lytic spectrum knowledge. Collaboration of phage KM16 and kanamycin sulfate have better anti-biofilm effect than alone of KM16 and kanamycin sulfate in low concentration of bacterial culture, KM16 has better anti-biofilm effect than kanamycin sulfate in high concentration of bacterial culture. The data of this study provided a strong evidence of application phage to reduce the growth of *Salmonella* biofilm of which were important for public health.

Methods

Bacterial strains and growth conditions

Salmonella paratyphi A NA3 was isolated from the patient in Yunnan first people's hospital, China, and it was used as host for phage isolation. The host strains were grown aerobically on LB plates or in LB broth (Difco, Detroit, MI, USA) that incubated at 37 °C. Soft top agar containing LB broth was prepared with 0.5% agar (M/M) for phage plaque confirmation and LB agar plates were prepared with broth that supplemented with 1.8% agar (M/M). All strains of *Salmonella* stock cultures were stored at -80 °C of LB broth (Difco, Detroit, MI, USA) which containing 20% (V/V) glycerol.

Phage isolation and purification

Phages were isolated from residential sewage sump and pig slaughterhouse sump samples collected from Kunming city, China in September 2018. The method of isolate phages was according to the following methods with brief modified [45]. Briefly, 10 g of each sample was mixed with 20 mL sterile normal saline (0.9% NaCl) buffered in 50 mL sterile centrifuge tube and then shake 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 µm 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 µm filter membrane.

The filtrate was diluted 10 times in series and mixing in 5 mL of molten 0.5% LB soft agar containing *Salmonella* (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

The phage stock was diluted to 1×10^8 pfu/mL with LB broth. Placed of 1 mL diluted phage in temperature controller of 4 °C, 25 °C, 37 °C, 42 °C, 50 °C, 60 °C and 90 °C for 1 h, respectively. Following determination of phage titer at different temperature. The experiment was repeated three times.

Optimum pH

The phage stock was diluted to 1×10^8 pfu/mL with LB broth. 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, added 0.01 mL diluted phage for titer of 1×10^8 pfu/mL to each tube. Place at room temperature for 1 h then determination of titer of phage for different pH buffer. 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 added phage stocks that diluted by LB broth into 10-fold series. Aliquots of each phage stocks were mixed with *Salmonella paratyphi* A NA3 cultures at 10^8 cfu/mL to each ratio and were shocked culture at 37 °C for 8 h. The cultures were centrifuged at $10\,000 \times g$ for 15 min at 4 °C, then the supernatant was filtered by 0.22 µm 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.

Adsorption rate and growth curve

For adsorption rate measure, co-culture of phage KM16 (1×10^8 pfu/mL) and logarithmic phase host bacterial suspension at the MOI of 0.1, mixing uniformly and adsorbed at 15 °C, sampling 100 µL at 0, 2, 4, 6, 8, 10, 15, 20 and 30 min, respectively. The supernatant was taken after centrifugation of $12\,000 g$ for 5 min. The titer of phage was determined by double - plate method. For growth curve measure, added phage KM16 of 1×10^8 pfu/mL to LB culture containing 1/250 seed fluid of host bacteria according the optimum MOI and shake 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 analyzed by transmission electron microscopy (TEM). Briefly, 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 [46].

Phage genomic DNA extraction, sequencing and bioinformatics analysis

Phage genomic extraction and restriction enzyme digestion

Firstly, phage was purified from concentrated to a high titer stock using 10 kDa filter (about 10^9 to 10^{10}). Purified phages were treated with RNase and DNase 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 *EcoRI*, *Hind III*, *Not I* and *Xho 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 phage KM16 were determined by the spot test method [47]. The isolated strains were tested for susceptibility of phage KM16 (ten strains of *Salmonella paratyphi* A and two strains of *Salmonella paratyphi* B). 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 °C for 24 h.

Antimicrobial susceptibility testing

Antibiotic sensitivity testing

Antibiotic sensitivity of the *Salmonella paratyphi* A and *Salmonella paratyphi* B were tested against seventeen antibiotics by minimal inhibitory concentration (MIC) method. The antimicrobials tested were: imipenem (IMI), ceftiofloxacin (FOX), gentamicin (GM), amoxicillin (AMC), ertapenem (ETP), ceftriaxone (CTR), piperacillin (TZP), ampicillin (AMP), amikacin (AN), cefepime (FEP), tobramycin (TOB), aztreonam (AZM), selectrin (SXT), macrodantin (FTN), tigecycline (TGC), ciprofloxacin (CIP) and levofloxacin (LEV).

Phylogenetic and genetically specific analysis of host

The genome of host *Salmonella paratyphi* were extracted by Takara minibest bacteria genomic DNA extraction kit. Then sequencing of 16S gene was performed with 9F upstream and 1512R downstream primers (F:GAGTTTGATCCTGGCTCAG; R:ACGGHTACCTTGTTACGACTT) in Sangon, China. Then biofilm and crisp-cas related gene sequencing was performed with primers in Sangon, China (Table 3) .The phylogenetic analysis of 16S, biofilm and crisp-cas related gene of *Salmonella paratyphi* were performed using Molecular Evolutionary Genetics Analysis (MEGA7.0) software. The gene specificity of biofilm and crisp-cas related gene was studied using the software of Boiedit.

Homology modelling of *Salmonella* fimA (pili) protein

Homology modelling of *Salmonella* fimA (pili) protein tertiary structure was performed using SWISS-MODEL online suit. The tertiary structure of *Salmonella* fimA among *Salmonella paratyphi* A-A, *Salmonella paratyphi* A-NA3 and *Salmonella paratyphi* B-H were predicted.

Table 3. Primers used for biofilm and crisp-cas related gene.

Primer name	Sequence 5'-3'	Length
16S-F	GAGTTTGATCCTGGCTCAG	1504
16S-R	ACGGHTACCTTGTTACGACTT	
Crisp 1-F	ATAATGCTGCCGTTGGTAA	845
Crisp 1-R	TTGATGAGTATGGTGGTTGTGGT	
SM-lsrK-F	GAABGTKCCGCCMAGMAC	807
SM-lsrK-R	ATGGCTCGACTCTKTACC	
SM-fimA-F	ATGACCTCTACTATTGCCGAG	525
SM-fimA-R	TTATTCGTATTTCATGATAAAGG	
SM-invA-F	GTGAAATTATCGCCACGTTTCGGGCAA	261
SM-invA-R	TCATCGCACCGTCAAAGGAACC	
SM-luxS-F	GCGACCACCTCAACGGTAA	116
SM-luxS-R	TTCAGCACATCCGCCATC	

2.6. 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 1/250. 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 µg/ml), Incubation (37 °C, 24 h). The other group, firstly culture for 12 h, 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 µg/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

Salmonella seed solution was inoculated in LB for the proportion 1/250 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, 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 µg/ml), incubation (37 °C, 24 h). The other group, firstly culture for 12 h, 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 µg/ml), incubation (37 °C, 12 h). The bacterial population density (OD₆₀₀ 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.

Abbreviations

MOI, multiplicity of infection; MDR, multidrug-resistance; TEM, transmission electron microscopy; AR, antibiotic resistance.

Declarations

Ethics approval and consent to participate

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.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

L. J and R. Z Performed the experiments; Acquisition and analysis of data; Drafting of the manuscript; L. J Technical or material support; R. Z Critical revision of the manuscript. All authors read and approved the final manuscript.

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Not Applicable

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Supplemental Legends

Table S1. Identified protein-coding genes (open reading frames (ORFs) of phage KM16 complete genome.

Figures

Figure 1

Adsorption rate and population dynamics of phage KM16 inoculate in *Salmonella paratyphi* A NA3.

Figure 2

Plaques formed by phage KM16, the host strains of *Salmonella paratyphi* A NA3 after an overnight incubation at 37°C.

Figure 3

Morphological features of phage KM16 with host of *Salmonella paratyphi* A NA3 by transmission electron microscopy (TEM).

Figure 4

Optimum temperature, pH and MOI of isolated phage KM16.

Figure 5

Restriction enzyme digests of phage KM16. Phage KM16 DNA was digested by EcoRI, Hind III, NotI and XhoI.

Figure 6

Line map of the phage KM16 genome. In the KM16 track, genes colored red instructions for lysozyme and genes colored yellow instructions for endonuclease. The arrows represent the ORFs and point the direction of transcription.

Figure 7

Phylogenetic tree of *Salmonella paratyphi* genes of 16S, crispr 1, fimA, invA, isrK and luxS. The phylogenetic tree were constructed using the Maximum Likelyhood phylogeny test and bootstrap values were set for 1 000 repetitions. The phylogenetic tree of 16S gene indicated that *Salmonella paratyphi* which can be lysed are more closely related. The results showed that lytic spectrum consistent with the gene of crispr 1 and fimA, but not correlated with the gene of invA, isrK and luxS. (Red square denotes the *Salmonella* strains lysed by KM16).

Figure 8

Predicted tertiary structure of *Salmonella* fimA protein. Superposition of *Salmonella* paratyphi A-A (blue), *Salmonella* paratyphi A-NA3 (purple) and *Salmonella* paratyphi B-H (green) (<https://www.rcsb.org/structure/6erj>).

Figure 9

Scanning electron micrograph (SEM) of *Salmonella paratyphi* A NA3 colonization before and after phage KM16 (MOI=0.1) and kanamycin sulfate (10 µg/mL) application to biofilms formed on round coverslip. (A) *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250 and culture for 24 h, (B) A added phage KM16 (MOI=0.1), (C) A added kanamycin sulfate (10 µg/mL). (D) *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250 and culture for 12 h, then phage KM16 (MOI=0.1) was added and cultured for 12 h, (E) *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250 and culture for 12 h, then kanamycin sulfate (10 µg/mL) was added and cultured for 12 h, (F) *Salmonella paratyphi* A NA3 seed solution inoculation at a rate of 1/250 and culture for 12 h, then phage KM16 (MOI=0.1) and kanamycin sulfate (10 µg/mL) were added and cultured for 12 h. (1, 000× magnification)

Figure 10

Effects of phages and kanamycin sulfate (10 µg/mL) on biofilms. (A,B) Effects of phage KM16 and kanamycin sulfate (10 µg/mL) on *Salmonella paratyphi* A NA3 seed solution (inoculation at a rate of 1/250) growth that culture for 12 h and 24 h (OD₆₀₀). (E, F) Effects of phage KM16 and kanamycin sulfate (10 µg/mL) on *Salmonella paratyphi* A NA3 seed solution (inoculation at a rate of 1/250) biofilm that culture for 12 h and 24 h (OD₅₇₀). (C, D) Effects of phage KM16 and kanamycin sulfate (10 µg/mL) on *Salmonella paratyphi* A NA3 seed solution (inoculation at a rate of 1/250) growth, first culture for 12 h, then phage KM16 and kanamycin sulfate (10 µg/mL) were added and cultured for 12 h and 24 h (OD₆₀₀). (G,H) Effects of phage KM16 and kanamycin sulfate (10 µg/mL) on *Salmonella paratyphi* A NA3 seed solution (inoculation at a rate of 1/250) biofilm, first culture for 12 h, then phage KM16 and kanamycin sulfate (10 µg/mL) were added and cultured for 12 h and 24 h (OD₅₇₀).

Figure 11

Effects of phages and kanamycin sulfate (10 $\mu\text{g/mL}$) on colony-forming unit of *Salmonella paratyphi* A NA3. (A) Effects of phage KM16 and kanamycin sulfate (10 $\mu\text{g/mL}$) on *Salmonella paratyphi* A NA3 seed solution (inoculation at a rate of 1/250) that culture for 24 h. (B) Effects of phage KM16 and kanamycin sulfate (10 $\mu\text{g/mL}$) on *Salmonella paratyphi* A NA3 seed solution (inoculation at a rate of 1/250), first culture for 12 h, then phage KM16 and kanamycin sulfate (10 $\mu\text{g/mL}$) were added and cultured for 12 h.

Supplementary Files

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