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Evaluation of Lytic Bacteriophages for Control of Multidrug-Resistant *Salmonella Typhi*

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Abstract:

The introduction of antibiotic-resistant bacteria can cause severe clinical and public health concerns. This research serves as an indication of using bacteriophages to control multidrug-resistant bacterial *Salmonella Typhi* as an alternative agent. Two anti-Salmonella lytic bacteriophages were isolated from sewage water, primarily called Phage 1 and Phage 2. The large host spectrum of Phage 1 was lytic against all Salmonella isolates included in the analysis. In comparison, phage 2 has a small host range since only 25 isolates could be inhibited, and there are no phages of Discouraged bacteria other than Salmonella spp. Adsorption rate (Phage 1) was (2.2 x 10-10) ml one minute, Adsorption rate (phage 2) was (1.8 x 10-10) ml one minute. Phage 1 eclipse and latent cycles were 5, 11 minutes and 6.5, 13 minutes respectively for phage 2; Phage 1 and 2 burst sizes were 78 ± 15 pfu\cell and 61 ± 10 pfu\cell, respectively. The complete Phage 1 particle lysis period was four hours, while the complete Phage 2 particle lysis time was 5 hours. Phage passage findings showed a substantial increase in PFU, which reached its maximum elevation in Phage 1 at six passages and in Phage 2 at the fourth passage. Phage 1 and Phage 2 fitness for six generations were 23 ± 5 pfu\cell and 20 ± 2 pfu\cell, respectively. The particles of Phage 1 and 2 were stable at a broad ph range (6-10) at (30-50) °C, with an optimum tem. of 37 °C for the two phages.

Keywords: Bacteriophages, *Salmonella Typhi*, Host Range, Phage kinetics models.

1- Introduction:

In addition to a prolonged overdose of antibiotics, this condition has intensified the appearance of antimicrobial-resistant germ. Compared to the lengthy and costly clinical trials [3], the rapid expansion of antibiotic resistance has abandoned new antibiotics. And Salmonella spp. Some of the major foodborne pathogens that cause salmonellosis have been recognized as being characterized by diarrhea, fever, abdominal pain, nausea, occasional vomiting or headache [4]. The antimicrobial resistance of the Salmonella spp is occurred due to the development of membrane permeability, antibiotic-analytic enzymes, and changes of efflux pumps [5]. The prevalence of multidrug-resistant of Salmonella was increased over time and leading to failure treatment by the antibiotics [6-8]. There is an annual rise in morbidity, and mortality rates of antibiotics resistance [9], multi antibiotic resistance Salmonella becomes a significant public health issue. The therapeutic weakness of currently available antibiotics has contributed to the challenge of treating multidrug-resistant bacterial infections. Therefore, the production of alternative therapy is essential for the treatment of multidrug-resistant microbes [8].

Because of its explicitness and self-duplicating property, Bacteriophage has gotten a lot of consideration as a likely option with no unsafe impacts on gainful microbiota and human cells [10].

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Target microbes explicitness are limits bacteriophages to bacterial surface receptors like flagella, case, ooze sheet, lipopolysaccharides, and proteins of the external layer. They bring about lysis of tainted microscopic organisms communicated as a lytic movement [11, 12]. The bacteriophage cell surface receptors are changed by adjusting external layer parts [13-15]. Nonetheless, there is moderately little data about the relationship between bacteriophages and multidrug microbes regarding the change of host cell surface receptors.

Our investigation is isolated and identified lytic bacteriophages from wastewater using S. Typhi as a host system, with the following goals:

1. Isolating and identifying *S. Typhi* and determination of the profile of antibiotic resistance.
2. Characterization and isolation of *S. Typhi* phages from sewage water.
3. Bacteriophage counting with the plaque with double agar.
4. Determination of the attachment rate between the Bacteriophage and bacterial cell.

2- Materials and Methods

2-1- Samples Collection:

One hundred seven specimens were collected from different clinical cases (in other age groups), from several health centers in Al-Diwanyia city (Al- Diwanyia General Teaching Hospital, Al-Diwanyia Maternity and Pediatrics Afak General Hospital, and Teaching Hospital) and thirty sewage water samples from hospital environmental sources.

2-2- Isolation and Identification of *Salmonella Typhi*:

The collected samples are stool and blood. The swab was kept in peptone (10) mL and keep (37) °C for 24 hours in the pre-enrichment in BPW. Part of the pre-enriched culture (0.1 mL) is transferred to Soy Peptone Rappaport-Vassiliadis broth (10) mL, then put in the incubator at (41.5) °C for (24) hours. After that, the loop is used to transfer from the broth and streaked on Xylose lysine deoxycholate media, Salmonella Shigella agar, Eiosin-Methylene Blue and MacConkey media. The petri dish incubated at (37) °C for one day, then collecting the suspected colonies to make the biochemical Identification and identified by VITEK 2 – Compact. The stock is a culture for storing in LB broth at (-20) °C that contain on % (20) glycerol.

2-3- Antibiotic Susceptibility Testing:

S. Typhi was examined for its sensitivity for twelve antibiotics using the disk diffusion method [17]. The findings were included measurements of the inhibition zone based on CLSI [18].

2-4- Bacteriophages Infecting *S. Typhi* isolation

The bacteriophages were taken from sewage water. The sample was clarified using centrifugation for twenty minutes at (6000) rpm then filtered by a special membrane (0.45) μm. Adding of the filtrate (50) mL with the same volume of LB broth and adding a culture of bacteria (2×108 cfu/mL) (1) mL then keep at shaker incubator for one day, at (37) °C at (120) rpm.

They were centrifuging the culture for ten minutes at (6000) rpm. The upper layer is filtered by membrane filter (0.45) μm and used as a phage source to determine the propagative bacteria. The bacteria are propagated on LB broth by a double agar overlay technique based on [19]. Adding of the mid-exponential phase of each bacterial culture (200) μL and optical density at 600 of 0.4) with semi-solid LB media (4) mL and poured it in solid nutrient media. Then, dry it, then add phage source previously prepared (10) μL droplets on the lawns, then dry. After that, incubation of the

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plates at (37) °C for one day then checked the lysing zones.

2-5- Plaque Assay and Spot Test:

The spot test is used and described by son et al. [20] to diagnose the upper solution phages. We were determining the phage titer by plaque techniques using the double agar overlay technique. All the phage suspension was diluted serially. We are mixing diluted phage 100 µl and host bacterium 100 µl (10^8 CFU/ml) with soft agar 5.0 ml (0.75 % agar, w/v) then pouring on nutrient media [21], then counting of the plaques after incubating at 37^oC for 24 hours.

2-6- Bacteriophages Purification and Propagation:

Bacteriophages were purified from plaque isolates (single) based on [21-22]. Pouring of the phages is done by three positive single-plaque isolation with a pasture pipette for the homogenous plaques. Picking of the single plaque and put it in nutrient broth (5) mL that contain bacterial host (100) µL then keep at (37) °C with shaking at (1200) rpm. After that, incubation and centrifuging of the phage-host mixture for ten minutes at (6000) rpm, then filtration of the supernatants by Millipore filter (0.45) µm for removing the bacteria, then storing of the purified phages at (4) °C.

2-7- Phage kinetics:

2-7-1- Passage:

S. Typhi isolates were suspended in a flask containing 10 ml of LB and incubated for 1 hr. They were allowed to obtain a density of 1–2 × 10^8 cfu/ml before phage addition. At this 1-h time point, 10^4–10^7 pfu/ml (almost always more than 10^5 pfu/ml) was added to the flask. The culture was incubated for 20–60 min (usually 30) before an aliquot of the infected culture, including both 10^4–10^7 pfu/ml (again usually more than 10^5) free Phage and infected cells, were transferred to the next flask in which cells were at the requisite density and had been incubated for 1 hour. After the sample is complete from the passage, treating the samples with chloroform and store them. They kept the free Phage and released phage particles that produced inside the cells and transferred the aliquots to other flasks without adding chloroform except between days. At the starting of each day's passages, the final stock provided the starting phage population for transfer [23].

2-7-2- Adsorption Rate:

Adsorption assay was included adding fresh phage lysate 1000.000 pfu/ml with cells suspended in LB (10^8 cfu/ml), after (5) min, circulation of the sample for absorption of pellet. At five minutes, all suspensions were plated for achieving free phage densities.

Calculation of the adsorption rate was done depending on:

$$N_{free} = N_{total}\, e^{-5}$$ **[23].**

2-7-3- Eclipse Period:

Eclipse time assay was done by adding 10.000.000 pfu/ml with cells for 60 minutes with 10^8 cfu/ml.

At the minute (5.5), the samples were taken over chloroform every half minute, for 1.5 min before the phage lysis time. Titers were calculated of the treated samples for an estimate of intracellular phages and free phages. The data fitting evaluates eclipse time to a simulation that modelled adsorption, eclipse, and a linear accumulation of Phage after eclipse over time. Empirical least-squares fitted parameter values for phage density, eclipse, and the linear phage increase slope. The

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observed phage density and model phage density was normalized by observed phage density for equal weighting of the squared deviations at all-time points [23].

2-7-4- Lysis Time:

The 10^7 pfu/ml was added to cells (10) ml at 10^8 cfu/ml and left for five minutes, then diluted 100.000 times and 1000 times in separate flasks to adsorption stopping. The infective centers are plated at many time points to know all the titers' changes [23-24].

2-7-5- Burst Size and Latent Period:

burst size assays 10^6 pfu/ml are adding with the suspensions with growing cells in flasks (10^8 cfu/ml). Diluting the mixture one thousand times after five minutes to curtail adsorption. At the minute (5.5) and (6.5), the phages were calculated before and after treatment with chloroform for kills cells because the minute (6.5) is the finishing of the eclipse. The infections are don't Succeed to leave progeny. The plaques only that treated by chloroform.

The essential thickness of contaminated cells is dictated by contrasting and these titers. At the minutes (15.5), (16.5), and (17.5), the examples treated by chloroform are plated to decide phage thickness. Burst sizes were determined as the titer of Phage created at a late time focuses/the quantity of at first tainted cells, determined from starting time focuses. The idle period is a period at that infection descendants are delivering into the climate [23].

2-7-6- Viral Fitness:

Measurement of viral fitness is by passaging method; the phages population were used at the same age after a few generations. So, the density of the Phage depended on exponential growth. Viral fitness was evaluated by a passage at low phage/cell ratios across (4-5) consecutive transfers, the increase of the phage level determined from the endpoint of the (1) or (2) passage to the last passage. The viral fitness is calculated according to:

$$\textbf{the } [\log_2(N_t/N_0)]/t,$$

N_t is the phage number in the flask at time
t is time in hours [23].

2-8- pH impact on Phage Stability:

PBS determined the S. Typhi phages stability at many pH values (4, 5, 6, 7, 8, 9, 10, 11 and 12). An aliquot (1 ml) of the lysate (6×10^9 pfu/ml) was added in tubes with the buffer (9) ml then incubated at (37) ^oC for 60 minutes. The tube's contents were diluted serially, and the plaque technique determined pfu by *S. aureus* [23].

2-10- Optimum temperature for Phage Stability:

The stability of *S. Typhi* phages was evaluated at different temperatures (4, 10, 20, 30, 37, 40 and 50^oC). A 1 ml aliquot of phage lysate (6×10^9 pfu/ml) was spread on screw tubes and incubated for one hour. The tube contents were diluted by using phosphate buffer after determining pfu on nutrient media [23].

2-11- Phage Host Range:

The *S. Typhi* phages can infect many bacterial spp such as *Staphylococcus aureus*,

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Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa and be isolated from the pathogenic cases and identify depending on Macfaddin [16]. They then determined by using the VITEK 2 – Compact technique. The mid-log phase of host bacteria (0.5) ml was added with soft agar (4) ml then poured on a nutrient media petri dish. After the suspension is gelled and dried, adding aliquot of phage lysate 100 µl (6×109 pfu/ml) to each plate's centre. After that, incubation of the plates at (37) ^{o}C for one day. After 24 hours, the clear zone on the plate are determined and evaluated [19].

2-12- Statistical Analysis:

Graph Pad Prism (V:5.04) was used for analyzing data. The data were expressed as mean ± standard error. Analysis of the groups is done using Turkey's posthoc analyses and one-way ANOVA after calculating LSD value at ($P \leq 0.05$) [25].

3- Results:

3-1- *Salmonella Typhi* (isolation and Identification):

The result proved that, out of 107 blood and fecal samples, 30 *S. Typhi* strains isolated from patients at different age groups.

3-2- Antibiotic Susceptibility Testing:

The findings showed that the antibiotics resistance rates of the thirty samples of *S. Typhi* were high for ampicillin, tetracycline, sulfonamide, and amoxicillin. It showed moderate resistance to tobacco and chloramphenicol and showed the lowest resistance for ceftriaxone and cefotaxime (table 3-1).

Table (3-1): Antibiotic susceptibility of *S. Typhi*

Total resistance of bacterial isolates	Antibiotics disk
(30) 100%	Ampicillin
(30) 100%	Amoxicillin-clavulanic acid
(26) 86.6%	Cefotaxime
(28) 93.3%	Ceftriaxone
(20) 66.6%	Streptomycin
(24) 80%	Gentamicin
(24) 80%	Amikacin
(16) 53.3%	Tobramycin
(20) 66.6%	Tetracycline
(26) 86.6%	Sulfonamide
(2) 6.6%	Ciprofloxacin
(20) 66.6%	Chloramphenicol

3-3- Isolation of *S. Typhi* Phages:

Two *S. Typhi* phages were mainly labelled Phage 1 and Phage 2 using four isolated from (30) wastewater samples. The phages were effectively checked against 30 *S. Typhi* isolates used as hosts for phages isolation by the plaque assay. As in table (3-2), the phase's characteristics were determined by the plaque appearance, size, turbidity, and halo in the cultural media. Infection with *S. Typhi* phage showed transparent plaques on the nutrient agar surface. From the plaque

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experiment. Plaques on media are large, hollow zone with irregular margins as figure (3-4), which displayed the plaques of *S. Typhi* phages were susceptible to phages' lytic infection.

Table (3-2): Characteristics of *S. Typhi* phages

Phage name	Plaque Size (mm)	Number of plaques	Turbidity	Halo
Phage 1	1-2	108	C	-
Phage 2	2-4	61	C	+

-: no halo, +: halo, C: clear

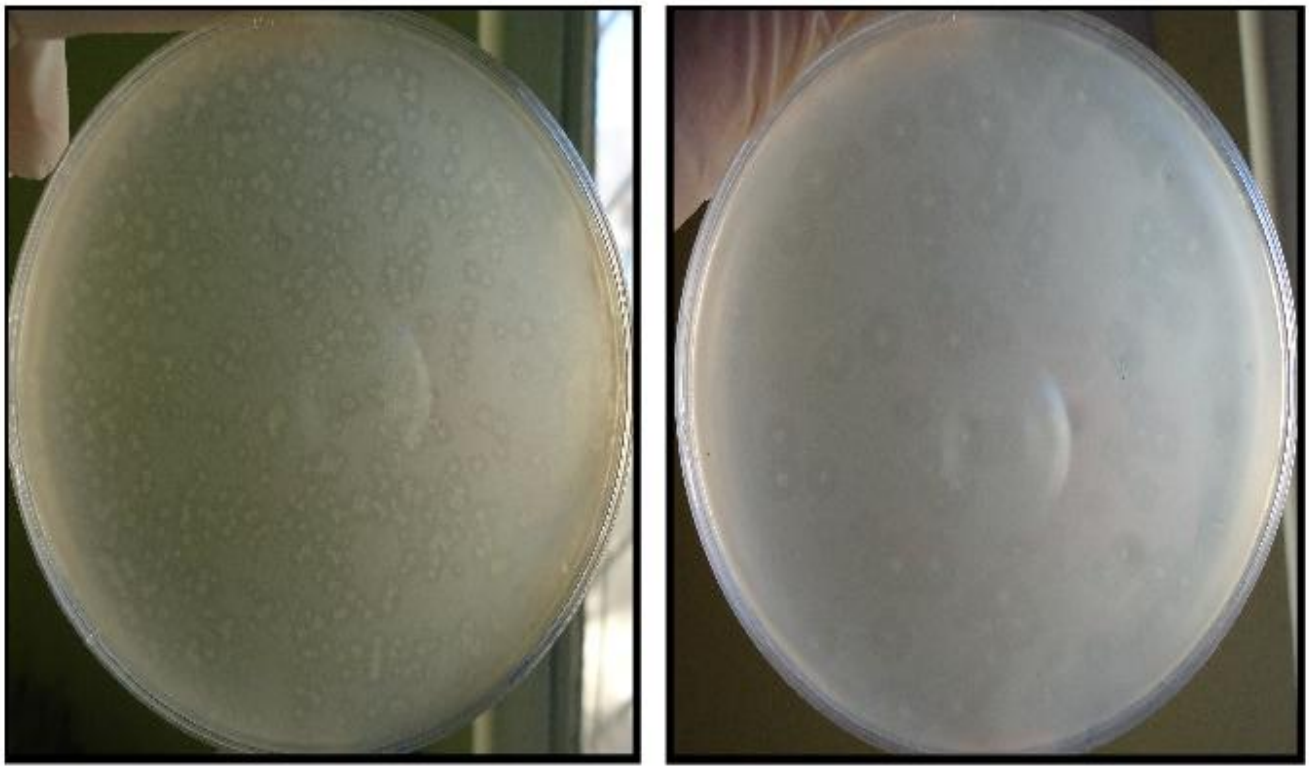

Figure (3-1): Plaques of *S. Typhi* phages at 37 ^{o}C on nutrient media after 24 hours

3-4- Phage kinetics:

3-4-1- Passages:

Phage passages were performed to spread and effectiveness of subculturing on Phage and fitness efficiently. The passage findings demonstrated a significant increase in PFU that achieved its maximum elevation at sixth passages in Phage 1 and fourth passage in Phage 2, while the absorption of tested samples showed a drastic decrease in optical density at two phages as in table (3-2).

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Table (3-2): Passages and PFU of *S. Typhi* phages

Phage 2		**Phage 1**		***Passages**
PFU	**Optical density (650 nm)**	**PFU**	**Optical density (650 nm)**	
20	0.722	32	0.855	**Passage 1**
48	0.611	48	0.592	**Passage 2**
61	0.471	72	0.362	**Passage 3**
80	0.233	102	0.172	**Passage 4**
80	0.215	128	0.89	**Passage 5**
80	0.215	139	0.11	**Passage 6**

* Each passage represents five replicate

3-4-2- Adsorption Rate:

The isolated two-phage particles can achieve a higher degree of adsorption over (4-6) minutes at (P>0.05) in raising the absorption of the phage particles on the bacterial cells, whereas dilution and moving decrease adsorption as shown in the chart (3-2). The absorption rate of the Phage 1 was (2.2×10^{10}) ml 0.1 minute, and Phage 2 was (1.8×10^{10}) ml 0.1 minutes.

Figure (3-2): The phage particles adsorption on bacteria surface

3-4-3- Eclipse Period:

The collected results demonstrated that there is an eclipse period of the isolated phage particles around

Phage 1= 5 minutes

Phage 2= 6:30 min.

3-4-3- Lysis period:

Our study demonstrated which the single phage particles would have a massively strong potential to induce microbial cells apoptosis (P > 0.05). Therefore, these actions achieved a higher incidence over one hour of integrating phage particles with microbial species. It has also shown that the percentage of bacterial cells that correspond with time is significantly reduced. The maximum Phage 1 particle lysis period was (4:20 hours) whereas the complete Phage 2 particle lysis time was 6 hours, as shown in the graph. (3-3).

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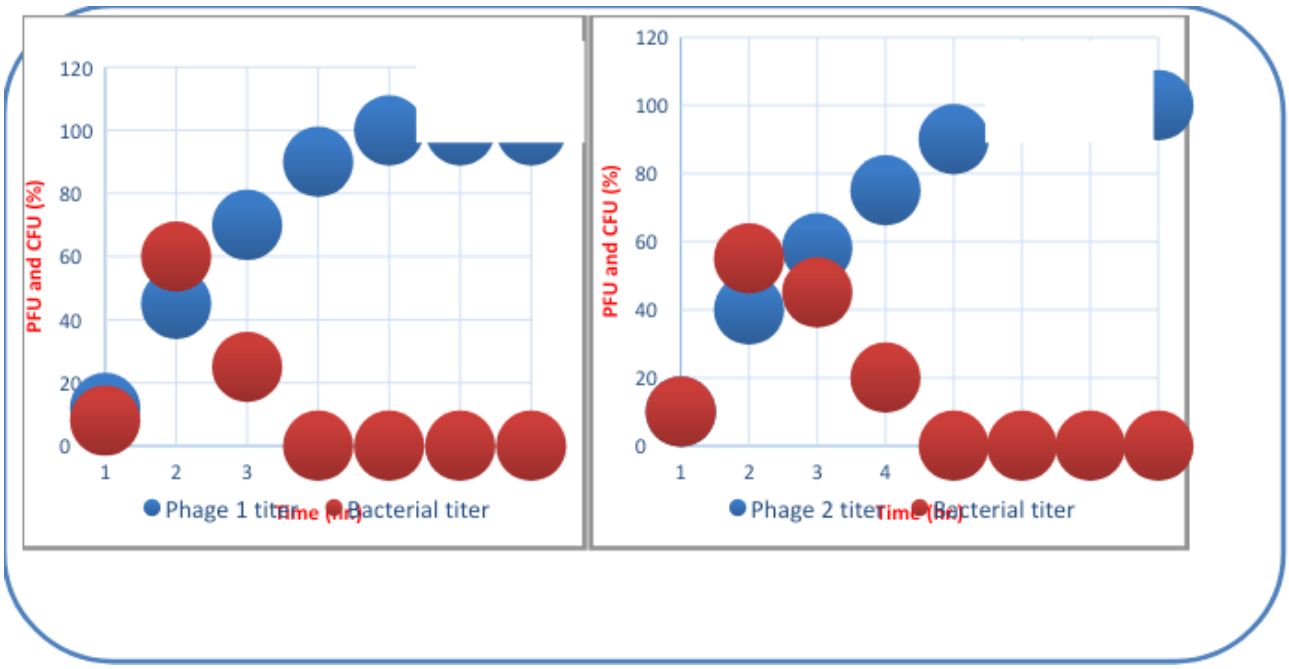

Figure (3-3): *S. Typhi* Lysis period

3-4-4- Latent Period and Burst Size:

They analyzed statistics revealed that the bursting scale (percentage of phages an infected cell produced) was:

Phage 1= 78±10 pfu\cell

During eleven min.

Phage 2= 61±10 pfu\cell

During thirteen min.

3-4-5- Fitness of the Phage:

The measured data revealed that phage strength (rate of expansion) was approximately:

Phage 1= 23 ± 5 pfu\cell sixth generations across

Phage 2= 20 ± 2 pfu\cell sixth generations throughout

3-5-6- pH Stability:

The pH has a significant impact at (P>0.05) on the efficacy of phage particles; these effects are done at the top level throughout the essential medium at pH around 6-8 and pH 9, effectively beginning to decrease drastically, at about pH < 4, effectively reducing them as shown in the graph (3-4).

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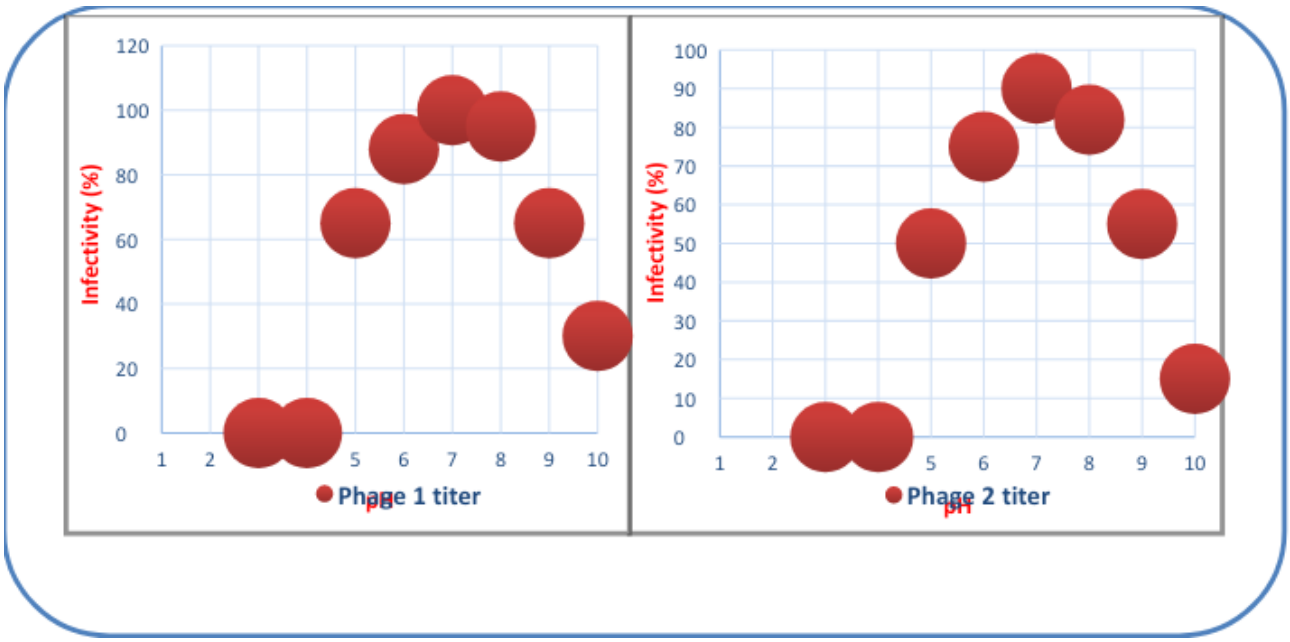

Figure (3-4): The effect pH on stability of *S. Typhi* phages

3-4-7- Thermal Stability:

The findings revealed that the efficacy of viral particles at temperatures about (35-40 °C) reaches their maximum level. The average temperature gets an incredible influence at (P>0.05) on elevated temperature phage particles' efficacy. After (8-10) minutes, the phage particles stopped successfully at 50 ° C, as in the figure (3-5).

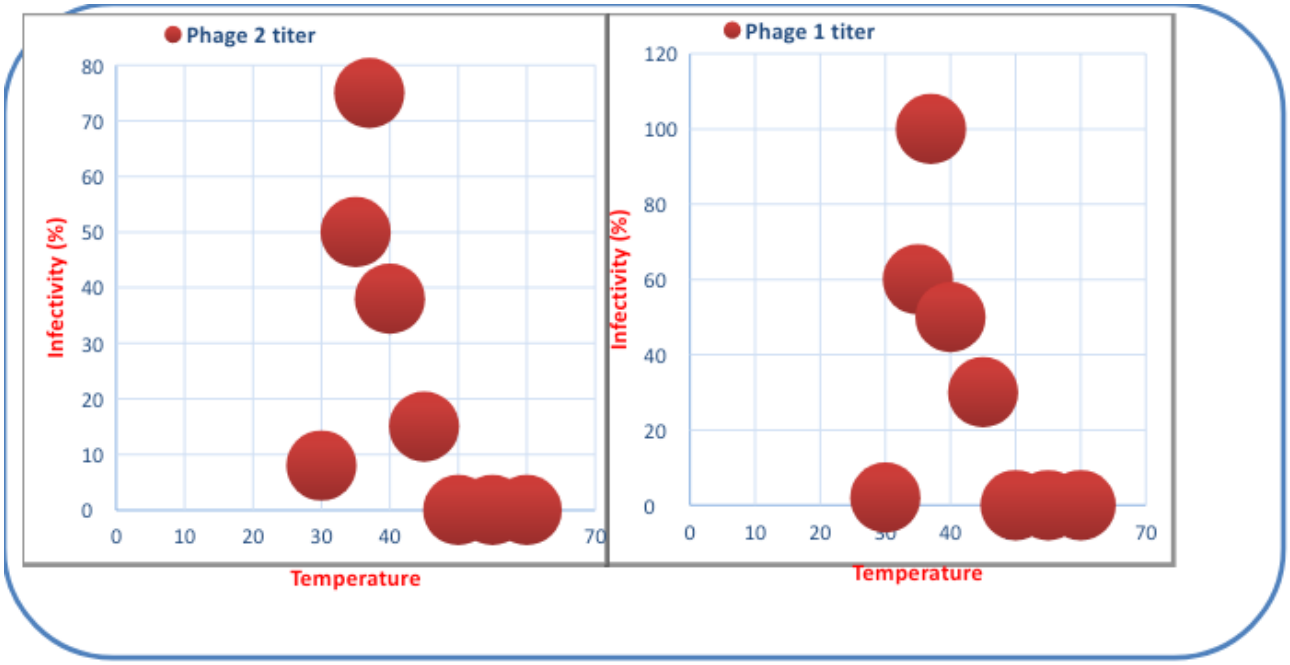

Figure (3-5): The temperature impact on *S. Typhi* phage stability

3-5- Host Range of *S. Typhi* Phages:

Phage 1 displayed a broad host range. It was lytic against all *S. Typhi* isolates used in this

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research. Alternatively, because they may inhibit only 25 isolates, phage 2 has a narrow host selection. As seen in the table, neither of these phages suppressed bacteria apart from Salmonella species (2).

Table (3-3): Host range of *S. Typhi* phages with bacterial isolates

Bacterial isolates	Phage infection	
	Phage 1	Phage 2
Salmonella typhimurium	+	+
Salmonella eneritidis	+	+
Staphylococcus epidermidis	-	-
Staphylococcus aureus	-	-
Escherichia coli	-	-
Streptococcus pyogenes	-	-
Klebsiella pneumoniae	-	-

(+) complete lysis, (-) no lysis

4- Discussion:

Currently, the World is riddled with pathogens, which are bacteria that are immune to the most recognized medicines [26]. Most notably, the biofilms improve their ability to withstand antibiotics, which further explains that many antibiotic-resistant infections are caused by bacteria living in biofilm populations [27]. Therefore, this research investigates the occurrence, and antibacterial resistance of *S. Typhi* isolates from the confirmed specimens in the Al-Qadisyia Province, Iraq. Throughout this research, we described *S. Typhi* with multidrug resistance, resulting in a high success rate of treatment. Hence, bacterial resistance. These bacteria that cause can be present in the blood in high quantities and extremely difficult to treat in conjunction with medications being taken to cure the *S. Typhi,* a greater desire to develop new drugs that combat and provide better therapies *S. Typhi* [28-29]. Therefore, the cause of the MDR *S.typhi* species is exceptionally urgent because the parasites that are present are a significant public health concern. When opposed to chemical pharmaceuticals, bacteriophages are regarded as a feasible option for chemical treatments against bacteria. [29].

We took in wastewater as a phage source because it has a wide range of phages in the past. Identification of two lytic phages (Phage 1 and Phage 2) against *Salmonella typhi* host bacterium like that of many animals showed that it is a rich source of this microorganism also, the anti-

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salmonella Phage. With such complex interactions, treatments like phage induction are likely to be more effective if there are several phages with powerful lytic capacities, which can be used in a complementary fashion with the phages present in a strain used in that process. Salmonella particular phage was also isolated from wastewater, close to what was used by previous researchers [30-31].

The adsorption rate of Phage 1 was (2.2×10^{-10}) ml $min^{-1,}$ and the Phage 2 was (1.8×10^{-10}) ml $min^{-1,}$ indicating that perhaps the Phage has a high affinity for the host target cells receptors with a very massive proportion of tail fiber protein found in the receptor binding to the unique cellular receptors. It was found earlier that the tail protein materials on the viral particles are essential in identifying and attaching to the host cell membrane. These findings are contrary to those previously stated about the adsorption rate of other T7 strains. The adsorption rate is highly variable, around 4.5×10^{-10} and 8.9×10^{-10} ml min^{-1}, with the infection rate based on whether or not bacteria live or die at the time [33].

After being mixed in a solvent, the two particles lived at pH values between 6 and 8. This research shows that these findings agree with AL Khozai et al. [34] Parra et al., [35] and ALKabii [36]. At defined circumstances, Phage 1 and Phage 2 can infect at a wide range of temperature at $(37)^{o}$C. After evaluating the isolated two phage particles' ability to regulate S. typhi in the environment, these phage particles are called "biological disinfectants". It made it possible for all phages to live at room temperature, with no noticeable loss in the amount of phages particles that existed, something very significant for phage particles used in therapeutic applications. The future use of Bacteriophage (Phage) therapy to manage *Salmonella serovar* infection is unknown. [35-37].

Phage 1 is known to kill bacterial strains of over 200 different species. It is a very effective antibacterial agent to use before severe illnesses occur in the correct quantities. Other than its genetic material, a host's phage repertoire varies depending on the host range of specific proteins, such as the phage receptors or the cellular defence mechanisms. So far, only minimal lytic efficacy of the phage 2 in the S. typhi extracts has been demonstrated and that too only the bacteria's already denatured cells. Therefore we do not know whether it has adverse effects on the normal flora of the host. This is possible because the various clinical bacterial isolates examined in this experiment show a varied surface appearance on their cell membranes' surface. Some bacteria isolated from the body make the receptor, but the immune system occludes these receptors. Still, other strains may have responses/modifications after their encounter/administration with the pathogen. [39].

Conclusions:

The need for new methods is evident, and antibiotics are no longer adequate to prevent several pathogens. Phages and lysines are available in various environments and can be impressively healthy, highly efficient and even reasonably cost-effective, with non-toxic techniques of unique pathogenic bacteria control. Phage 1 and 2 were individual to Salmonella and could not infect bacteria used in this study, such as (*Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa*). The findings would pave the way for the development of a new multidrug-resistant pathogens bacteriophage control system. However, more research is required to elucidate changes in lytic efficacy and bacteriophage receptors for using the Bacteriophage as an alternative factor for kill multidrug-resistant *S. Typhi*.

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5- References:

1. Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS. Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. Microb Pathog. 2016;95(1):32–42.
2. Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial World? Clin Microbiol Rev. 2013;26(2):185–230.
3. Hede K. Antibiotic resistance: an infectious arms race. Nature. 2014;509(7498):S2–3.
4. Coburn B, Grassl GA, Finlay B. 2007. *Salmonella*, the host and disease: a brief review. Immunol. Cell Biol. 85: 112-118.
5. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med. 2006;119(6A):S3–10.
6. Mulvey MR, Finley R, Allen V, Ang L, Bekal S, El Bailey S, et al. Emergence of multidrug-resistant *Salmonella enterica* serotype involving human cases in Canada: results from the Canadian Integrated Program on Antimicrobial Resistance Surveillance (CIPARS). J Antimicrob Chemother. 2013;68(9):1982–6.
7. Dionisi AM, Lucarelli C, Benedetti I, Owczarek S, Luzzi I. Molecular characterization of multidrug-resistant *Salmonella enterica* serotype Infantis from humans, animals and the environment in Italy. Int J Antimicrob Agent. 2011;38(5):384–9.
8. Lu Y, Zhao H, Sun J, Liu Y, Zhou X, Beier RC, et al. Characterization of multidrug-resistant Salmonella enterica serovars Indiana and Enteritidis from chickens in Eastern China. PLoS ONE. 2014;9(5):e96050.
9. Eng S-K, Pusparajah P, Ab Mutalib N-S, Ser H-L, Chan K-G, Lee L-H. *Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci. 2015;8(3):284–93.
10. Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, et al. Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother. 2005;11(5):211–9.
11. Le S, He X, Tan Y, Huang G, Zhang L, Lux R, et al. Mapping the tail fiber as the receptor binding protein responsible for differential host specificity of *Pseudomonas aeruginosa* bacteriophages PaP1 and JG004. PLoS ONE. 2013;8(7):e68562.
12. Rakhuba DV, Kolomiets EI, Dey ES, Novik GI. Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. Pol J Microbiol. 2010;59(3):145–55.
13. Qimron U, Marintcheva B, Tabor S, Richardson CC. Genome wide screens for *Escherichia coli* genes affecting growth of T7 Bacteriophage. Proc Natl Acad Sci. 2006;103(50):19039–44.
14. Madaras-Kelly KJ, Daniels C, Hegbloom M, Thompson M. Pharmaco-dynamic characterization of efflux and topoisomerase IV-mediated fluoroquinolone resistance in Streptococcus pneumoniae. J Antimicrob Chemother. 2002;50(2):211–8.
15. Giraud E, Cloeckaert A, Kerboeuf D, Chaslus-Dancla E. Evidence for active efflux as the primary mechanism of resistance to ciprofloxacin in *Salmonella enterica* serovar *Typhimurium*. Antimicrob Agent Chemother. 2000;44(5):1223–8.
16. MacFaddin, J.F. (2008). Biochemical tests for Identification of Medical Bacteria. 4th ed.,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 11359 - 11372
Received 25 April 2021; Accepted 08 May 2021.

Waverly press, *Inc.*, *Baltimore*, U.S.A.

17. Perilla, M.J., Ajello, G., Boop, C., Elliott, J., Facklam, R., Popovic, T. and Wells, J. (2012). Manual for laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in developing World.CDC, Atlanta, Georgia, USA.
18. Clinical and Laboratory Standards Institute (CLSI)(2014). Performance standards for antimicrobial susceptibility testing. 20th informational supplement. M 100-S20., Wayne, Pennsylvania; 30 (1).
19. Clokie, M.R. and Kropinski, A.M. (2009). Bacteriophages. Methods and Protocols: Vol.1: Isolation, Characterization and Interactions. Humana Press.
20. Son, J. S.; Lee, S. J.; Jun, S. Y.; Yoon, S. J.; Kang, S. H.; Paik, H. R.; Kang, J. O.; and Choi, Y. J. (2011). Antibacterial and biofilm removal activity of a Podoviridae *Staphylococcus aureus* bacteriophage SAP-2 and a derived recombinant cell-wall-degrading enzyme. *Appl. Microbiol. Biotechnol. 86*:1439–1449.
21. Adams, M.H., 1959. Bacteriophages. Interscience Publishers, New York.
22. Hoshiba, H.; Uchiyama, J.; Kato, S.; Ujihara, T.; Muraoka, A.; Daibata, M.; Wakiguchi, H. and Matsuzaki, S. (2012). Isolation and characterization of a novel *Staphylococcus aureus* bacteriophage, phiMR25, and its therapeutic potential. *Arch. Virol. 155*:545–552.
23. Heineman, R.H. and Bull, J.J. (2010).Testing optimality with experimental evaluation: lysis time in Bacteriophage .J. Evolution, Vol.61, No.7: 1695-1709.
24. Vandersteegen, K.; Mattheus, W.; Ceyssens, P. J.; Bilocq, F.; De Vos, D.; Pirnay, J. P.; Noben, J. P.; Merabishvili, M.; Lipinska, U.; Hermans, K. and Lavigne, R. (2011). Microbiological and molecular assessment of bacteriophage ISP for the control of *Staphylococcus aureus*. *PLoS One 6*:e24418.
25. Paulson, D.S. (2010). Biostatistics and Microbiology: A Survival Manual. Springer Science, Business Media, LLC.
26. Fair, R.J., Tor, Y., 2014. Antibiotics and bacterial resistance in the 21st century. Perspect. Med. Chem. 6, 25–64.
27. Bjarnsholt, T., Givskov, M., Molin, S., Ciofu OHøiby, N., 2010. Antibiotic resistance of bacterial biofilms. Int. J. Antimicrob. Agents 35 (4), 322–332.
28. Coates, A.R., Halls, G., Hu, Y., 2011. Novel classes of antibiotics or more of the same? Br. J. Pharmacol. 163 (1), 184–194.
29. Ventola, L., 2015. The antibiotic resistance crisis. Pharmacol. Ther. 40 (4), 277–283.
30. Merabishvili, M.; Pirnay, J.P.; Verbeken, G. et al (2009). Quality controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One 4(3): 4932-4944.
31. Kim J, Jo A, Ding T, Lee H-Y, Ahn J. Assessment of altered binding specificity of Bacteriophage for ciprofloxacin-induced antibiotic-resistant *Salmonella Typhimurium*. Arch Microbiol. 2016;198(6):521–9.
32. Wong CL, Sieo CC, Tan WS, Abdullah N, Hair-Bejo M, Abu J, et al. Evaluation of a lytic bacteriophage, Φ st1, for biocontrol of *Salmonella enterica serovar Typhimurium* in chickens. Int J Food Microbiol. 2014;172(2):92–101.
33. Akhtar, M., Viazis, S., Diez-Gonzalez, F., 2014. Isolation, Identification and characterization of lytic, wide host range bacteriophages from waste effluents against Salmonella enterica serovars. Food Control 38, 67–74.
34. Al-khozai, Z.M; Al-Khafaf; D.M.R and Al-Hamdani, A.H.O. (2010). Isolation and

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 11359 - 11372
Received 25 April 2021; Accepted 08 May 2021.

Studying of Cholera phage in Al-Diwanyia city and possibility of using it to control *Vibrio cholerae* 01 in laboratory. J. of Al-Qadisiya-Pure science, Vol.4, No. 16: 10-22.

35. Parra, B., Robeson, J., 2016. Selection of polyvalent bacteriophages infecting *Salmonella enterica serovar Choleraesuis*. Electron. J. Biotechnol. 21, 72–76.
36. AL-Kabii, M.N. (2013). Isolation and Characterization of Lytic Bacteriophages Infecting *Pseudomonas aeruginosa*. M.sc. Thesis. College of Sciences. Al-Qadisiya University.
37. Nikkhahi, F., SoltanDallal, M.M., Alimohammadi, M., RahimiForoushani, A., Rajabi, Z., Fardsanei, F., Imeni, S.M., TorabiBonab, P., 2017. Phage therapy: assessment of the efficacy of a bacteriophage isolated in the treatment of salmonellosis induced by *Salmonella enteritidis* in mice. Gastroenterol. Hepatol. Bed Bench. 10 (2), 131–136.
38. Fineran, P.C.; Blower, T.R.; Foulds, I.J.; Humphreys, D.P.; Lilley, K.S. and Salmond, G.P. (2012). The phage abortive infection system, ToxIN, functions as a protein RNA toxin antitoxin pair. *Proc Natl Acad Sci USA 106*: 894–899.
39. Labrie, S.J.; Samson, J.E. and Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nat Rev Microbiol 8*: 317–327.
40. Jassim SAA, Limoges RG. Natural solution to antibiotic resistance: bacteriophages 'The Living Drugs'. World J Microbiol Biotechnol. 2014;30(8):2153–70.