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Original Article

Isolation, Characterization, and Efficacy of Three Lytic Phages Infecting Multidrug-Resistant *Salmonella* Serovars from Poultry Farms in Egypt

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Abstract

Multidrug-resistant (MDR) *Salmonella* serovars are considered a significant threat to veterinary and public health. Developing new antimicrobial compounds that can treat the infection caused by these notorious pathogens is a big challenge. Bacteriophages can be adsorbed on and inhibit the growth of bacteria, providing optimal and promising alternatives to chemical antimicrobial compounds against foodborne pathogens due to their abundance in nature and high host specificity. The objective of the current study was to isolate and characterize new phages from poultry farms and sewage and to evaluate their efficacy against *S.* Enteritidis isolates. The study reports three lytic phages designated as ϕSET1, ϕSET2, and ϕSET3 isolated from poultry carcasses and sewage samples in Qalubiya governorate Egypt. The effectiveness of phages was evaluated against multidrug-resistant *S.* Enteritidis strains. Electron microscopy showed that these phages belong to the Siphoviridae family. Phages were tested against 13 bacterial strains to determine their host range. They could infect four *S.* Enteritidis and one *S.* Typhimurium; however, they did not infect other tested bacterial species, indicating their narrow infectivity. The bacteriophage's single-step growth curves revealed a latent period of 20 min for ϕSET1 and 30 min for ϕSET2 and ϕSET3. The isolated *Salmonella* phages prevented the growth of *S.* Enteritidis for up to 18 hrs. The findings revealed that *Salmonella* phages could be used as alternative natural antibacterial compounds to combat infection with MDR *S.* Enteritidis in the poultry industry and represent a step forward to using large panels of phages for eliminating *Salmonella* from the food chain.

Keywords: *S.* Enteritidis, bacteriophage, Siphoviridae, poultry

Isolement, Caractérisation et Efficacité de Trois Phages Lytiques Infectant des Sérovars de *Salmonella* Multirésistants Provenant d'Elevages Avicoles en Égypte

Résumé: Les sérovars de *Salmonella* multirésistants (MDR) sont considérés comme une menace importante pour la santé vétérinaire et publique. Le développement de nouveaux composés antimicrobiens capables de traiter l'infection causée par ces agents pathogènes notoires est un grand défi. Les bactériophages peuvent être adsorbés et inhiber la croissance des bactéries, offrant des alternatives optimales et prometteuses aux composés antimicrobiens chimiques contre les agents pathogènes d'origine alimentaire en raison de leur abondance dans la nature et de leur spécificité élevée pour l'hôte. L'objectif de la présente étude était d'isoler et de caractériser de nouveaux phages provenant de fermes avicoles et d'eaux usées et d'évaluer leur efficacité contre les isolats de

S. Enteritidis. L'étude rapporte trois phages lytiques désignés comme ϕSET1, ϕSET2 et ϕSET3 isolés de carcasses de volaille et d'échantillons d'eaux usées dans le gouvernorat de Qalubiya en Égypte. L'efficacité des phages a été évaluée contre des souches multirésistantes de *S.* Enteritidis. La microscopie électronique a montré que ces phages appartiennent à la famille des Siphoviridae. Les phages ont été testés contre 13 souches bactériennes pour déterminer leur gamme d'hôtes. Ils pourraient infecter quatre *S.* Enteritidis et un *S.* Typhimurium; cependant, ils n'ont pas infecté d'autres espèces bactériennes testées, ce qui indique leur infectivité étroite. Les courbes de croissance en une étape du bactériophage ont révélé une période de latence de 20 min pour SET1 et de 30 min pour ϕSET2 et ϕSET3. Les phages de *Salmonella* isolés ont empêché la croissance de *S.* Enteritidis jusqu'à 18 heures. Les résultats ont révélé que les phages de *Salmonella* pourraient être utilisés comme composés antibactériens naturels alternatifs pour lutter contre l'infection par MDR *S.* Enteritidis dans l'industrie avicole et représentent un pas en avant vers l'utilisation de grands panels de phages pour éliminer *Salmonella* de la chaîne alimentaire.
Mots-clés: *S.* Enteritidis, bactériophage, Siphoviridae, volaille

1. Introduction

Salmonellosis is a significant foodborne disease transmitted to humans through contaminated raw foods, including poultry meat and eggs (1). It has been reported that around 20% of the world's poultry products are contaminated with *Salmonella* spp. (2). Among several *Salmonella* spp., the non-typhoidal *Salmonella* (NTS) strains, e.g., *Salmonella enterica* serovars Enteritidis and Typhimurium, are the most significant serovars that can remain for a long time in human and animal environments. Subsequently, they are the most common causes of foodborne illness in humans (3). Emerging multidrug resistant (MDR) *Salmonella* strains, resistant to most antibiotics used to treat humans and animals, are now widespread in developing and developed countries (4). Diseases caused by NTS such as *S.* Enteritidis and *S.* Typhimurium often stem from consuming contaminated poultry products, resulting in foodborne illness that may lead to hospitalization and require long-term antibiotic treatment (5), eventually resulting in the development of drug resistance and mortality. MDR strains of *Salmonella* serovars are a severe threat to public and animal health, and there is an urgent need to discover new antimicrobials that can mitigate these resistance mechanisms or biofilm formation (6). In Egypt, *S.* Typhimurium, *S.* Kentucky, and *S.* Infantis were isolated from healthy and diseased chickens flocks (7), while *S.* Enteritidis and *S.* Kentucky were the most dominant serovars isolated from retail chicken meat shops (8). *S.* Enteritidis, *S.* Virchow, *S.* Typhimurium, and S. Kentucky were the serovars most isolated from broiler chicks during the first week of age from different Delta and north coastal governorates of Egypt (9).

In poultry farms, up to 90% of the antibiotics administered are given orally. Nevertheless, they are not completely absorbed in the chicken's intestinal tract and are most often excreted unchanged in fecal matter. The poultry industry produces large amounts of manure and litter used in agricultural lands (10). Contaminated poultry litter may transmit zoonotic pathogens like *S.* Enteritidis into the environment (11). Therefore, novel and effective strategies to control *S.* Enteritidis and reduce the risk of antimicrobial resistance dissemination among animals and humans are needed. The appearance of drug residue and resistant bacteria in food animals has initiated interest in using probiotics and bacteriophages as alternative treatments (12). In recent years, bacteriophages have received attention as a potential antimicrobial alternative to eliminate harmful bacterial infections, particularly in treating foodborne pathogens (13). Lytic bacteriophage infects the bacterial cell and multiplies, producing new bacteriophage particles (14). Bacteriophages are host-specific (15), widely distributed, and isolated from water, soil, and sewage (16). Thus, they have minimal impacts on the natural microbiota populations in humans and animals (12). They are also used in treating

and classifying bacterial strains, including *Salmonella* (14, 17). The application of bacteriophages in therapy and prophylaxis is considered safe. Therefore, this study aimed to isolate and characterize new lytic phages from different poultry farms and sewage and evaluate their potential antimicrobial action against *S.* Enteritidis.

2. Material and Methods

2.1. Isolation of *Salmonella*

Fifty-six samples were collected from diseased living and freshly dead young chicks, ducks, broilers, and laying hens on different farms in Qalubiya governorate, Delta region of Egypt, in 2019. Samples were collected aseptically from the intestine, liver, spleen, vent, excreta, oviducts, and unabsorbed yolk sac. The classical microbiological tools were conducted for detecting different *Salmonella* serovars as previously described (18). Briefly, samples of 25 g from poultry composite were homogenized in buffered peptone water (BPW) (225 ml) for 2 min and then incubated for 16 - 20 hrs at 37 ℃ under aerobic conditions. Next, 0.1 ml was enriched in 10 ml of Rappaport–Vassiliadis (RV) broth, and the samples were incubated at 42 ℃ for 18-24 hrs. After that, the content was subcultured onto Xylose Lysine Deoxycholate Agar (XLD) agar and was incubated for 18 - 24 hrs at 37 ℃. Presumptive positive colonies were identified based on colony morphologically, classical biochemical techniques, and serologically using slide agglutination test by virulence (Vi), polyvalent and monovalent somatic (O), and tube agglutination test for flagellar (H) antigens at the Serological Department of Animal Health Research Center, Cairo. The stock cultures were kept in nutrient broth containing 20% V/v glycerol in the deep freezer.

2.2. Antibiotic Susceptibility Testing

Based on the National Committee for Clinical Standards guidelines, the antibiotic susceptibility testing (AST) of isolated *Salmonella* strains was performed using the disk diffusion method (19). The isolates were tested against 15 antibiotics, i.e. doxycycline, chloramphenicol, norfloxacin, amoxicillin, ampicillin, trimethoprim/sulphamethoxazole, gentamycin, ciprofloxacin, imipenem, colistin, nalidixic acid, neomycin, tetracycline, vancomycin, and streptomycin (Oxoid, England). A solution corresponding to a McFarland of 0.5 was applied to the surface of the Mueller-Hinton agar plate. All antibiotic disks were situated aseptically on the inoculated agar. The plates were kept at 37 ℃ for 16–24 hrs. The growth inhibition zone was evaluated to the nearest millimeter and interpreted based on the criteria reported by the European Committee on AST.

2.3. Isolation and Purification of *S.* Enteritidis Phages

Chicken carcass and sewage water samples were collected to isolate bacteriophages using the previously described enrichment technique (20, 21). All samples were obtained from the same region in the Qalubiya governorate of Egypt. Sewage samples were centrifuged at 6000 xg for 20 min and then filtered by a 0.45-µm-membrane filter. One mL of filtrate was incubated with preferred bacterial strains at 37 ℃ overnight. Chloroform (10 ml) was combined with the culture, and centrifugation at 6000 xg for 10 min was done to remove bacteria debris. Next, 10 µl of the supernatant was spotted onto different strains of bacteria, and phages were isolated from lysis zones and purified from single plaques. A single plaque was picked up and added into a log phase culture of *S.* Enteritidis. After incubation at 37 ℃ for 12 hrs, the phage-host mixture was centrifuged for 10 min at 10000 xg. The content was filtered by a membrane of 0.45 µm. Pellets produced by centrifugation of 10000 xg at 4 ℃ for 30 min were collected and dissolved in SM buffer (10 mM $MgSO_4$, 50 mM Tris-HCl at pH 7.5, 100 mM NaCl, and 0.01% gelatine), and the purified phages were kept at 4 ℃ until use.

2.4. Determination of Host Range of *S.* Enteritidis Phages

To determine their host range, phages were tested against 13 bacterial strains. Bacteriophage lysis assay was performed based on the double-layer method: 100 µl of a log-phase culture of tested bacteria was added to 3.5 ml of 50 ℃ molten soft agar (0.7% agar). After solidification, 10 µl of the phage suspension was spotted on the lawn of bacteria. The plates were left at room temperature for half an hour and then incubated for 24 hrs at 37 ℃ before checking for the presence of a clear zone on the plate.

2.5. Morphological Characterization of Phages by Transmission Electron Microscopy (TEM)

Purified particles of bacteriophages were stained by Na-phosphotungstate or uranyl acetate and analyzed using an electron microscope as previously described (15, 20). A total of 10^{10} pfu/ml (one drop) was placed on 200 mesh copper grids with carbon-coated formvar films and stained with Na-phosphotungstate or uranyl acetate. Finally, samples were examined by TEM in the electron microscope unit at the Faculty of Agriculture, Mansoura University.

2.6. Single-Step Growth Experiment

Growth patterns were determined by single-step growth curves as previously described (22). Briefly, phages were added at a MOI < 0.1 to the cells of *S.* Enteritidis and allowed to adsorb for 10 min at 37 ℃, and then the content was centrifuged at 10000 xg. Ten pellets containing infected cells were chosen and suspended in 10 ml nutrient broth and incubated at room temperature. After that, samples were harvested in a timely manner at 15-min intervals for 90 min, diluted, and titrated using the double-layer technique. One set of samples was immediately diluted before titration, while another was treated with 1% (v/v) chloroform to release the intracellular phages.

2.7. Characterization of Nucleic Acids from Phage Particles

The DNA of phages was extracted as previously reported through the phenol/chloroform method (23). According to the supplier's instructions, DNA was digested with two restriction enzymes, the EcoRI and the HindIII (Takara Bio Inc., Japan).

2.8. Evaluation of the Lytic Activity of Isolated Bacteriophages *in-vitro*

The efficiency of the growing culture of *S. Enteritidis* in a liquid medium in the presence of ϕSET1, ϕSET2, and ϕSET3 phages at multiplicities of infection (MOI) of 1, and 10 PFU/CFU were estimated in comparison to phage-free samples (uninfected bacterial control) at 37 ℃ at time intervals varying from 0 to 36 hrs by optical density measurement at (OD 600). Next, 0.1 mL of fresh bacterial culture and each phage filtrate were mixed with 3 mL of sterilized nutrient broth at MOI =1, alone and in a mixture. The optical densities at (OD 600) were determined over a time from zero to 36 hrs.

2.9. Effects of pH and Different Temperatures on Phage Stability

The viability of phages was determined at different pH values by enumerating the phage titer after 1 hr incubation of a 10 log10 PFU/mL suspension in a range of SM buffers. Phage suspension containing (1 × 107 pfu) was kept at various temperatures, i.e. 30, 40, 60, 55, 70, 80, 90, and 100 ℃ for 10 min. Phage infectivity was assessed by the spot test and double overlayer agar technique.

3. Results

3.1. Identification and Antibiotic Susceptibility Testing of *Salmonellae* Isolates

Seven strains out of 56 samples were isolated and properly identified as *Salmonella*e spp. (5 *S.* Enteritidis, 1 *S.* Typhimurium, and 1 *S.* Taksoni) based on biochemical identification and growth on XLD agar specific for *Salmonella* at 37 ℃ for 12 hrs (Figure 1A). The antibiotic sensitivity test showed that all strains (n=7) were resistant to ampicillin, gentamycin, vancomycin, and neomycin (100%), 85.7% (n=6) were resistant to doxycycline and amoxicillin, and more than 50% were resistant to colistin, nalidixic acid, and tetracycline. However, 100% of isolates were sensitive to imipenem (Table 1). The susceptibility of isolated serovars was tested for phage infection by using them individually as indicators.

3.2. Identification and Morphological Characterization of *S*. Enteritidis Phages by TEM

Three single clear plaques with different diameters designated as ϕSET1, ϕSET2, and ϕSET3 were isolated and selected for further characterization and purification (Figure 1B). TEM identified the morphotype of all phages. The TEM micrograph showed that all three identified phages had a long non-contractile thin tail and belonged to the Siphoviridae family. The head diameter of ϕSET1 was 38 nm and a long tail of 74 nm (Figure 2A), while ϕSET2 had an icosahedral head with a diameter of 41 nm and a long tail of 78 nm (Figure 2B), and ϕSET3 had a head diameter of 36 nm and a long tail of 68 nm (Figure 2C). The morphological characteristics of *S.* Enteritidis phages (ϕSET1, ϕSET2, and ϕSET3) are shown in Table 2.

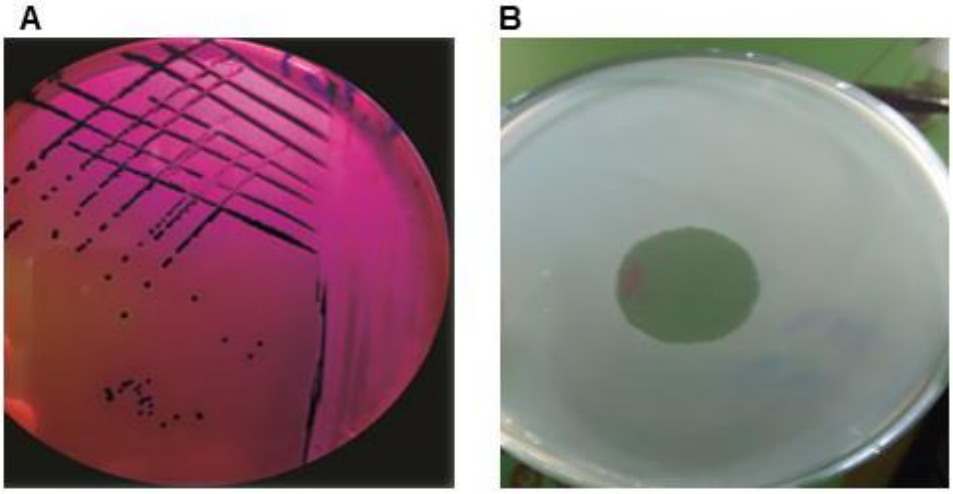

Figure 1. *Salmonella* organism on XLD agar appeared as smooth red colonies with a black center (A); clear plaque of isolated phage (B).

Table 1. Susceptibility testing of isolated *salmonellae* strains to different antimicrobials using the disc diffusion method

Antibiotics	DO	AX	AM	SXT	C	NOR	GN	CIP	IMP	CL	NA	TE	VA	S	N
S. Enteritidis 1	R	R	R	S	S	S	R	S	S	R	R	R	R	S	R
S. Enteritidis 2	R	R	R	S	S	S	R	S	S	S	R	R	R	S	R
S. Enteritidis 3	R	R	R	S	S	S	R	S	S	R	R	R	R	S	R
S. Enteritidis 4	R	R	R	R	R	S	R	S	S	R	R	S	R	S	R
S. Enteritidis 5	R	R	R	S	S	S	R	S	S	R	S	S	R	S	R
S. Typhimurium1	R	S	R	S	S	S	R	S	S	R	S	S	R	S	R
S. Taksoni	S	R	R	R	R	R	R	R	S	S	R	R	R	S	R

S = sensitive; R = resistant; DO = Doxycycline; AX = Amoxicillin; AM = Ampicillin; SXT = Trimethoprim/Sulphamethoxazole; C = Chloramphenicol; NOR = Norfloxacin; GN = Gentamycin; CIP = Ciprofloxacin; IMP = Imipenem; CL = colistin; NA = Naldixic acid; TE = Tetracycline; VA = Vancomycin; S = Streptomycin; N = Neomycin.

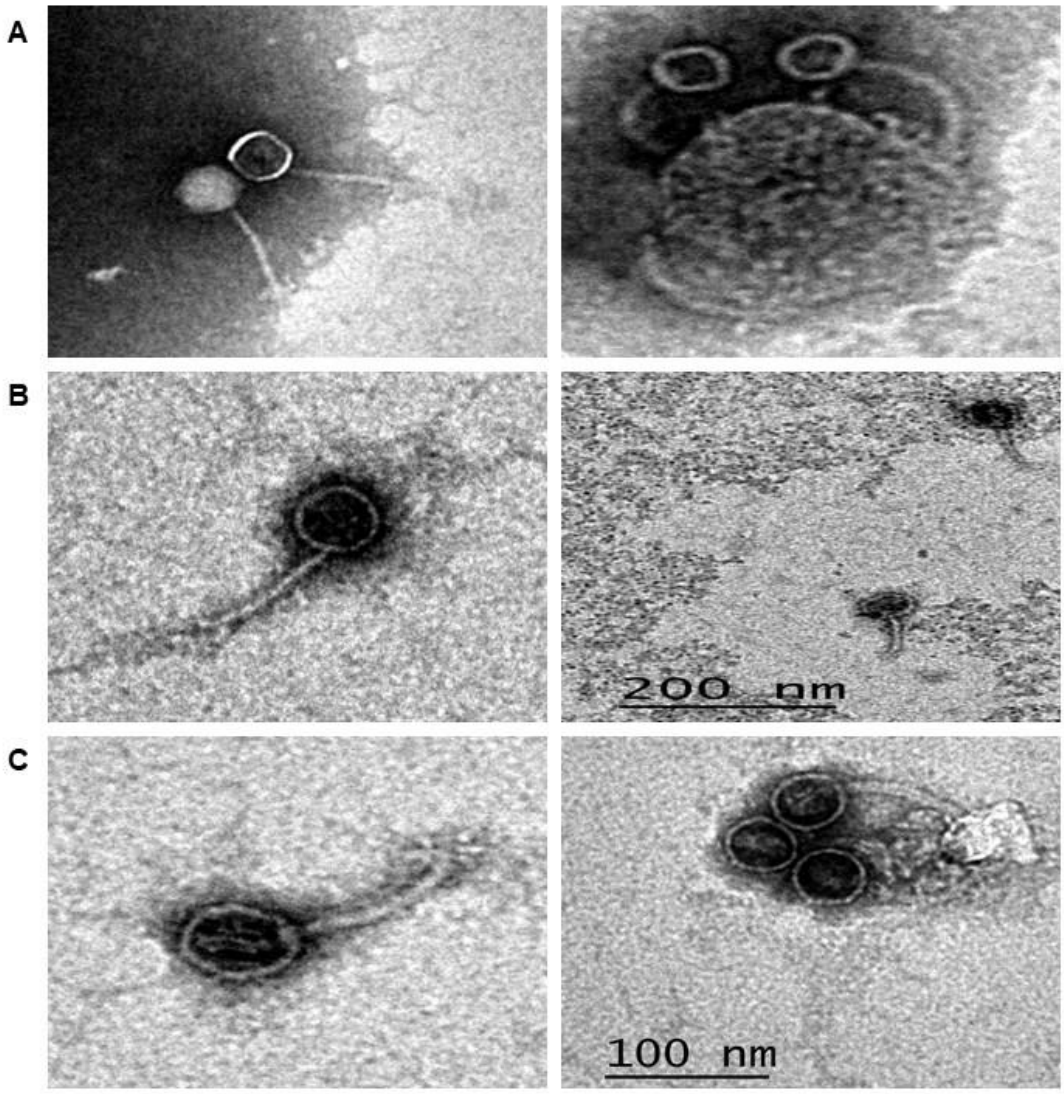

Figure 2. Electron micrograph shows *S*. Enteritidis phage particles under TEM: (A) ϕSET1, (B) ϕSET2, and (C) ϕSET3.

Table 2. Morphological characteristics of heads and tails of *S*. Enteritidis phages (ϕSET1, ϕSET2, and ϕSET3)

Phage	Head		Tail	
	Shape	Diameter (nm)	Shape	Length (nm)
ϕSET1	Icosahedral (appears spherical)	38	Long non-contractile	74
ϕSET2	Icosahedral (appears spherical)	41	Long non-contractile	78
ϕSET3	Icosahedral (appears spherical)	36	Long non-contractile	68

3.3. Host Range of *S*. Enteritidis Phages and Phage Growth Characteristics

The host range of phages ϕSET1, ϕSET2, and ϕSET3 was determined against 13 bacterial strains. The results showed a quite broad host range of these phages, as all phages were able to infect most of the tested *Salmonella* strains (Table 3). For phage ϕSET1, the latent period was 20 min followed by a rise period of 15 min. Approximately ~35 min was required for one round cycle of infection, and the average burst size was~ 100 pfu per infected cell. In phages ϕSET2 and ϕSET3, approximately 60 min was required for the one-round cycle of infection with a latent period of 30 min, and the average burst size was estimated around ~ 100 pfu per infected cell.

Table 3. Host range specificity of the isolated phages against 13 different bacterial species

Bacterial Strain 3	ϕSET3	ϕSET2	ϕSET1
S. Enteritidis 1	+	+	+
S. Enteritidis 2	+	+	+
S. Enteritidis 3	+	+	+
S. Enteritidis 4	+	+	+
S. Enteritidis 5	-	-	-
S. Taksoni	-	-	-
S. Typhimurium 1	+	+	+
S. Typhimurium 2 (standard)*	+	+	+
Escherichia coli O157	-	-	-
Pseudomonas aerigonosa	-	-	-
Pasteurella multocida	-	-	-
Klebsiella	+	+	+
Staphylococcus aureus (MRSA)	+	+	+

Positive indication (+) means that the strain is susceptible to the phage, while a negative indication (-) means that no plaques were observed.

3.4. Genomic Properties of *Salmonella* Bacteriophages

The genomic DNAs of all phages were sensitive to the different tested restriction enzymes, revealing double-stranded DNA (Figure 3). Restriction with EcoRI and HindIII resulted in the formation of multiple fragments in the three viral genomes.

3.5. Detection of Antibacterial Activity of Bacteriophages *in-vitro*

The lytic activity of ϕSET1, ϕSET2, and ϕSET3 phages was evaluated using MDR serovars *S. enteritidis* as an example of a host strain by measuring the optical density (OD) of the liquid medium during the growth of host bacteria at 37 °C and MOI 1 (Figure 4). Different patterns of inhibition were seen when *S.* Enteritidis was used as a host with the isolated three phages. All phages slightly delayed the growth of S. Enteritidis, and complete inhibition activity was observed after incubation for 24 hrs, showing promising results in minimizing the growth of *S.* Enteritidis.

3.6. Effect of Temperature and pH on the Infectivity of Isolated Phages

As shown in Table 4, the ϕSET1, ϕSET2 and ϕSET3 phages were stable at pH (5-12). On the other hand, ϕSET2 and ϕSET3 phages were relatively heat-stable throughout 1 hr in a range of 30–70 °C, while ϕSET1 was stable in a range of 30–60 °C (Table 5). The efficiency of the phage activity was not -significantly altered (Figure 5).

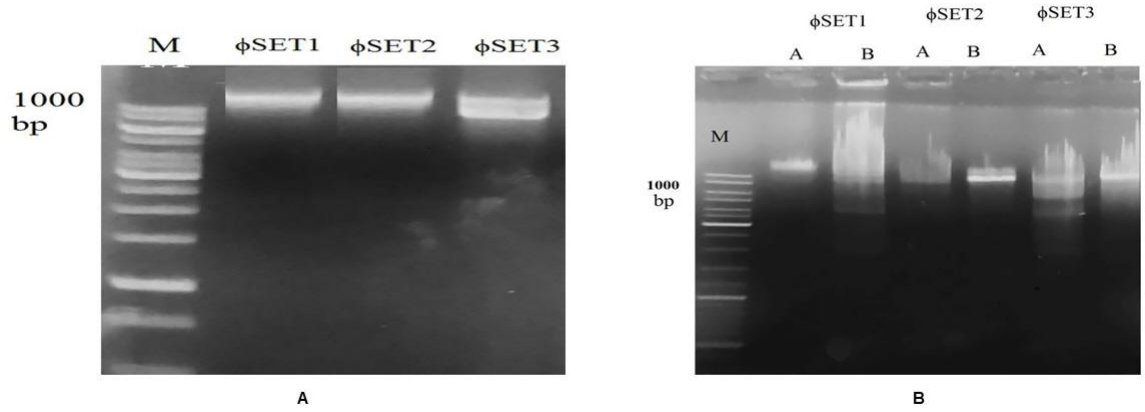

Figure 3. Restriction enzyme digestion patterns for the genomic DNAs of all phages after treatment with different restriction enzymes (Lane 1–3) ϕSET1, ϕSET2, and ϕSET3 undigested. DNA restriction endonuclease digestion of ϕSET1, ϕSET2, and ϕSET3, digested with EcoRI (A) and HindIII (B); and (M) 1 kb DNA ladder.

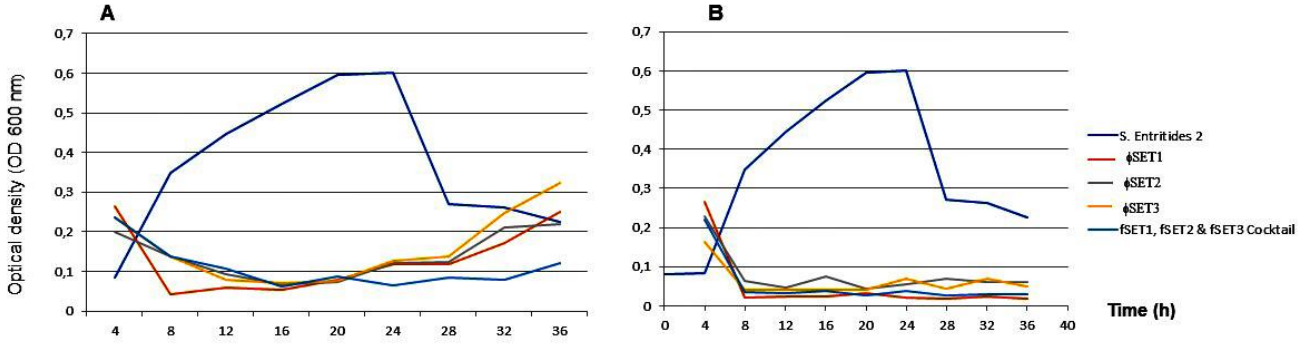

Figure 4. Growth of *S.* Enteritidis strain 2 at 37 °C in the presence of each of ϕSET1 or ϕSET2 or ϕSET3 alone or in a mixture. The growth of S. Enteritidis 2 was represented by measuring optical density at (OD 600 nm) at MOI = 1 (A) and in a mixture at MOI = 10 (B).

Table 4. Effect of pH on the infectivity of isolated phages

PH	ϕSET1		ϕSET2		ϕSET3	
	PFU/ml × 10⁷	***%***	***PFU/ml × 10⁷***	***%***	***PFU/ml × 10⁷***	***%***
3	0	0	0	0	0	0
5	1.25	89.2	2.9	78.4	1.03	33.2
7 (control)	1.4	100	3.7	100	3.1	100
9	1.2	85.7	3.5	94.6	1.64	52.9
12	1.12	80	2.8	75.6	1.17	37.7

Table 5. Effect of temperature on the infectivity of isolated phages

Temperature (°C)	ϕSET1		ϕSET2		ϕSET3	
	PFU/ml × 10^8	*%*	*PFU/ml × 10^8*	*%*	*PFU/ml × 10^8*	*%*
4	8.3	100	7.9	100	7.6	100
37-40	7.9	95.2	7.8	98.7	7.3	96
50	3.6	43.4	7.6	96.2	6.7	88.1
60	1	12	3.1	39.2	2.8	36.8
70	0	0	0.8	10.1	0.4	5.2
80	0	0	0	0	0	0

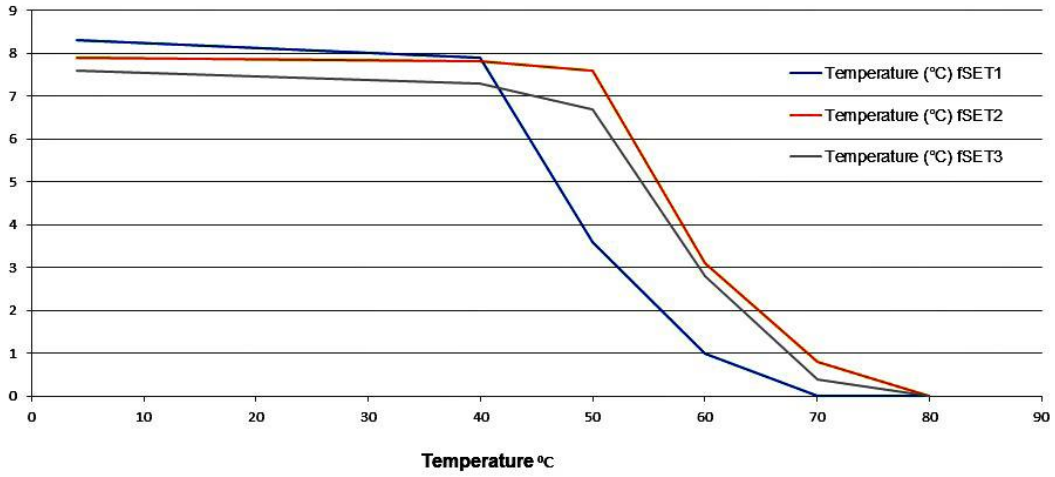

Figure 5. Effect of heat on the *S.* Enteritidis phages stability measured by Log (PFU/ml) showed no significant loss in phage activity.

4. Discussion

It is presumed that AMR will be the leading cause of death worldwide by 2050, exceeding other causes of death such as cancer or traffic road accidents (24). The emergence of antibiotic resistance in *S.* Enteritidis and concerns about the misuse of antibiotics have provided strong motivation to find optimal alternatives for eradicating S. Enteritidis from poultry. *Salmonella* is considered one of the leading causes of foodborne outbreaks in Egypt, and poultry and poultry products are mainly considered the most important human salmonellosis vehicles (25-27). It has been shown that applying phages to contaminated food inhibits bacterial growth and ensures food safety (28-30). Phage therapy has been shown to have many advantages compared to traditional antibiotics and has a bactericidal effect against both Gram-negative and Gram-positive bacteria (31, 32). It is used as an alternative or a supplement to antibiotics during treatment (33). Moreover, phages can be isolated rapidly due to their wide distribution in nature, which reduces the cost of development compared to other antimicrobial compounds such as antibiotics. Bacteriophages have been applied widely in food preservation to extend the shelf life of foods (14, 17). Mainly when used in a cocktail, they have been shown to have a higher impact on targeted bacteria (32). Herein, the isolation, morphological characteristics, and antibacterial activity of lytic bacteriophages that infect *S.* Enteritidis have been described.

Using lytic phages to control salmonellosis in the food chain has become a prominent biocontrol agent method (34). Lytic phages have been reported to be more suitable than lysogenic phages (35). In this

study, seven strains of *Salmonellae* (5 *S.* Enteritidis, 1 S. Typhimurium, and 1 S. Taksoni) initially isolated from diseased living and freshly dead avian species showed MDR patterns and resistance to three or more tested antibiotics in the experimental settings. In Egypt, the current method for controlling S. Enteritidis in poultry depends on applying a prophylactic dose of antibiotics, in addition to farm biosecurity, but these strategies are increasingly unreliable. Therefore, further approaches to combatting such deadly pathogens are required to save our local poultry industry. Furthermore, poultry consumers' concerns regarding contamination of poultry meat with harmful concentrations of drug residues are increasing (32, 33). Three bacteriophage-infecting strains of *Salmonella* were isolated from chicken carcasses and sewage tanks in Qalubiya governorate, Egypt. Importantly, electron microscopy analyses showed that the three phages were characterized as siphoviruses. Bacteriophages ϕSET1, ϕSET2, and ϕSET3 showed high efficacy in reducing viable bacteria counts of various *Salmonella* serovars *in-vitro*. This is consistent with previous findings (36), which confirmed that the obtained phages could combat these antibiotic-resistant strains, aiming to control AMR *Salmonella.* Research on phage therapy has just begun in Egypt, and only a few studies have been published (37-40). Two broad-spectrum lytic *Salmonella* phages, namely SPHG1 and SPHG3, were isolated from the same governorate (38). They had high lytic activities against MDR S. Typhimurium, and lysis time was estimated as one hour, while burst sizes were 104 and 138 PFU/cell, respectively.

One of the crucial factors that affect phage activity is temperature. The current results showed that ϕSET1, ϕSET2, and ϕSET3 phages could resist high temperatures and were thermostable in a range of 30–70 °C. Interestingly, all phages survived at 37-40 °C without remarkable loss in phage particle number. This is one of the significant parameters taken into consideration for phages during therapeutic application. Different latent period lengths and burst sizes have been found among *Salmonella*-specific phages. Previous reports have shown small burst sizes and long latent periods in some *Salmonella*-specific phages. For example, ϕ *st*1 (22 pfu cell^{-1}, 40 min) (41), and phage Felix 01 (14 pfu cell^{-1}, 60 min) (42). In contrast, others possess shorter latent periods and large burst sizes ranging from 100 to 200 pfu cell^{-1} (43). The latent period and burst size differences may result from differences in medium, pH, temperature, or host cell (44). It is worth mentioning that optimum latent periods and burst sizes have not yet been identified. However, there is evidence that these parameters can be changed considerably according to physiological and metabolic status and host density (45-47). Few studies have reported the use of phages in the treatment of infection with *S. Enteritidis* (48-50). In the current study, most strains examined were susceptible to ϕSET1, ϕSET2, and ϕSET3; thus, a phage cocktail containing a mixture of these phages may create a better effect.

Poultry are highly susceptible to *Salmonella* infection, and decreasing the incidence of infection before processing is necessary. Several alternative compounds such as probiotics, prebiotics, and organic acids were applied to reduce infection (51). Using a phage cocktail is advisable to cover a broader inhibitory spectrum and to avoid, to some extent, the development of resistance. In conclusion, *Salmonella* phages can be used to treat MDR S. Enteritidis infection, and they may be alternative natural antibacterial compounds for reducing S. Enteritidis in the poultry industry. Further studies are recommended to investigate the possible use and application of bacteriophages in poultry farms in routine treatment programs.

Authors' Contribution

Methodology, H.S., E.A.S., and AA.; Software, W.A.; Validation, A.A.A., F.I.E, and G.W.; Investigation, E.M.E.; Writing—Original Draft Preparation, H.S.; Writing—Review and Editing, W.A and G.W.; Supervision, A.A.A., F.I.E., and A.A. All authors have read and agreed to the published version of the manuscript.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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