

ISOLATION AND INITIAL CHARACTERIZATION OF A *Myoviridae* PHAGE FOR CONTROLLING ZONOTIC *Salmonella* Typhimurium AND *Salmonella* Enteritidis FROM BROILERS IN EGYPT

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Abstract: This study targeted isolation and characterization of phage against multidrug resistant (MDR) *Salmonella* Typhimurium and *Salmonella* Enteritidis recovered from broilers and to evaluate the lytic effect of the phage on growth of *Salmonella* serovars. *Salmonella* isolates were recovered from caecal contents, liver and breast meat of broiler chickens from retail outlets at Sharkia Governorate, Egypt. *Salmonella* Typhimurium (n=14) and *S. Enteritidis* (n=11) were tested for their antimicrobial susceptibilities against 15 antimicrobials by disc diffusion method. Isolates of *S. Typhimurium* and *S. Enteritidis* were 100% resistant to seven antimicrobial agents. The phage was isolated from Zagazig sewage water by spot test and double over layer agar assay. The phage designated as phiSalmchick1 showed an icosahedral head and contractile tail structure in electron microscopy, indicating a member of the family *Myoviridae*. The phage was a polyvalent infecting a wide host range of all MDR strains of *S. Typhimurium*, *S. Enteritidis*, *S. Paratyphi*, *E. coli* serotypes O26 and O168 and *Klebsiella pneumoniae*. Myovirus phage had burst size of 100 plaque forming unit (PFU)/cell with latent period of 60 min. The phage genome had double-stranded DNA by molecular analysis. The lytic effect of phiSalmchick1 phage was *in vitro* assessed on growth of *S. Typhimurium* and *S. Enteritidis* isolates by measuring the optical density (OD) of the liquid media during *Salmonella* growth at 37 °C and the multiplicity of infection (MOI) was equal to 1.0. Significant reductions were observed in OD of *S. Typhimurium* and *S. Enteritidis* treated with the phage after 24 hrs incubation compared to the controls (P<0.05). The myovirus has a high potential for phage application to control zoonotic and MDR *Salmonella* serovars isolated from broiler chickens in Egypt.

Key words: multidrug resistant; *Salmonella* serovars; *Myoviridae* phage; broiler chickens; *in vitro* control

Introduction

Salmonellosis is an occupational anthro-poozoonoses and constitutes economic and public health concern (1). *Salmonella* is the main cause of foodborne illness in human as sixteen

million human cases had typhoid fever, approximately 1.3 billion cases had gastroenteritis and annually deaths of 3 million were recorded (2). The raw eggs, poultry meat and other chicken products are responsible for the sporadic and epidemic salmonellosis in

humans (3). In Egypt, the contamination of poultry meat is derived from infected poultry, cross-contamination with bird dropping, instruments and hands of workers during processes of slaughtering, defeathering and scalding (4). The genus *Salmonella* comprises more than 3000 serovars; and the most important serovars are *S. Pullorum*, *S. Gallinarum*, *S. Enteritidis* and *S. Typhimurium*. In Egyptian poultry farms, *S. Typhimurium* and *S. Enteritidis* have been distinguished as the most predominant serovars (5). The antibiotics were inappropriately used in chicken farms in Egypt, which resulted in the spread of multidrug resistant (MDR) *S. Typhimurium* and *S. Enteritidis* (6). The increase of *Salmonella* resistance to most antimicrobials utilized for human therapy creates a zoonotic threat (7). Therefore, bacteriophages are used as potential therapeutic agents for biocontrol of MDR bacteria in poultry (8). Bacteriophages are viruses that have the ability to attack only bacterial cells with rapid killing and self-replication and are safe for eukaryotic cells (9,10). Three phages (Salmacey1, Salmacey2 and Salmacey3) were completely inhibiting the growth of *S. Kentucky* isolated from broilers in Egypt (11). Thereby, this study was aimed to isolate and characterize phages targeting MDR *S. Typhimurium* and *S. Enteritidis* isolated from retailed broiler chickens in Sharkia Governorate, Egypt, as well as to assess the lytic efficacy of the isolated phage for controlling *Salmonella* serovars in broilers.

Material and methods

Collection and preparation of chicken samples

A total of 150 samples including swabs from caecal contents (n=60), liver (n=50) and breast meat (n=40) were collected from broiler chickens marketed at different retail outlets at Sharkia Governorate, Egypt. The samples were individually collected in sterile plastic bags, then kept in an insulated box with packs of ice and sent immediately to the laboratory.

Isolation and identification of Salmonella

The swabs from caecal contents were pre-enriched in buffered peptone water (BPW, Oxoid, UK); while liver and breast meat (25 g/each) were mixed with 225 ml of BPW then homogenized using a stomacher at 230 rpm for 5 min. The swabs and tissue mixtures were incubated at 37 °C for 18 hrs. The isolation of *Salmonella* from caecal contents, liver and breast meat of retailed broilers was performed according to the method of ISO-6579 (12). In brief, the pre-enriched cultures (0.1 ml) was added to 10 ml of Rappaport-Vassiliadis soy peptone broth (Oxoid, UK) then incubated at 41.5 °C for 18-24 hrs. A Loopful from enriched broth was streaked on the surface of Xylose Lysine Desoxycholate (XLD, Oxoid CM0469, UK) agar plates, and then incubated at 37 °C for 24 hrs.

The presumptive *Salmonella* isolates were biochemically identified using catalase, oxidase, indole, triple sugar iron, methyl red, Voges-Proskauer, citrate and urea hydrolysis, H₂S tests according to the described protocols in Bacteriological Analytical Manual (13). The isolates of *Salmonella* were serotyped by using rapid diagnostic *Salmonella* antisera sets (Denka Seiken CO., Japan) at Serology Unit, Animal Health Research Institute, Dokki, Giza, Egypt as previously described (14). Stock cultures of *Salmonella* isolates were stored in 20% glycerol at -80°C.

Antimicrobial susceptibility testing

Twenty-five *Salmonella* isolates including *S. Typhimurium* (n=14) and *S. Enteritidis* (n=11) were tested for their antimicrobial susceptibility against 15 antibiotics by Kirby-Bauer agar disc diffusion method (15). The antibiotic panels and their concentrations including amoxicillin-clavulanic acid (AMC, 30 µg), ampicillin (AMP, 10 µg), gentamycin (CN, 10 µg), amikacin (AK, 30 µg), tetracycline (TE, 30 µg), imipenem (IPM, 10 µg), sulphamethoxazole (RL, 25 µg), streptomycin (S, 10 µg), rifampin (RD, 5 µg), trimethoprim / sulphamethoxazole (SXT, 25 µg), cefoxitin (FOX, 30 µg), ciprofloxacin (CIP, 5 µg), ceftriaxone (CRO, 30 µg) and

cephazolin (KZ, 30 µg) were obtained from Thermoscientific Oxoid (USA); while trimethoprim (W, 5 µg) were obtained from Bio-Rad (France). The bacterial isolates were enriched in brain heart infusion broth (Oxoid CM0337, UK) then incubated at 37°C for 24 hrs. A loopful of bacterial growth was mixed with 5 ml of tryptone soya broth (Oxoid, UK), followed by incubation at 37°C for 24 hrs till reaching the turbidity of 0.5 McFarland standard as previously described (16). The bacterial suspensions were inoculated on Mueller Hinton agar plates, then discs of antibiotics were placed on the plates, and subjected to incubation at 37°C for 18 hrs. The diameters of inhibition zones and interpretation of *Salmonella* isolates were in accordance to the guidelines of the relevant CLSL document (17).

Bacterial strains

Thirteen bacterial isolates including *S. Paratyphi* from blood of humans (n=3), *Escherichia coli* O168 from dropping of ducks (n=6); *Proteus vulgaris* from urine of patients (n=2) and *Klebsiella pneumoniae* from sputum of patients (n=2) were kindly obtained from Microbiology Department, Faculty of Science, Zagazig University. Also, twelve bacterial strains comprising *E. coli* O26 from milk of dairy cows (n=5), *Staphylococcus aureus* from edible parts of fish (n=4) and *Listeria monocytogenes* from litters of broilers and meat products (n=3) were obtained from Zoonoses Department, Faculty of Veterinary Medicine, Zagazig University.

Phage isolation

The bacteriophage was isolated from sewage water samples from different stations at Zagazig City, Egypt using the enrichment technique (18). Briefly, a sewage sample was centrifuged at 10,000 xg for 15 min, then filtered using a sterile 0.45 µm Millipore filter (Steradisc, Kurabo Industries LTD., Japan). Fifty milliliters of the filtrate was added to an equal volume of Luria-Bertani (LB, Oxoid, UK) broth, which was inoculated with 1 ml of *Salmonella* serovars, then subjected to incubation with shaker at 120 rpm at a

temperature of 37°C/24 hrs. The centrifugation of bacterial cultures was performed at 10,000 xg/10 min. The supernatant was filtered using a sterile 0.45 µm Millipore filter, then used as a phage source to be found on the propagative *Salmonella* isolates. The bacterial lawns of *S. Typhimurium* and *S. Enteritidis* were subjected to propagation on LB plates using the double agar overlay method as previously explained (18). Two hundred µl of each *Salmonella* serovar (OD of 0.4 at 600 nm) were added to semi-solid LB agar (4 ml) then poured over nutrient agar plates. The droplets of formerly prepared bacteriophage source (10 µl) were spotted on the bacterial lawns, and then were left for drying. Incubation of plates was carried out at 37 °C overnight and checked for existence of the lytic zones.

Purification and propagation of phage

The bacteriophage was purified by three single plaque isolation assays using sterile pasture pipette (11,18). In brief, a single plaque was picked up, then placed in 0.5 ml of nutrient broth harboring 100 µl of *Salmonella* Typhimurium, then subjected to incubation at 37°C with shaking at 1200 rpm. Afterwards, centrifugation of phage-bacteria mixture was done at 10,000 xg for 10 min. The supernatant was filtered via a sterile 0.45 µm Millipore filter to exclude any bacterial cells. Storage of purified phages was done at 4°C.

Determination of phage host range

The isolated bacteriophage was tested against 50 isolates of pathogenic bacteria including *S. Typhimurium* (n=14), *S. Enteritidis* (n=11), *S. Paratyphi* (n=3), *E. coli* O26 (n=5), *E. coli* O168 (n= 6), *P. vulgaris* (n=2), *K. pneumoniae* (n=2), *S. aureus* (n=4), *L. monocytogenes* (n=3) to detect the host range as previously explained (19). The propagation of bacterial lawns was achieved on LB agar plates, followed by addition of 10 µl of phage droplets (1×10^7 plaque forming unit, PFU/ ml). These plates were incubated at 37°C/ 24 hrs then tested for existence of plaques (lytic zones). Efficient phage was selected on the bases of lysis profile, clarity of plaque and size.

Transmission electron microscopy analysis

A drop of phage suspension (10^7 PFU/ml) was located on 200 mesh copper grids with the carbon-coat films, and the excess was removed with a filter paper. The uranyl acetate as a saturated solution was placed on grids, and the excess was removed by filter paper. The purified phage particle was stained with uranyl acetate, and then was examined using an electron microscope (Hitachi H600A, Japan) at Mansoura University, Egypt as previously described (20).

One -step growth experiment

The single-step growth curve of the isolated bacteriophage was determined as previously explained (21). The phage was added at a multiplicity of infection (MOI) = 1.0 to *S. Typhimurium* cells then the phage was allowed to be adsorbed on host cells at 37° C/ 10 min. The phage-host mixture was centrifuged at 10,000 xg for 10 min, and the pellet harboring infected cells was suspended in 10 ml of nutrient broth then incubated at room temperature. The specimens were taken in duplicate at 15 min interval for a period of 150 min. The titer of phage was estimated by the double-layer plaque assay. The first sample set was subjected to dilution before titration; while, 1% chloroform (V/V) was used to treat the second sample set for the release of intracellular phages and determination of the eclipse period. Three independent assays were carried out. The means of relative burst size were calculated $\pm$ standard deviation (SD).

Nucleic acids characterization of phage particle

The genomic DNA of isolated phage was extracted by the phenol/chloroform method as previously described (22). Three restriction enzymes (*Eco*R1, *Hind*III and *Bam*H1) were used to digest phage DNA according to the manufacturer's instructions (Takara Bio Inc., Japan). The restriction required a time ranging from 2-4 hrs. The analysis of DNA digestion mixtures was performed by electrophoresis (at 100 V) in agarose gel (1.0%) stained with the ethidium bromide using a DNA ladder.

Effects of phiSalamchick1 on the growth of Salmonella serovars

The efficacy of isolated phiSalamchick1 were evaluated on the growth of MDR *S. Typhimurium* and *S. Enteritidis* isolated from retailed broilers in a liquid media over a time period from zero time (starting point of host-phage incubation) to 42 hrs by measurement of the optical density (OD 600) by using UV-Spectrophotometer (UNICO 1200, USA). The freshly prepared bacterial culture of each *Salmonella* serovar (0.1 ml) and phage filtrate (0.1 ml) were added to sterile nutrient broth (3 ml) at MOI equals to 1.0 starting from zero time to 42 hrs. The optical density at OD 600 was measured each 6 hrs for the control (bacterial culture alone) and for the treated bacterial broth (bacteria+phage) as previously described (11). This experiment was performed for independently three times. The means of bacterial OD were calculated $\pm$ standard deviation (SD).

Statistical analysis

The data for *Salmonellae* distribution in different samples of broilers was analyzed by IBM SPSS Statistics (vers.22) to compute p-values for Pearson chi-square. The analysis of data for single step growth curve experiment was done by one way analysis of variance (ANOVA) followed by the post hoc Duncan's test (IBM SPSS Statistics, version 22). Also, the data concerning *in vitro* assessment of phiSalamchick1 on the growth of *Salmonella* serovars was analyzed using independent sample T. test (IBM SPSS vers.21).

Results

Isolation and serotyping of Salmonella isolates from broilers

In this study, only 25 *Salmonella* isolates were serotyped into *S. Typhimurium* (n=14) and *S. Enteritidis* (n=11) out of 150 samples from broilers at various retail outlets at Sharkia, Egypt. *Salmonella Typhimurium* showed insignificant distribution ($P>0.05$): 11.7% (7/60) in caecal contents, 8% (4/50) in liver and 7.5% (3/40) in breast meat. The distribution of

S. Enteritidis was significantly higher (12.5%, 5/40) in breast meat followed by 10 % (6/60) in caecal contents ($P < 0.05$), while it was not detected in liver of retailed broilers.

Antimicrobial susceptibility testing

The antibiotic resistance patterns of recovered *S. Typhimurium* and *S. Enteritidis* to 15 antimicrobials of various classes were determined by the disc diffusion method (Table 1). *Salmonella* Typhimurium strains showed moderate sensitivity (64.2%) to cephazolin followed by ceftriaxone (57.1%), while *S. Enteritidis* isolates illustrated higher sensitivity (81.8%) to cephazolin followed by cefoxitin (72.2%). Moreover, *S. Typhimurium* showed higher resistance (85.7%) to streptomycin followed by ampicillin (71.4%) and then a moderate resistance (64.2%) to amikacin and rifampin. The isolates of *S. Enteritidis* expressed higher resistance (81.8%) to streptomycin then followed by a similar resistance percentage (72.7%) to ampicillin, amikacin and rifampin. Of interest, both isolates of *S. Typhimurium* and *S. Enteritidis* were 100% resistant to seven antimicrobial agents (amoxicillin-clavulanic acid, gentamycin, tetracycline, imipenem, trimethoprim, sulphamethoxazole, trimethoprim / sulphamethoxazole) as shown in Table (1). This result proved that the two isolated *Salmonella* serovars were MDR to several classes of antimicrobials.

Isolation and morphology of phage

The bacteriophage against MDR *Salmonella* serovars was detected from Zagazig sewage water by spot test and double over layer agar technique. We picked up single plaque for propagation and characterization (Figure 1A). Four phage particles were observed during electron micrograph. The isolated phage was designated as phiSalmchick1. Under electron microscopy, the phage had an icosahedral head (130 ± 5 nm in diameter, $n=3$) and long contractile tail with length of (133 ± 5 nm in diameter, $n=3$). The phage (phiSalmchick1) appeared to be a member of *Myoviridae* family based on its morphological features (Figure 1B).

Host range of phage

Our results showed that phiSalmchick1 could infect all MDR strains of *S. Typhimurium*, *S. Enteritidis*, *S. Paratyphi*, *E. coli* serotypes O26 and O168 and *Klebsiella pneumoniae*; and had lytic effects on such strains; while this phage could not infect *Proteus vulgaris* strains or other Gram positive bacteria (*S. aureus* & *L. monocytogenes*) as shown in Table (2). This finding indicated that phiSalmchick1 was a polyvalent phage and had a wide host range.

Characteristics of phage growth

From the curve (Figure 2), the latent period of phiSalmchick1 was 60 min and one cycle took 120 min. The average burst size of this myovirus was 100 PFU per infected cell (Figure 2). There was a significant increase in average burst size at 105 min compared to those burst sizes at time of 90 and 75 min ($P < 0.001$).

Genomic digestion with restriction enzymes

The genomic DNA of phage was sensitive to digestion with *EcoR*I and *Hind*III restriction enzymes. This indicated that the myovirus was a double stranded DNA. However, the phage DNA was resistant to digestion with *Bam*H1 enzyme.

Effects of phiSalmchick1 on the growth of MDR salmonellae

The lytic effects of phiSalmchick1 on the growth of *S. Typhimurium* and *S. Enteritidis* isolates were assessed by measuring OD₆₀₀ of the bacterial culture infected with the phage at MOI of 1.0 and incubated at 37 ° C (Figure 3). The values of standard deviation (SD) were small ranging from 0.005 to 0.01, and were not appeared in Figure (3). From zero to 12-18 hrs post infection, the growth of bacterial cells were inhibited by phage infection (OD values were much lower compared with uninfected controls) and after 12-18 hrs post infection, the bacterial lysis was significantly apparent ($P < 0.05$). The net reduction of bacterial density was significantly detected after 24 hrs from the infection point ($P < 0.05$). The growth of MDR salmonellae was inhibited by phiSalmchick1

infection and the bacterial populations were (P<0.05) and reached to complete lysis at 42 hrs significantly reduced 24 hrs post treatment incubation (Figure 3).

Table 1: Antimicrobial resistance patterns of *S. Typhimurium* and *S. Enteritidis* isolates from retailed broilers by disc diffusion method

Antimicrobials (disc content/ μ g)	S. Typhimurium (no=14)			S. Enteritidis (no=11)		
	R	I	S	R	I	S
AMC(30)	14(100)	-	-	11 (100)	-	-
AMP(10)	10(71.4)	3(21.4)	1(7.1)	8(72.7)	2(18.1)	1(9.09)
CN(10)	14 (100)	-	-	11 (100)	-	-
AK(30)	9(64.2)	2(14.2)	3 (21.4)	8 (72.7)	1 (9.09)	2 (18.1)
TE(30)	14 (100)	-	-	11(100)	-	-
IPM(10)	14 (100)	-	-	11(100)	-	-
W(5)	14(100)	-	-	11(100)	-	-
RL(25)	14(100)	-	-	11(100)	-	-
S(10)	12(85.7)	2 (14.2)	-	9 (81.8)	1 (9.09)	1 (9.09)
RD(5)	9 (64.2)	2 (14.2)	3 (21.7)	8 (72.7)	2 (18.1)	1(9.09)
SXT (25)	14 (100)	-	-	11(100)	-	-
FOX(30)	3 (21.4)	4 (28.5)	7 (50)	1 (9.09)	2 (18.1)	8 (72.7)
CIP(5)	4 (28.5)	4 (28.5)	6(42.8)	2 (18.1)	2 (18.1)	7 (63.6)
CRO(30)	3 (21.4)	3 (21.4)	8 (57.1)	2 (18.1)	3 (27.2)	6(54.5)
KZ(30)	3 (21.4)	2 (14.2)	9 (64.2)	1(9.09)	1(9.09)	9 (81.8)

R: resistant; I: intermediate; S: sensitive.

Data were represented by No (%).

Where AMC: amoxicillin-clavulanic acid; AMP: ampicillin; CN: gentamycin; AK: amikacin; TE: tetracycline; IPM: imipenem; W: trimethoprim; RL: sulphamethoxazole; SXT: trimethoprim/sulphamethoxazole; S: streptomycin; RD: rifampin; FOX: cefoxitin; CIP: ciprofloxacin; CRO: ceftriaxone; KZ: cephalazolin.

Table 2: The host range of isolated phage phiSalmchick1

Strains of Bacteria	No. of isolates	PhiSalmchick1	Source of strains
<i>Salmonella</i> Typhimurium	14	+	This study
<i>Salmonella</i> Enteritidis	11	+	This study
<i>Salmonella</i> Paratyphi	3	+	MSZU
<i>Escherichia coli</i> O26	5	+	ZVZU
<i>Escherichia coli</i> O168	6	+	MSZU
<i>Proteus vulgaris</i>	2	-	MSZU
<i>Klebsiella pneumoniae</i>	2	+	MSZU
<i>Staphylococcus aureus</i>	4	-	ZVZU
<i>Listeria monocytogenes</i>	3	-	ZVZU

+: Bacterial strain was susceptible to the bacteriophage phiSalmchick1 and the plaques were produced.

-: Bacterial strain was resistant to the bacteriophage phiSalmchick1 and the plaques were not produced.

MSZU: Microbiology Department, Faculty of Science, Zagazig University, Egypt.

ZVZU: Zoonoses Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

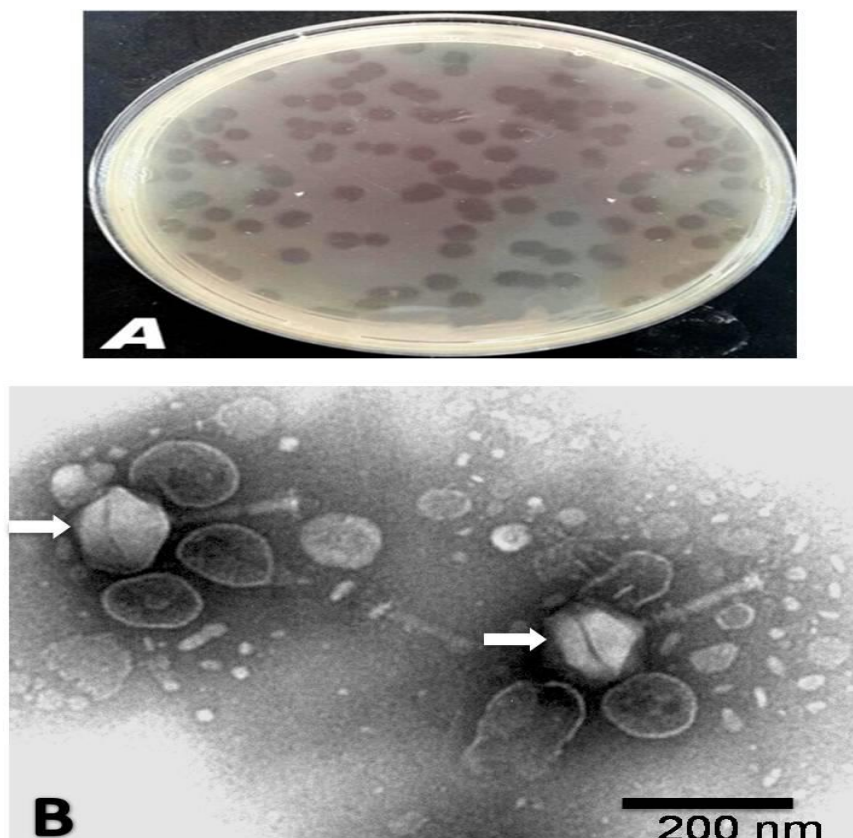


Figure 1: Plaque morphology and electron micrograph of phage phiSalmchick1. 1A: Plaque morphology produced on *S. Typhimurium* as a bacterial host. 1B: Electron micrograph of *Myoviridae* phage (phiSalmchick1) under transmission electron microscope

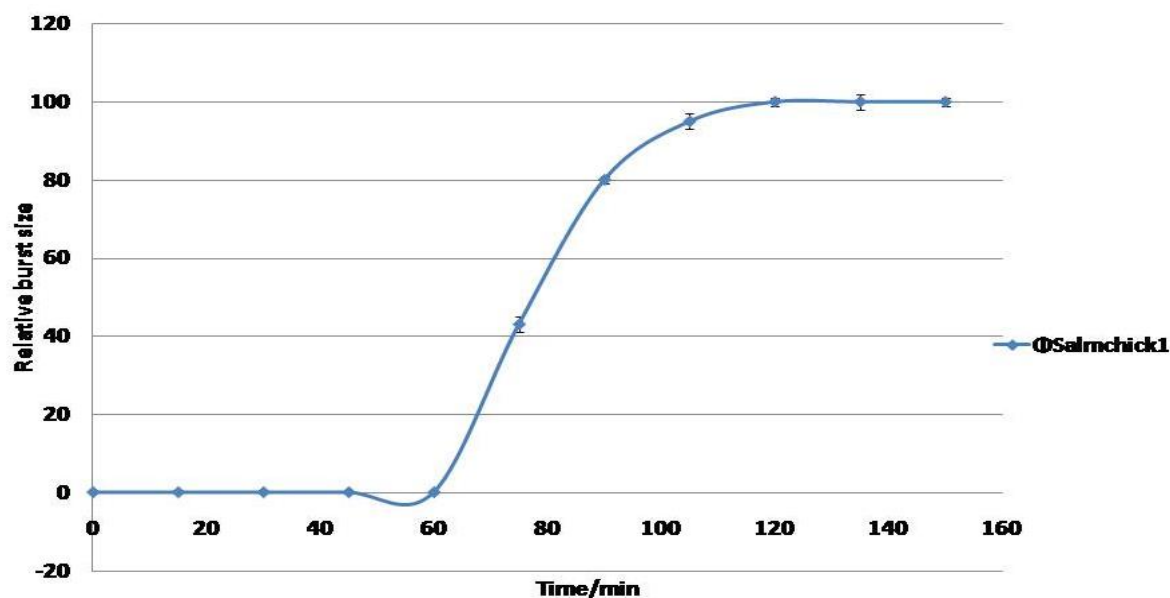


Figure 2: Single-step growth curve of the phage phiSalmchick1. The plaque forming Units (PFUs) per infected cell in *S. Typhimurium* cultures were calculated at different times after treatment. The samples were taken with an interval of 15 min. The means of relative burst size were calculated $\pm$ standard deviation (SD)

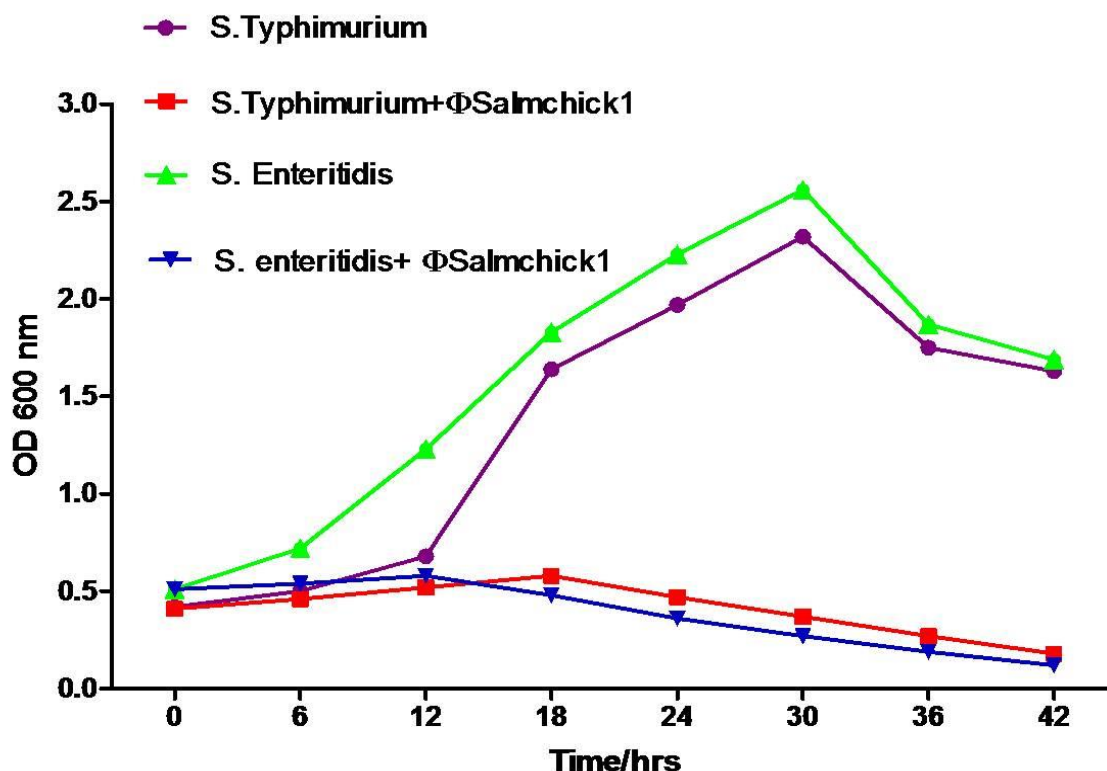


Figure 3: Effects of phage phiSalmchick1 on the growth of *S. Typhimurium* and *S. Enteritidis* at 37°C at a MOI of 1.0. Each data point was representing means of bacterial OD $\pm$ standard deviation (SD). The values of SD were small ranging from 0.005 to 0.01; and were not appeared on the curves

Discussion

Our study showed that all recovered isolates of *S. Typhimurium* and *S. Enteritidis* from retailed broilers showed 100% resistance to seven antimicrobial agents. So, this study was handled with the isolation and characterization of specific bacteriophages targeting those MDR isolates of *S. Typhimurium* and *S. Enteritidis* derived from caecal contents, liver and breast meat of retailed broilers in Sharkia Governorate, Egypt. In this regards, one bacteriophage infecting *Salmonella* serotypes was firstly isolated from sewage water at Zagazig City, Sharkia Governorate, Egypt. It was appeared that the plagues formed by the isolated bacteriophage were clear, indicating that phiSalmchick1 is a lytic phage as previously supported (23). The isolated phage (phiSalmchick1) was belonging to family *Myoviridae* under electron micrograph. Of

interest, the recovered myovirus phage was polyvalent and had a broad host range. Similarly, three phages belonging to *Siphoviridae* and *Myoviridae* (Salamacey1, Salamacey2 and Salamacey3) were polyvalent and had lytic effects on *S. Typhimurium*, *S. Enteritidis* and *S. Kentackey* originated from broilers and could infect other *Enterobacter cloacae* and *E. coli* isolates of broiler chicken (11). Moreover, polyvalent phages having ability to infect *E. coli* and *S. Choleraesuis* were reported in previous studies (24,25). However, phage SES8 recovered from chicken skin posed the narrowest host range as it induced lysis for only 2 out of 22 *Salmonella* isolates in Japan (26). The degree of phage specificity or susceptibility relies on phage attachment or adsorption on bacterial cell in the initial stage of phage infection cycle (27). The process of phage adsorption often includes specific binding of receptor-binding proteins (RBPs) in

bacteriophages and the binding of specific carbohydrate receptors located on cell wall of host bacteria (28).

Regarding one-step growth curve of myovirus, phiSalmchick1 phage had a large burst size 100 PFU per infected cell with latent period of 60 min. Similarly, the phages Salmacey3 and STS9 had large burst size of 110 and 209 PFU/ infected cell, respectively (11,26). On the other hand, the *Salmonella*-bacteriophage Felix 01 showed a small burst size of 14 PFU/cell (29). The differences in latent period and burst size of various bacteriophages could be augmented for the variations in bacterial host cell, temperature and pH (30,31). Interestingly, the isolated phage (phiSalmchick1) had a short latent period and a large burst size verifying its applicability for the control and treatment of salmonellosis in poultry and humans as previously supported (32). The genome of phiSalmchick1 was sensitive to digestion with *EcoR*I and *Hind*III enzymes. This was corroborated by a previous study, where the genome of myovirus Salmacey3 was digested by *EcoR*I and *Hind*III (11).

Phages were used to control food poisoning or zoonotic pathogens (33). Several studies have demonstrated the potential use of lytic phages to treat infections with zoonotic *Salmonella* serovars (25,34). In this study, there were significant reductions in the OD of *S. Typhimurium* and *S. Enteritidis* treated with phage phiSalmchick1 at MOI/1.0 and at 37°C after 24 hrs incubation when compared to OD values of non-treated *S. Typhimurium* and *S. Enteritidis* ($P < 0.05$). Similar studies evaluated *in vitro* the lytic activity of different phages on various *Salmonella* serovars from broiler chickens: Mahmoud et al. (11) cited OD reduction of treated *S. Kentucky* with three phage cocktails, Salmacey1, Salmacey2 and Salmacey3, after 24 hrs; Duc et al. (25) reported a significant reduction in the count of *S. Enteritidis* co-incubated with phages SEG5, SES8, STG2, STG5, and STS9 to less than 10 CFU/ml after 24 hrs; Atterbury et al. (35) observed significant reductions in counts of *S. Enteritidis*, *S. Typhimurium* and *S. Hadar* co-

treated with the phages $\Phi 151$, $\Phi 10$ and $\Phi 25$ by means of 2.2, 5.9 and 2.5 log, respectively after 24 hrs incubation.

Conclusion

This study provided polyvalent, lytic phage (phiSalmchick1) belonging to family *Myoviridae* against MDR isolates of *S. Typhimurium* and *S. Enteritidis* recovered from retailed broilers. Our data confirmed that this phage has a high potential for phage application to control zoonotic and MDR *Salmonella* serovars derived from broilers in Egypt. Further molecular characterization of phiSalmchick1 genome is required for future applications.

Conflict of interest

None of the authors have any conflict of interest to declare

Acknowledgments

The authors would like to thank the staff members of Serology Unit, Animal Health Research Institute, Dokki, Giza, Egypt for serotyping of *Salmonella* isolates; and also we would like to thank Dr. Ahmed Askora, Assistant Professor of Microbiology, Faculty of Science, Zagazig University for providing us with some bacterial isolates and helping us to isolate and characterize the recovered phage. Also, we would like to acknowledge Dr. Mahmoud Salah El-Tarabany, Professor and Head of Animal Wealth Development Department for his support in the statistical analysis of data.

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