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Isolation, Identification, and Characterization of Antibiotic-Resistant *Salmonella* Strains from Poultry Farms and Evaluation of Bacteriophage Therapy as a Potential Control Strategy

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ABSTRACT

Salmonella infection represents a significant public health threat, particularly within the poultry industry, due to its widespread prevalence and potential for contamination. The rise of antibiotic-resistant *Salmonella* strains has exacerbated this challenge, making the need for alternative control strategies, such as bacteriophage therapy, increasingly urgent. This study aimed to isolate *Salmonella* strains from poultry farms in six Egyptian governorates and characterize their antibiotic resistance profiles. The strains were further identified using both biological and molecular methods, and their antibiotic susceptibility was evaluated. Bacteriophages specific to *Salmonella* were isolated, characterized by transmission electron microscopy, and encapsulated in alginate beads for enhanced stability. The efficacy of these encapsulated phages was assessed *In Vitro* through stability and bactericidal assays, as well as *In Vivo* via a controlled study involving infected poultry groups. Eighteen *Salmonella* strains were identified, several of which demonstrated multi-drug resistance, particularly to commonly used antibiotics, such as chloramphenicol and kanamycin. Phage Sal-1, a Myovirus with high specificity against *Salmonella*, was successfully isolated and encapsulated. *In Vitro*, encapsulated phages displayed robust stability and effectively lysed the *Salmonella* strains. *In Vivo*, phage-treated poultry exhibited significant reductions in bacterial colony-forming units (CFU), especially in water-based phage treatments, which achieved reductions to 10^2 CFU, compared to 10^4 CFU in feed-treated groups. Phage-treated groups showed no mortality, and organ histology was comparable to healthy controls, unlike the severe pathological changes observed in untreated and partially recovered antibiotic-treated groups. In conclusion, encapsulated phage therapy proved more effective than antibiotics, both in terms of efficacy and overall systemic recovery. This study underscores the potential of phage encapsulation as a sustainable alternative to antibiotics in combating *Salmonella* infections in poultry.

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INTRODUCTION

Salmonella is considered one of the most frequently identified emerging pathogenic bacteria globally (Newell *et. al.,* 2010; Lee *et. al.,* 2015; Velasquez *et. al.,* 2018; Jajere, 2019). It is a Gram-negative bacterium characterized by a thin peptidoglycan layer and an outer membrane composed of lipopolysaccharides. *Salmonella* is primarily classified into two species: *Salmonella bogori* and *Salmonella enterica*.

In poultry, *Salmonella* causes the disease salmonellosis at dosages ranging from approximately 10^7 to 10^9 CFU/g; however, these dosages may vary depending on factors such as the composition of the food and the health status of the animal (Chen *et. al.*, 2013). The infection spreads among poultry through lateral transmission, involving contaminated feces, litter, food, water, and feathers. Previous studies have shown that *Salmonella* can develop an acid tolerance response, enabling it to survive the acidic environment of the stomach (Bearson *et. al.*, 1998; Bearson *et. al.*, 2006).

In the gastrointestinal tract (GIT) of poultry, *Salmonella* strains that can withstand acidic pH conditions progress to the small intestine, cecum, and colon. The primary defense against *Salmonella* in the GIT is provided by the epithelial and immune cells lining these organs. To colonize the GIT, *Salmonella* competes with the gut microbiota for initial contact with enterocytes or M cells (Velge *et. al.*, 2012; Ruby *et. al.*, 2012; Micciche *et. al.*, 2018).

Antibiotics remain the most common and effective solution for treating salmonellosis in poultry. However, the widespread and often indiscriminate use of antibiotics has led to the emergence of *Salmonella* strains resistant to multiple commonly used antibiotics. This growing resistance poses a significant public health threat, particularly when resistant strains contaminate poultry products. Such strains can lead to infections that are more difficult to treat with conventional antibiotics, increasing the risk to consumers and highlighting the urgent need for better antibiotic stewardship in agriculture and food production (Munita & Arias, 2016; Penha Filho *et. al.,* 2016).

Bacteriophages offer a promising alternative to traditional antibiotics. Unlike antibiotics, phages can replicate at the site of infection by using bacterial hosts for replication, which allows them to achieve higher concentrations at infection sites (Górski *et. al.*, 2016; Manohar *et. al.,* 2020). This self-replicating ability helps overcome some of the limitations of antibiotics, such as their inability to sustain high local concentrations. Furthermore, phages have unique physicochemical properties that enable them to reach infection sites where chemical agents cannot. Phage therapy also offers several advantages over antibiotics, including high bactericidal efficacy, minimal intrinsic toxicity, and the ability to target specific pathogens without disrupting the normal microbiota (Hagens & Loessner, 2007; Górski *et. al.,* 2009; Huang *et. al.,* 2018).

The aim of this study is to isolate, identify, and characterize antibiotic-resistant *Salmonella* strains from poultry farms in Egypt, evaluate their antibiotic resistance profiles, and assess the potential of bacteriophage therapy as an alternative treatment strategy for controlling *Salmonella* infections in poultry. Additionally, the study seeks to explore the efficacy of encapsulated phages in reducing bacterial load, improving poultry health, and offering a sustainable solution to the growing problem of antibiotic resistance.

MATERIALS AND METHODS

Sample Collection:

A total of 51 samples were collected from the digestive systems of poultry suspected to be infected with *Salmonella*. The selection of these samples was based on external clinical signs, including pasty diarrhea, conjunctivitis, reduced body

weight, high mortality, whitish diarrhea, and decreased feed intake, along with anatomical abnormalities. Sampling took place at poultry farms housing approximately 30,000 birds, ranging in age from 3 to 28 days, across six Egyptian governorates (as detailed in Table 1). Each organ sample was aseptically placed into sterile Falcon tubes and stored at 4°C until further analysis.

Salmonella strains were isolated under aseptic conditions as follows: One Gram of each collected sample was homogenized in 10 mL of Buffered Peptone Water (BPW, Merck) and incubated aerobically at 37°C with agitation at 80 rpm overnight. After incubation, a loopful from each sample was streaked onto two selective media: Xylose Lysine Deoxycholate agar (XLD, Merck) and Bismuth Sulfite agar (BS, Oxoid). The plates were incubated aerobically at 37°C, and colonies suspected to be *Salmonella* were collected and transferred to fresh Tryptic Soy Broth (TSB, Oxoid) for further identification.

Table 1. Number of samples collected from each governorate.

No.	Poultry farm locations	Number of collected samples
1	Beni Suef	01
2	Fayoum	07
3	Giza	18
4	New Valley	04
5	Qalyuobia	13
6	Tanta	08

Biochemical Identification:

To confirm the identity of the *Salmonella* isolates based on their metabolic activities, two biochemical tests—the Urease test (Merck) and Triple Sugar Iron (TSI) test (Merck)—along with Gram staining, were performed on eight isolates. Urease and TSI slants were prepared in test tubes for each isolate. A loopful of each suspected colony was streaked onto the surface of the Urease and TSI slants, with the butt of the TSI slants also being stabbed. The slants were incubated overnight at 37°C. For Gram staining, day cultures of each isolate were prepared in TSB, and a drop of each culture was subjected to Gram staining using a Gram staining kit.

***In Vitro* Pathogenicity Test:**

The pathogenicity of eight *Salmonella* isolates was assessed using the Congo Red (CR) binding assay. Day cultures of each isolate were inoculated onto Congo Red agar plates and incubated at 37°C overnight. The isolates' ability to bind Congo Red dye was then evaluated to determine pathogenic traits.

Molecular Identification of *Salmonella* Virulence:

The eight *Salmonella* isolates, confirmed by biochemical tests and selective media, were streaked onto fresh XLD plates and incubated overnight at 37°C. A colony from each plate was then suspended in sterile, nuclease-free water for colony PCR. The virulence gene targeted in this study was the *invA* gene, which is essential for the pathogenicity of *Salmonella* species.

For the 25 µL PCR reaction, 1 µL of the *invA* forward primer (sequence: 5'-TCATCGCACCGTCAAAGGAACC-3') and 1 µL of the *invA* reverse primer (sequence: 5'-GTGAAATTATCGCCACGTTCGGGCAA-3') were added to 12.5 µL of Master Mix (Biolab), along with 2 µL of the suspended *Salmonella* colony. The remaining volume was adjusted with nuclease-free water to reach a final volume of 25 µL. Each PCR reaction was prepared separately for each isolate. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of

denaturation at 95°C for 30 s, annealing at 64°C for 30 s, and extension at 72°C for 30 s. The PCR amplicons were stored at −20°C.

Gel Electrophoresis of PCR Products:

A 2% agarose gel electrophoresis was prepared by dissolving 2 Grams of agarose powder (Sigma-Aldrich) in 100 mL of 1X TAE buffer (containing 40 mM Tris base, 20 mM acetic acid, and 1 mM EDTA). Prior to solidification, 5 µL of ethidium bromide was added to the gel solution. The mixture was poured into a casting tray and allowed to solidify at room temperature. Once set, the gel was placed in an electrophoresis chamber filled with 1X TAE buffer. Subsequently, 7 µL of each PCR product, along with a 100 bp DNA ladder (GeneDirex), was loaded into the wells. Electrophoresis was performed at 100 volts for 40 minutes, and the gel was visualized under a UV transilluminator.

Bacterial DNA Extraction & 16S rRNA Gene Sequencing:

For 16S rRNA sequencing, overnight cultures of each *Salmonella* isolate were prepared in Luria-Bertani (LB) broth (Oxoid). The bacterial samples were sent to a sequencing facility for DNA extraction and 16S rRNA sequencing using Sanger sequencing technology and capillary electrophoresis. Phylogenetic analysis was performed using MEGA X software. The closest 11 sequences to each isolate's sequence were identified using BLASTn on NCBI. These sequences were aligned with the test sequences in MEGA 11 using the CLUSTALW algorithm, and phylogenetic trees were constructed using the Maximum Likelihood method.

Antibiotic Susceptibility Test:

The disk diffusion method was used to assess the antibiotic resistance profile of eight *Salmonella* isolates. Antibiotic discs were impregnated with the following antibiotics: Amikacin (30 µg), Amoxicillin (25 µg), Colistin (10 µg), Doxycycline (30 µg), Chloramphenicol (30 µg), Piperacillin (100 µg), Vancomycin (30 µg), Kanamycin (30 µg), and Clindamycin (2 µg). Mueller-Hinton agar plates were inoculated with each bacterial isolate to achieve an optical density (OD600) of approximately 0.1. The antibiotic discs were placed on the surface of the agar, and the plates were incubated at 37°C for 16 hours. The diameters of the inhibition zones were measured and analyzed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Phage Isolation:

Mixed soil samples were collected from various farms for phage isolation. Five Grams of each soil sample were added to 25 mL of 5× TSB, along with 1 mL of an overnight culture of the *Salmonella* strain. The mixture was incubated at 37°C for 18 hours with agitation. After incubation, the solution was centrifuged at 6000 rpm for 20 minutes, and the supernatant was transferred to a new sterile Falcon tube. Chloroform was added to the supernatant, which was then filtered through a 0.45 µm syringe filter (MCA, CHMLAB, USA) into another sterile tube. The plaque assay technique was employed to isolate phages from the filtered supernatant. A 10 µL aliquot of the phage supernatant was spotted onto a bacterial lawn of *Salmonella* strain in a top agar layer and incubated at 37°C overnight.

Phage Purification & Amplification:

Single plaques were collected using a sterile cork borer and suspended in 300 µL of CM buffer (containing 2.5 g/L $MgSO_4 \bullet 7H_2O$, 0.05 g/L gelatin, 6 mL/L 1M Tris buffer, 0.735 g/L $CaCl_2$; pH 7.5). Phages were purified three times using the plaque assay technique.

For phage amplification, a liquid culture of the bacterial strain at OD600 of 0.2 was infected with an appropriate volume of phage Sal-1 at a multiplicity of infection (MOI) of 1.0. The culture was incubated at 37°C with shaking in a water bath for 5 hours. Chloroform was added to the mixture, which was allowed to stand at room temperature for 20 minutes. The chloroform was removed, and the mixture was centrifuged at 6400×g for 15 minutes. The supernatant was then centrifuged at

15,300×g for one hour at 4°C. Phage titers were measured using the plaque assay technique.

Phage Morphology:

Phage Sal-1 morphology was examined using Transmission Electron Microscopy (TEM) at the National Research Institute. A 100 µL aliquot of phage suspension with a titer of approximately 10^{10} PFU/mL was placed onto a copper grid. The phage was negatively stained with saturated Uranyl Acetate and visualized under a high-resolution transmission electron microscope (JEOL JEM-1400 Plus).

Phage Host Range:

The host specificity of phage Sal-1 was evaluated against a panel of isolated *Salmonella* strains, as well as reference strains *Salmonella* Typhimurium ATCC 14028 and *Salmonella* Typhimurium Seftenberg. Additional bacterial species, including *Escherichia coli* O157, *Shigella flexneri*, and *Shigella boydii*, were also tested. These bacterial strains were kindly

Phage Propagation:

The isolated phage *Sal-1* was propagated in 1000 mL of Tryptic Soy Broth (TSB). An overnight culture of *Salmonella* was inoculated into the broth, and the culture was incubated at 37°C with agitation at 130 rpm for 4 hours. After incubation, the mixture was centrifuged at 5000 rpm for 15 minutes to separate the bacterial cells. The supernatant was then further centrifuged to pellet the phages. The resulting phage pellet was suspended in CM buffer, and plaque assays were performed to determine the plaque-forming units (PFU). Phage titers reached up to 10^{9} PFU/mL.

Bacteriophage Encapsulation:

Phage encapsulation was achieved by forming alginate beads, as described by Ahmadi *et. al*. (2018). A solution containing 3% alginate and 1% gelatin was prepared, and a mixture of phages was added to this solution. The mixture was then dispensed dropwise using a syringe to form beads. This process consistently produced uniform beads that effectively encapsulated the phages.

Performance Evaluation of Encapsulated Phages:

The stability and bactericidal efficacy of the encapsulated phages were evaluated both *In Vitro* and *In Vivo*.

***In Vitro* Experiments**: These assessed the performance of alginate beads under three conditions: direct exposure to bacterial culture in broth, evaluation of phage release into broth, and application of the beads to agar plates containing bacterial culture.

***In Vivo* Experiments**: The therapeutic efficacy of encapsulated phages was tested in poultry (Gomez-Garcia *et. al.*, 2021). Six experimental groups were established, including a negative control group (infected with bacteria but no treatment). The treatment groups were as follows:

-Poultry fed alginate beads before infection.
-Poultry fed alginate beads after infection.
-Poultry treated with alginate beads in water after infection.

For the water-based treatments, the dosage was standardized at 1 mL of phage solution per 100 mL of water. For feed-based treatments, the dosage was 5 g of alginate per 100 g of feed. Samples were collected via swabs on the first day of the experiment and by harvesting organ tissues at 24-hour intervals for two weeks to monitor bacterial loads and phage performance.

Gastric Juice Stability Assay:

To investigate the stability of encapsulated phages under varying pH conditions, both encapsulated and non-encapsulated phages were exposed to gastric juice. The gastric juice was prepared by adding 0.1% bile salt and 0.4% pancreatin (Sigma-Aldrich, MO, USA) to a 50 mM KH_2PO_4 solution, and the mixture was incubated for 72 hours (Abdelsattar *et. al*., 2019a). Survival rates of the phages were monitored at specific intervals. Non-encapsulated phages were assessed at 10-hour intervals, while encapsulated phages were analyzed over the entire 72-hour

period to assess their resilience in acidic and variable pH environments.

RESULTS

Isolation and Identification of *Salmonella* Strains:

Salmonella spp were identified in 18 of the 51 samples collected from various poultry farms. The identification was based on growth characteristics on selective media, including Xylose Lysine Deoxycholate (XLD) agar and Bismuth Sulfite (BS) agar. On XLD media, *Salmonella* spp ferment xylose, creating an acidic environment that turns the medium yellow. The decarboxylation of lysine results in alkaline conditions, turning the medium back to red or dark pink. Additionally, *Salmonella* spp reduce sodium thiosulfate to hydrogen sulfide (H_2S), which reacts with ferric ions to form ferrous sulfide, producing the characteristic black colonies (Fig. 1A). On BS agar, *Salmonella* spp formed black colonies due to H_2S production, with the selective properties of Bismuth Sulfite Indicator and Brilliant Green inhibiting the growth of Gram-positive bacteria and coliforms, thereby favoring *Salmonella* growth. Ferrous Sulfate in the medium promotes H_2S production, resulting in a distinctive brown to black coloration with a metallic sheen (Fig. 1B).

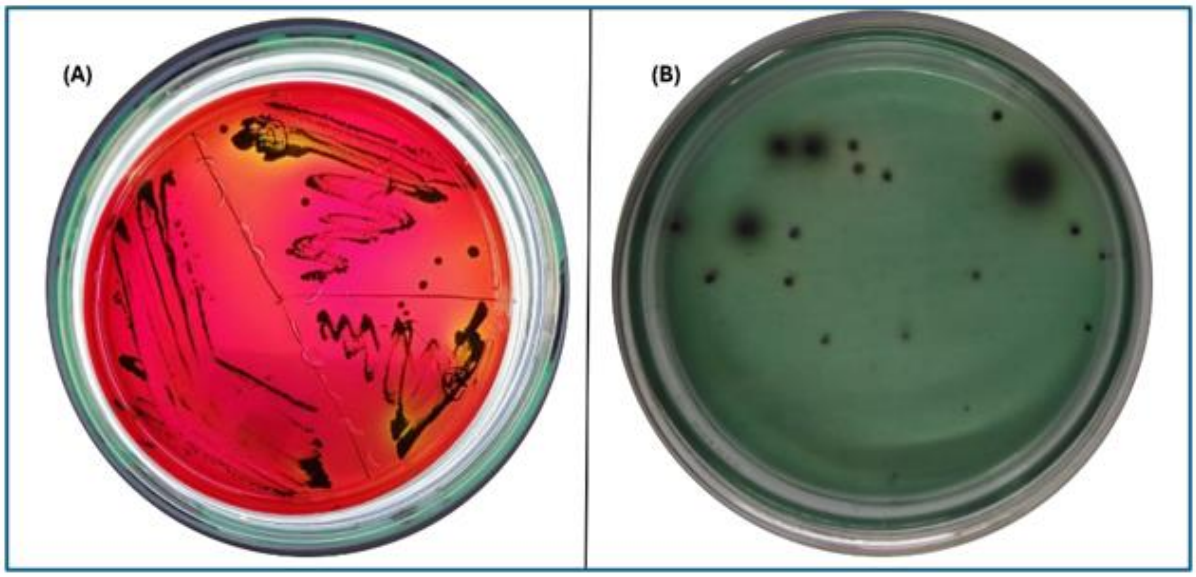

Fig. 1: Morphology of *Salmonella* colonies on XLD agar (A) and BS agar (B).

Biochemical Identification of *Salmonella* Isolates:

The urease test was conducted to determine whether the *Salmonella* isolates could hydrolyze urea into ammonia and carbon dioxide via urease activity. The test yielded a negative result, indicated by the lack of color change in the medium (Fig. 2A), consistent with the typical urease-negative trait of *Salmonella* species. The Triple Sugar Iron (TSI) test was used to assess the isolates' ability to ferment glucose, lactose, and sucrose, and to produce hydrogen sulfide (H_2S) and gas. The slant turned red, indicating alkaline conditions (K), while the butt exhibited black coloration with visible gas production, consistent with *Salmonella* activity under anaerobic conditions (Fig. 2B). Gram staining revealed the isolates as Gram-negative, rod-shaped bacteria (Fig. 2C).

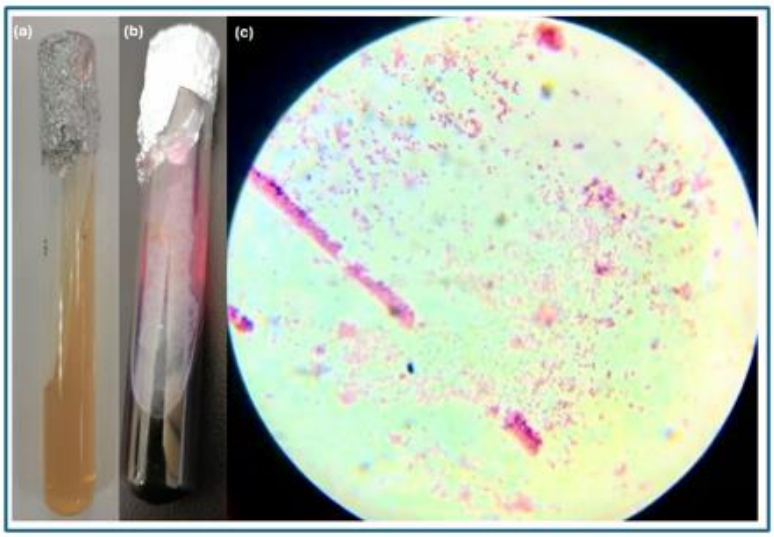

Fig. 2: Representative Congo Red agar plate showing the growth and Congo Red dye binding of a pathogenic *Salmonella* strain**.**

***In Vitro* Pathogenicity Test:**

The Congo Red (CR) binding assay demonstrated that all tested *Salmonella* isolates were pathogenic, as indicated by their strong binding affinity for CR dye. This result suggests that the isolates possess significant virulence factors, consistent with pathogenic *Salmonella* strains (Fig. 3).

Fig. 3. Representative Congo Red agar plate showing the growth and Congo Red dye binding of a pathogenic *Salmonella* strain.

Molecular Identification of *Salmonella* Isolates:

Molecular identification was carried out using 16S rRNA sequencing, and phylogenetic analysis confirmed that all isolates were *Salmonella*. The sequences have been deposited in GenBank with the following accession numbers: PQ507950.1, PQ505138.1, PQ505421.1, PQ507875.1, PQ507917.1 (Fig. 4). PCR amplification of the invA gene yielded a distinct band at approximately 284 bp for all isolates, confirming the presence of *Salmonella* (Fig. 5). No non-specific bands were observed, further validating the specificity of the PCR assay.

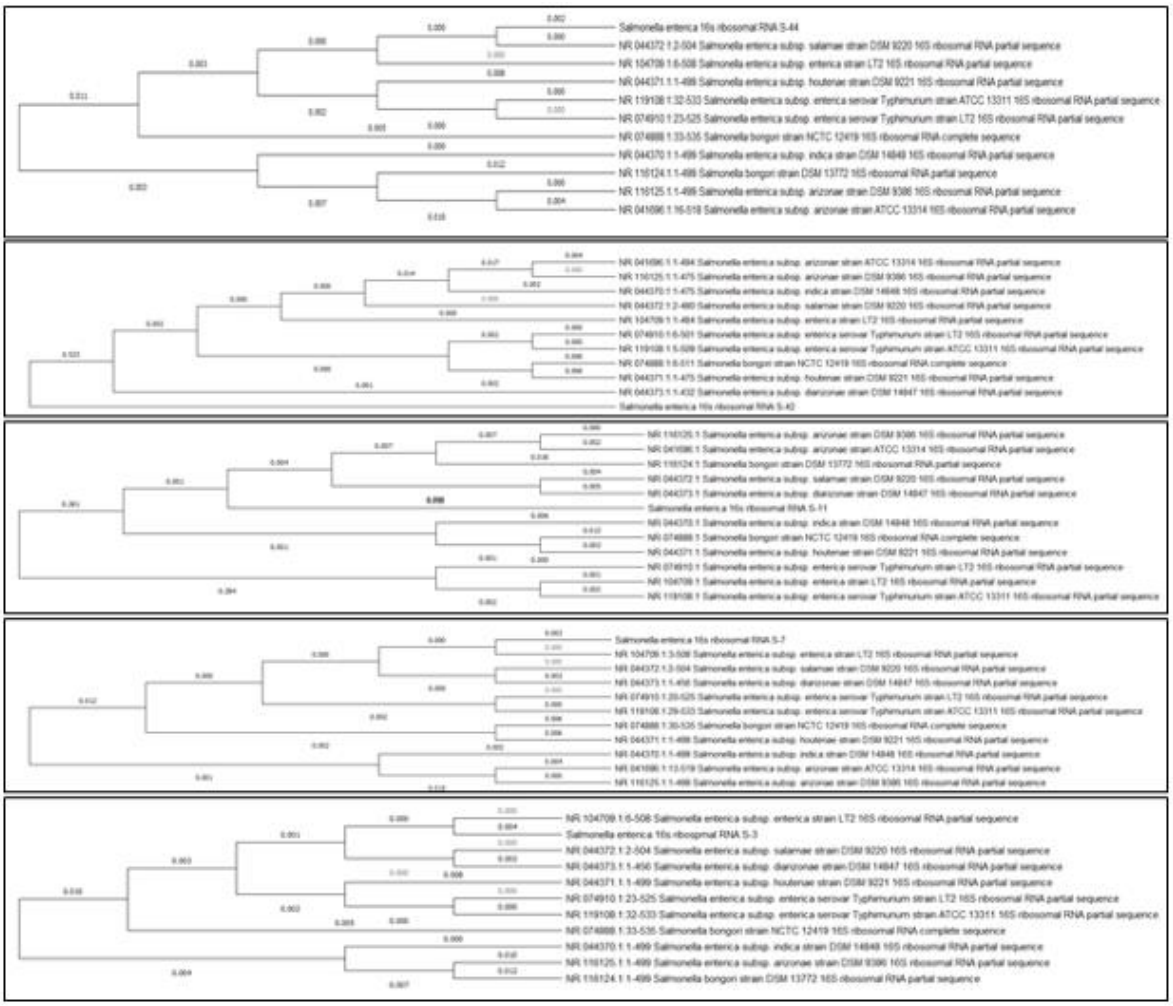

Fig. 4: Phylogenetic tree of the sequenced *Salmonella* isolates constructed using the Maximum Likelihood method.

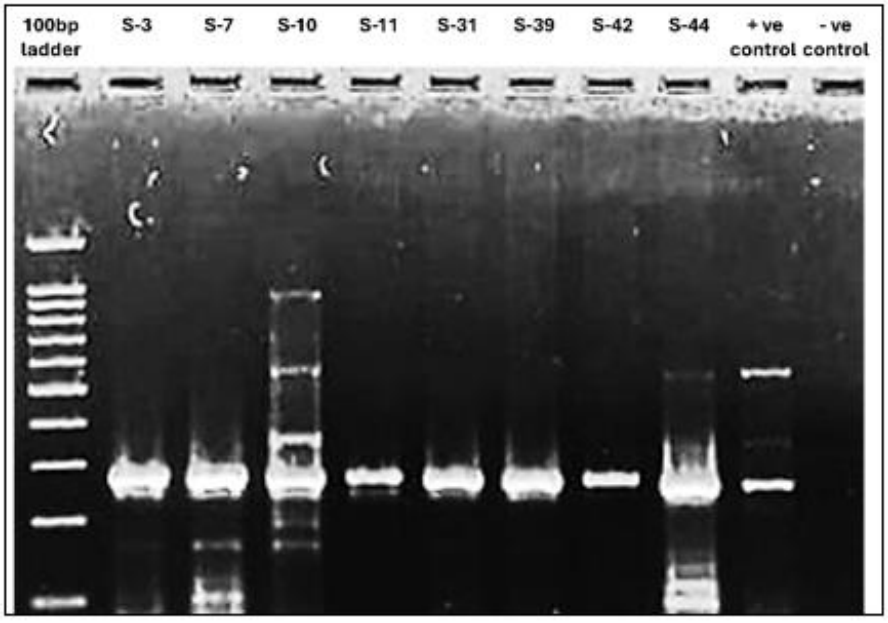

Fig.5: Agarose gel electrophoresis under UV light. Lanes 2–9 display distinct 284 bp bands, corresponding to the amplified *invA* gene from each isolate. Lanes 10 and 11 show positive and negative controls, respectively.

Antibiotic Sensitivity:

The antibiotic susceptibility testing revealed that 62.5% of the isolates were resistant to Chloramphenicol and Kanamycin, 25% were resistant to Amoxicillin and Clindamycin, 37.5% were resistant to Colistin, and 12.5% were resistant to Piperacillin and Doxycycline. No resistance was observed to Amikacin or Vancomycin (Table 2). *Salmonella* strains S-44, S-42, and S-11 were found to be multidrug-resistant, while isolate S-7 was highly sensitive to all tested antibiotics.

Table 2. The antibiotic profile of *Salmonella* isolates.

Used antibiotics	µg	Poultry farm locations							
		Bani-Suef	Fayoum		Giza		Qalyuobia		Tanta
		S-11	S-10	S-3	S-44	S-39	S-31	S-42	S-7
Amikacin	30	S	S	S	S	S	S	S	S
Amoxicillin	30	S	S	S	R	S	S	R	S
Chloramphenicol	30	R	R	R	S	S	R	R	S
Clindamycin	02	S	S	S	S	R	S	R	S
Colistin	10	R	S	S	S	R	S	R	S
Doxycycline	30	R	S	S	S	S	S	S	S
Piperacillin	100	S	S	S	R	S	S	S	S
Vancomycin	05	S	S	S	S	S	S	S	S

R: Resistant. S: Sensitive.

Phage Isolation and Morphological Analysis:

A lytic bacteriophage, Sal-1, was successfully isolated. Transmission Electron Microscopy (TEM) revealed that phage Sal-1 has an isometric head (diameter 90.6 nm) and a long, non-contractile tail (diameter 115.7 nm), classifying it as a Myovirus according to the International Committee on Taxonomy of Viruses (ICTV) (Fig. 6).

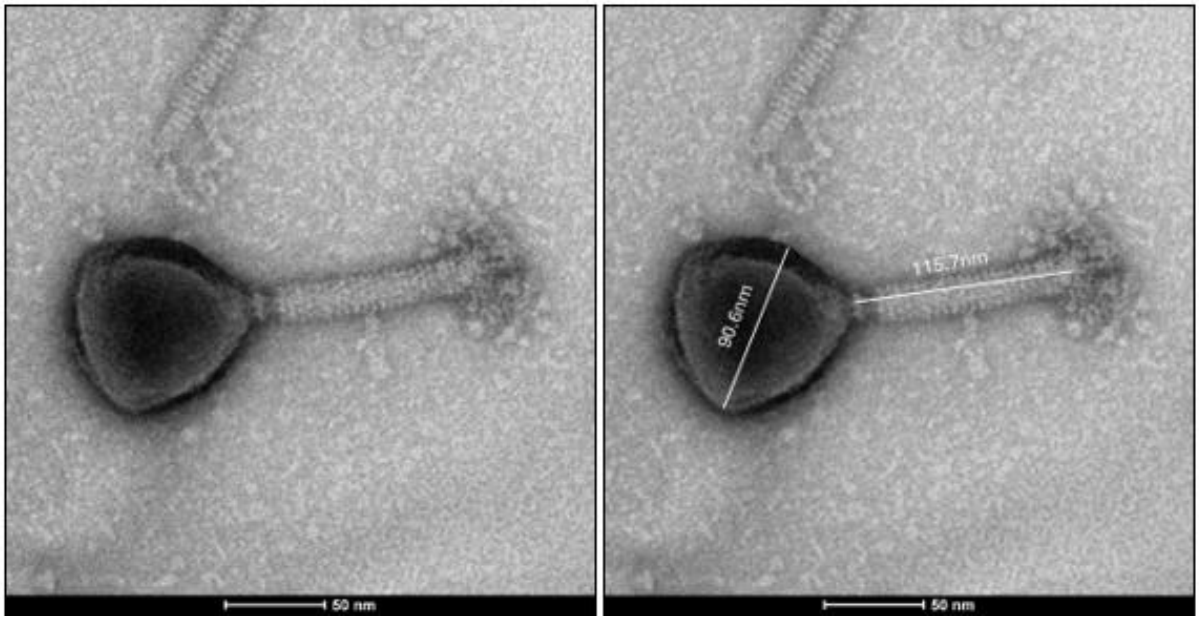

Fig. 6: Morphology of phage Sal-1 as observed under TEM. Purified Sal-1 phage particles were visualized at a scale bar of 50 nm.

Phage Amplification and Host Range:

Phage Sal-1 was amplified using the liquid propagation method, achieving a titer of approximately 10^{10} PFU/mL. The phage's lytic activity was tested against a panel of *Salmonella* strains using the spotting method. Results showed that phage Sal-1 exhibited high specificity towards certain *Salmonella* strains (Table 3).

Table 3. The host range of phage sal-1.

Bacterial strains	**Phage sal-1**
Escherichia coli O157:H7	-
Salmonella Typhimurium ATCC 14028	++
Salmonella Typhimurium Seftenberg	++
Shigella boydii	-
Shigella flexneri	-
Salmonella spp. S12	-
Salmonella spp.S13	+
Salmonella spp. S14	-
Salmonella spp. S15	-
Salmonella spp. S16	-
Salmonella spp. S17	+
Salmonella spp .S20	+
Salmonella spp. S21	-
Salmonella spp. S22	-
Salmonella spp. S18	-
Salmonella spp. S19	-
Salmonella spp. S1	-
Salmonella spp. S2	-
Salmonella spp. S3	-
Salmonella spp. S4	-
Salmonella spp. S5	-
Salmonella spp. S6	-
Salmonella spp. S7	-
Salmonella spp. S8	-
Salmonella spp. S9	-
Salmonella spp. S10	-
Salmonella spp. S11	-

(++) Strong lysis, (+) Moderate lysis, (-) No lysis.

Propagation of Bacteriophages:

Phages were successfully propagated with titers of approximately 10^{9} PFU/mL, demonstrating efficient amplification and recovery through optimized centrifugation and plaque assay techniques.

Encapsulation Efficacy:

Alginate bead encapsulation successfully preserved phage stability and viability. The alginate beads exhibited a uniform texture and structure, confirming the feasibility of this technique for stabilizing bacteriophages.

Performance Evaluation of Encapsulated Phages:

In Vitro experiments demonstrated that alginate beads enhanced phage titers when applied directly to broth media or bacterial cultures. Direct application of alginate beads to agar plates showed no phage release, indicating that the beads effectively encapsulated the phages (Fig. 7). In the *In Vivo* study, poultry treated with

encapsulated phages showed significant reductions in bacterial colony-forming units (CFU) compared to the untreated control group. Water-based administration of phages led to the most pronounced reduction in bacterial counts (102 CFU), outperforming feed-based administration, which resulted in a reduction to 10^4 CFU.

Fig. 7: A notable difference in fecal discoloration is observed between the antibiotic-treated poultry group (A) and the phage-treated poultry group (B). Feces from the phage-treated group closely resemble normal color, while the antibiotic-treated group shows marked discoloration.

Extrinsic Symptoms and Mortality:

No mortality was observed in phage-treated poultry, while untreated control poultry experienced 100% mortality. Healthy, uninfected poultry exhibited no fatigue, normal fecal coloration, and no signs of illness. In contrast, the untreated infected group showed severe fatigue, discolored feces (ranging from yellowish to greenish), and 100% mortality. Antibiotic-treated poultry showed partial recovery, with approximately 70% appearing healthy, although residual fatigue and occasional fecal discoloration persisted, indicating incomplete infection control.

Anatomical Examination:

Anatomical examination of organs from different groups confirmed the findings (Figs.8-10). In the untreated group, intestinal tissues showed severe inflammation, necrosis, and hyperemia, while liver tissues exhibited congestion and discoloration indicative of septicemia, and heart tissues were pale and degenerated. In contrast, poultry treated with phages showed intact intestinal tissues, normal liver appearance, and structurally healthy heart tissues, similar to the healthy control group.

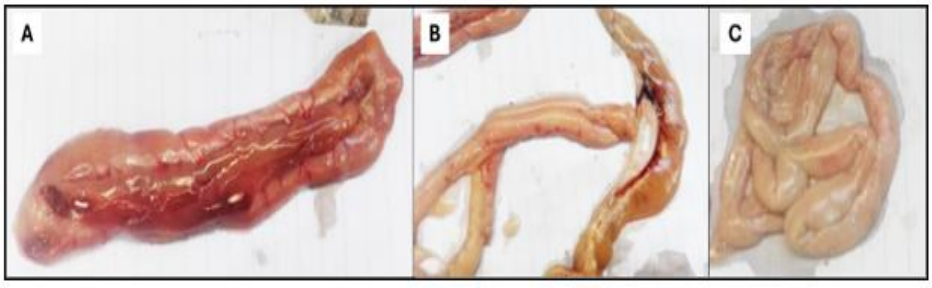

Fig. 8: Ccomparative Histological Examination of Intestinal Tissues across Poultry Groups:

(A) Intestinal tissues from the untreated, infected group exhibit severe inflammation, necrosis, and hyperemia, characteristic of advanced bacterial infection.

(B) The antibiotic-treated group shows a reduction in inflammation but still presents with mild necrosis and hyperemia.

(C) The phage-treated group demonstrates intact intestinal tissues, with no signs of inflammation or necrosis, resembling the healthy baseline."

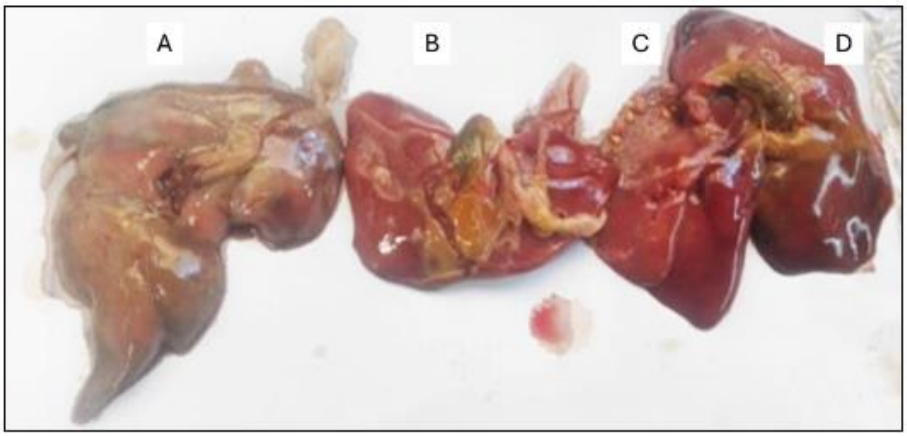

Fig. 9: Histological Differences of Liver Tissues Across Poultry Groups:
(A) Liver tissues from the infected group show severe degeneration, hepatocellular necrosis, and pronounced hyperemia, indicative of bacterial infection.
(B) The antibiotic-treated group exhibits moderate improvement, with reduced necrosis but persistent mild degeneration and hyperemia.

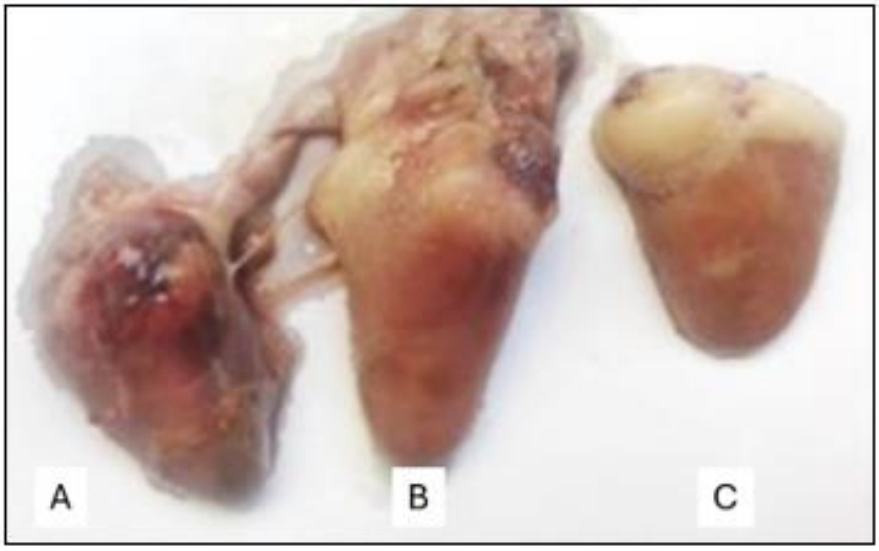

Fig. 10: Comparison of Histology of Heart Tissues across Poultry Groups:
(A) Heart tissues from the infected group exhibit severe myocardial degeneration, necrosis, and prominent inflammatory infiltration, characteristic of bacterial infection.
(B) The antibiotic-treated group shows partial improvement, with reduced necrosis but persistent mild inflammation.
(C) The phage-treated group displays intact myocardial structure with minimal signs of damage, closely resembling the normal appearance of healthy cardiac tissue.

Gastric Juice Stability:

Encapsulated phages remained stable and viable under acidic conditions, surviving for up to 72 hours in low pH environments. In contrast, non-encapsulated phages were inactivated within 10 hours, highlighting the protective role of encapsulation in maintaining phage functionality under harsh gastric conditions.

DISCUSSION

In this study, we successfully isolated and identified *Salmonella* strains from various poultry farms in Egypt. The results reveal a significant prevalence of antibiotic-resistant *Salmonella* strains in poultry and demonstrate the feasibility and effectiveness of bacteriophage-based treatments.

Of the 51 samples collected across six governorates, *Salmonella* was isolated from 18 samples, highlighting the widespread contamination of poultry farms and the substantial public health risks associated with it. The morphological and biochemical tests used for identification were consistent with established methods, and the presence of pathogenic strains was confirmed through PCR amplification of the invA gene, further validating the virulence of the isolated *Salmonella* strains.

Antibiotic susceptibility testing revealed that nearly all isolated strains were resistant to multiple antibiotics, particularly *Salmonella* strains S-44, S-42, and S-11, which showed multi-antibiotic resistance. Resistance to commonly used antibiotics such as chloramphenicol, kanamycin, and amoxicillin underscores the growing concerns regarding antibiotic misuse in the poultry industry. This misuse accelerates the development of resistant strains, which can then enter the human food chain, presenting serious health risks.

Bacteriophage therapy presents a promising solution for treating antibiotic-resistant *Salmonella* infections (Olawade *et. al.*, 2024; Kortright *et. al.*, 2019; Cui *et. al.*, 2024; Anjay *et. al.*, 2022; Huang *et. al.*, 2018). In this study, the isolation and characterization of phage Sal-1, a Myovirus with high specificity for *Salmonella* strains, including *Salmonella Typhimurium*, is a significant finding. Phage therapy's ability to selectively target bacteria without disrupting the host's microbiota or inducing resistance offers clear advantages over traditional antibiotics (Lin *et. al.*, 2017).

The absence of lytic activity against non-*Salmonella* species, such as *E. coli* and *Shigella*, indicates the high specificity of Sal-1, minimizing the risk of off-target effects. These findings are consistent with previous research that highlights the potential of phages for controlling *Salmonella* in food and agricultural settings (Haq *et. al.*, 2024).

The results of this study emphasize the potential of encapsulated bacteriophages as a robust therapeutic tool for combating bacterial infections (Choińska-Pulit *et. al.*, 2015; Yang *et. al.*, 2023; Loh *et. al.*, 2020). The successful propagation and encapsulation of phages into alginate beads demonstrate the feasibility of this technique in preserving phage stability and viability, aligning with previous studies (Abdelsattar *et. al.*, 2019b). The alginate beads, with their uniform structure, not only effectively encapsulate phages but also exhibit controlled release and strong retention properties, as confirmed by the *In Vitro* studies (Moghtader *et. al.*, 2017; Malik *et. al.*, 2017).

In Vivo findings further support the efficacy of encapsulated phages in reducing bacterial loads in poultry (Yin *et. al.*, 2021; Loh *et. al.*, 2020). Notably, water-based phage delivery outperformed feed-based administration, likely due to improved phage accessibility and distribution in the gastrointestinal tract. The complete absence of mortality in phage-treated groups, coupled with superior performance compared to antibiotic treatments, underscores the therapeutic advantage of encapsulated phages.

These findings also highlight the practical implications of phage stability during gastrointestinal transit. Encapsulation proved critical in ensuring phage survival in acidic environments, extending their functionality and therapeutic window compared to non-encapsulated phages, which rapidly lost viability.

Overall, the integration of encapsulation technology with bacteriophage therapy represents a promising advancement in antimicrobial strategies, particularly in the context of rising antibiotic resistance (Choińska-Pulit *et. al.*, 2015; Barber *et. al.*,2021; Cui *et. al.*, 2024; Richards & Malik, 2021). Further optimization of encapsulation techniques and exploration of other delivery matrices could enhance the versatility and

application of phage-based treatments in both veterinary and human medicine.

In conclusion, this study highlights the persistent issue of antibiotic-resistant *Salmonella* in poultry farms in Egypt and underscores the importance of phage therapy. The strong lytic activity of phage Sal-1 against *Salmonella* strains, demonstrated by the host range experiment, supports its potential application in poultry farms and offers hope for reducing reliance on antibiotics, while mitigating the public health risks associated with resistant pathogens.

Declarations:

Ethical Approval: Not applicable.

Conflicts of Interest: The author declares no conflicts of interest.

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