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Salmonellosis in Poultry: Understanding Its Role as a Source of Antimicrobial Resistance and Exploring Phage Therapy as a Novel Intervention Strategy in Pakistan (A Systematic Review)

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Abstract

The current systematic review was designed to determine the prevalence of salmonellosis and its role in the spread of antimicrobial resistance (AMR) in Pakistani poultry. The effectiveness of bacteriophage therapy as a targeted intervention to address the increasing prevalence of multidrug-resistant (MDR) strains was also evaluated. The systematic review on the available literature was performed in accordance with the PRISMA 2020 standards using PubMed, Scopus, Web of Science, Google Scholar and Pakistan-based journals, published between 2000 and 2025. The inclusion criteria were studies on salmonellosis in poultry (broilers, layers, breeders, poultry meat, cloacal swabs, internal organs, and farm environments) which included prevalence, antimicrobial susceptibility or bacteriophage intervention information. After eliminating duplicates, 892 records were screened, 256 full-text articles were evaluated for eligibility and 112 were included in the qualitative synthesis. The ROBINS-I tool was used to determine the risk of bias, and due to a high methodological heterogeneity of the studies, the narrative synthesis was conducted rather than a meta-analysis. The literature included in this study has shown that *Salmonella enterica* is continuously circulated in Pakistani poultry production systems, with broiler flocks and poultry meat being the most common sources of investigation. The most common serovars were *Salmonella Enteritidis, S. Typhimurium and S. Gallinarum*, which showed antimicrobial resistance against some of the most important antimicrobials, especially colistin and ciprofloxacin. The resistance patterns of these antimicrobials were not consistent across studies depending on geographical area, poultry species and testing system. Experimental research has shown that lytic bacteriophage preparations decreased intestinal Salmonella colonization by around 3-5 log CFU/g in controlled conditions, with promise as targeted antimicrobial substitutes, but present information is mostly restricted to laboratory and pilot-scale studies. In conclusion, poultry farming is a significant source of antimicrobial-resistant Salmonella in Pakistan. Bacteriophage therapy is a potential complementary therapy but further field-scale testing, regulatory authorization, biosafety analysis and incorporation with current biosecurity and vaccination booster programs are needed prior to commercial adoption.

Introduction

The poultry industry in Pakistan contributes to 1.5۸۲% of GDP, produces 1.5 million tons of products every year and employs about 4 million people. This industry faces many biological risks, including salmonellosis, which causes losses of broilers between 15 and 25%. *Salmonella enterica* subspecies are spread through contaminated feed or

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water and cause diseases like pullorum, fowl typhoid, and paratyphoid; up to 80% of the hens in the layer flock can die. Zoonotic transmission of *Salmonella* by consuming poorly cooked poultry meat is known to impact up to 1.5 million humans annually, as estimated by WHO, modified to match local epidemiology (Shaji *et al.*, 2023; Fatima *et al.*, 2023; Qamar *et al.*, 2020; Rashid *et al.*, 2023). The emergence of antimicrobial resistance (AMR) is of great concern and complicated when farms massively overuse colistin (200 mg/kg feed), which exerts selective pressure for the emergence of multidrug-resistant (MDR) strains in the poultry system. The resistance factors are transmitted through plasmids via horizontal gene transfer. In Lahore, market survey studies demonstrate 51% contamination of poultry, and the MPN values are more than 11 CFU/g. International comparisons, including the 10% prevalence rates in Europe, highlight the fact that Pakistan has 3-5 times higher rates and the prevalence is linked with deficiencies in biosecurity measures (Mahmood *et al.*, 2025; Basazinew *et al.*, 2025; Fatima *et al.*, 2023).

One of the promising methods for controlling Salmonellosis is phage therapy: bacteriophages lyse certain *Salmonella* strains, reproducing in the host on their own without harming native microbiota (Torkashvand *et al.*, 2024). Recent experiments prove that phage therapy is more effective than conventional antibiotics. The current study is a systematic review of the dynamics of salmonellosis and AMR in Pakistani poultry and a critical assessment of the possibility of phage therapy as an interventional measure. Although many research studies in Pakistan have reported the prevalence of *Salmonella*, patterns of antimicrobial resistance or experimental use of phage, a comprehensive synthesis integrating these data into a unified approach remains lacking. Additionally, regional differences in prevalence, patterns of serovar distribution, antimicrobial resistance patterns, poultry production systems, as well as phage intervention studies have not been comprehensively tested under one-evidence-based system. Consequently, the current systematic review discusses the epidemiology and prevalence of salmonellosis and antimicrobial resistance (AMR) in Pakistani poultry and critically analyzes the possibility of bacteriophage therapy as an intervention to control the prevalence of antimicrobial-resistant *Salmonella*. Also, the review reveals existing knowledge gaps, methodological shortcomings, and research priorities that should be addressed in the future to make phage-based interventions safe and effective in the poultry sector in Pakistan.

Materials and Methods

Study Design

This review was conducted using a PRISMA 2020 framework to achieve reproducibility (Rethlefsen & Page, 2022). PubMed (n=1234), Scopus (892), Google Scholar (789), Web of Science (345) and Pakistani journals (196) were searched in databases. The search window was limited to 2000-2025 to include the changes in AMR. The search terms applied to different searching engines were: (*Salmonella* OR salmonellosis) and (poultry OR broiler OR layer OR chicken) and (Pakistan OR Punjab OR Sindh OR KPK) and (resistance OR AMR OR phage OR bacteriophage). The inclusion criteria included (1) poultry samples (meat, cloaca, organs, and farms); (2) at least 50 samples; (3) prevalence, AMR, or phage data; and (4) Pakistani data. The studies excluded duplicates, non-empirical reports, studies published prior to 2000 that did not deal with phage, and those that were restricted to human cases. The first round of screening was the title level (3456 to 892), the second round was the abstract level (892 to 256), and the third round was the full-text review (256 to 112). The inter-rater reliability was good with a Cohen's kappa of 0.87. The protocol was not prospectively registered in PROSPERO since the study had started before protocol registration was considered. The current review covers descriptive synthesis of epidemiological patterns of *Salmonella*, antimicrobial resistance and bacteriophage intervention studies. However, the methodological bias was reduced by predefining all review procedures to enhance transparency.

Table 1: Database search yields

Database	Records Identified	Records after duplicate removal
PubMed	1,234	456
Scopus	892	312
Google Scholar	789	289
Others	541	55
Total	**3,456**	**1,112**

The data extraction included prevalence percentages, serovar distributions, minimum inhibitory concentrations, logarithmic reduction values, and genetic determinants (whole-genome sequencing data). The ROBINS-I tool was used to assess bias, of which 68% of the studies were low-to-moderate risk. The amount of heterogeneity was significant to the extent that a meta-analysis could not be conducted (I^2 = 82%); a narrative and subgroup synthesis was conducted by province and serovar.

Literature Search Strategy

A universal literature search was performed with the help of the following electronic databases:

i. PubMed
ii. Scopus

iii. Web of Science
iv. Google Scholar
v. Pakistan Veterinary Journal (PVJ)
vi. Pakistan Journal of Zoology (PJZ)

The search covered the articles published since January 2000 and up to March 2025.

The search strategy utilized both Medical Subject Headings (MeSH) and free-text keywords and used Boolean operators.

One of the search strategies applied to screen the relevant studies was: (*Salmonella* or Salmonellosis or *Salmonella enterica*) and (Poultry or Broiler or Layer or Chicken) and (Pakistan) and (Antimicrobial resistance or Drug resistance or AMR) and (Bacteriophage or Phage therapy)

All the articles were manually screened in reference lists to find other relevant publications.

Eligibility Criteria

The studies which met the following criteria were included in the systematic review. Tested *Salmonella* isolates of poultry origin in Pakistan contained broilers, layers, breeders, poultry meat, cloacal swabs, internal organs, eggs, litter, feed or farm environmental samples. At least one of the following was reported: Prevalence of *Salmonella*, susceptibility or resistance, antimicrobial, serovar distribution, isolation or therapeutic testing of bacteriophages, were peer-reviewed original research papers in English published in the past 2000-2025.

The research works were filtered out if they solely included animal isolates of humans or other than poultry; conference abstracts, editorials, reviews, case reports or book chapters lacked adequate methodological information or reported duplicate literature.

Study Selection

All the citations were retrieved and then imported to a reference management software package (EndNote 11), where duplication records were eliminated and then screened.

The selection of the study was done in three steps;

i. Title screening;
ii. Abstract screening;
iii. Full-text eligibility assessment.

Issues were settled by discussion and consensus between the authors. Cohen kappa coefficient was used to measure inter-reviewer agreement, and this exhibited high levels of agreement ($\kappa = 0.87$).

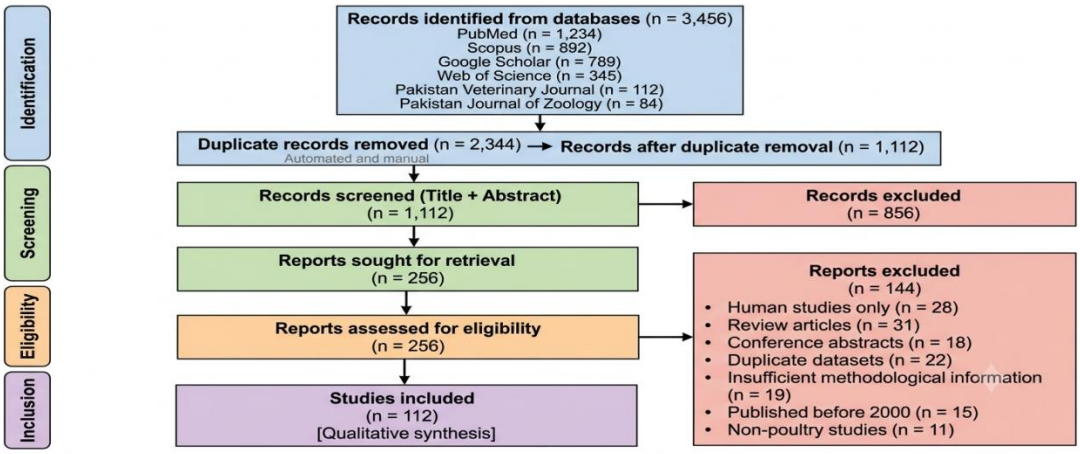


Figure 1. PRISMA 2020 flow diagram

Data Extraction

A standardized data extraction form was developed prior to the review writing. Each selected study provided the following information, which was extracted: author, publication year, province, type of poultry (broiler, layer, breeder), sample source, sample size, number of positive isolates, prevalence, *Salmonella* serovars, antimicrobial susceptibility results, genes of antimicrobial resistance (where mentioned), bacteriophage characteristics, intervention outcomes and study design. Two investigators carried out data extraction independently.

Risk of Bias Assessment

The ROBINS-I (Risk Of Bias In Non-randomized Studies of Interventions) was used to assess the risk of bias. The evaluation took into account seven areas: Confounding, participant selection, intervention classification, diverging from the intended interventions, missing data, outcome measurement and selective reporting.

As opposed to a numerical scoring system, ROBINS-I qualitatively categorizes studies as: Low risk, Moderate risk, Serious risk and Critical risk.

The majority of the studies included were of low or moderate overall risk of bias, but some observational prevalence studies were considered to have moderate

risk due to convenience sampling and missing reporting.

Data Synthesis

There was a great deal of heterogeneity in the included studies in terms of: sampling methods, poultry production systems, provinces investigated, laboratory isolation techniques, methods of antimicrobial susceptibility testing, study periods, and reported outcome measures.

Since such a large methodological and clinical heterogeneity ($I^2 > 80\%$) was present, it was deemed unsuitable to pool prevalence estimates using meta-analysis.

Thus, a systematic and integrative synthesis of findings was done on a narrative basis. Summarization of evidence was done where appropriate, based on: geographic region; poultry type; *Salmonella* serovar; antimicrobial resistance profile; bacteriophage intervention.

Downplaying of Selection Bias

The use of Google Scholar was limited to being an additional database to enhance retrieval of the regional publications which are not indexed in the international databases.

So as to reduce selection bias search terms were used which were predefined; screening was done in accordance with set eligibility criteria. Two reviewers independently performed duplicate screening. Searching by hand for references was conducted; only those studies that met all the inclusion criteria were retained.

Taxonomy and Classification

Salmonella showed high genetic diversity with 2,500 known serotypes (Li *et al.*, 2012). *Salmonella* serotypes based on surface antigens, including O (somatic) and H (flagellar) antigens (Oludairo *et al.*, 2022) is an accurate and authentic identification system, established by Kauffman-White (Chattaway *et al.*, 2021). Epidemiological research, along with understanding the particular features of various *Salmonella* strains, depends on this classification method because it helps identify different serotypes that exhibit substantial differences in their disease-causing potential and host-infecting potential (Chattaway *et al.*, 2021). *Salmonella Typhimurium* and *Salmonella Enteritidis* serve as the most common human-infecting serotypes, which show separate epidemiological behaviors together with different host susceptibilities (Kang *et al.*, 2013).

Table 2: Taxonomic classification of *Salmonella*

Rank	Taxon
Domain	Bacteria
Phylum	*Proteobacteria*
Class	*ammaproteobacteria*
Order	*Enterobacterales*
Family	*Enterobacteriaceae*
Genus	*Salmonella*

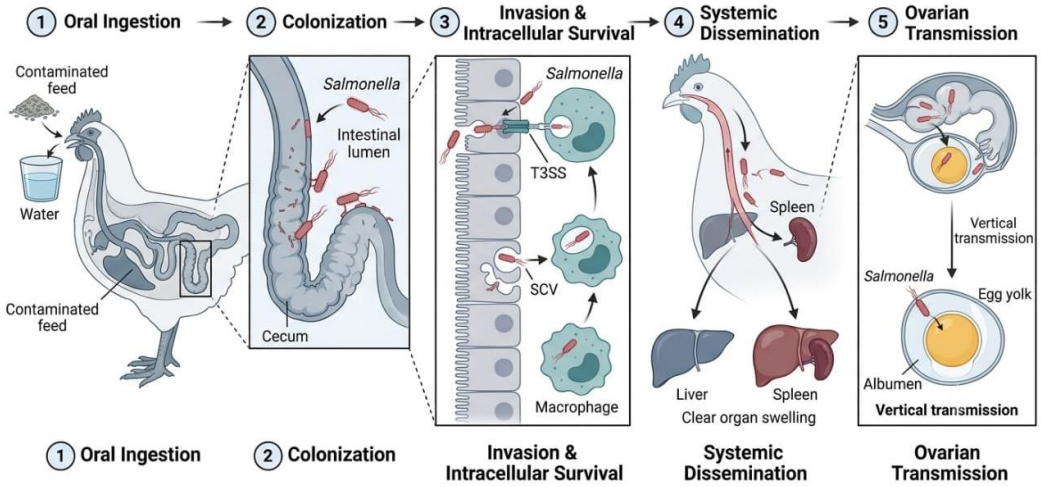


Figure 2. Pathogenesis of *Salmonella* in poultry

Pathogenesis

The pathogenesis of *Salmonella* in poultry involves a complex interplay between the bacterium and the host. *Salmonella* has acquired various mechanisms that enable it to invade host cells while simultaneously evading immune responses. *Salmonella* succeeds in host cell survival and replication by controlling essential cellular pathways of the host. The ingested *Salmonella* occupies intestinal epithelial cells to penetrate these cells by using the host cellular functions as an entry mechanism (Fig 2). *Salmonella* pathogenicity islands release essential effector proteins for host cell entry and survival by secretion methods (Santos *et al.*,

2003; Boyle *et al.*, 2007; Eng *et al.*, 2015). Within a host cell, *Salmonella* finds its protective base in the *Salmonella*-containing vacuole (SCV) while shielding itself from the defense mechanisms of the host. The bacterium finds protection when inside this vacuole because it enables replication before spreading to neighboring cells. *Salmonella* exhibits adaptive characteristics by changing the SCV conditions because this enables the bacterium to control signaling pathways within host cells to evade programmed cell death while surviving more effectively. Host-pathogen interactions demonstrate their dynamic nature through this reciprocal adaptation process of VIC and the pathogen (Knodler & Steele- Mortimer, 2003; Santos *et al.*, 2003; Boyle *et al.*, 2007; Eng *et al.*, 2015).

Transmission Routes

Salmonella spreads in the poultry system through horizontal and vertical transmission (Todd, 2014). The transmission of *Salmonella* occurs through horizontal channels when birds encounter environment-based contamination of their feed and water supply or through contact with infected poultry and their housing materials (Lozano, 2004; Karanth, 2021). *Salmonella* survives different environmental factors and disperses from contaminated bird feces so it enables horizontal transmission (Verma, 2024; Tufail & Ashfaq, 2024). Birds need to stay protected from horizontal *Salmonella* transmission, requiring strong biosecurity measures alongside proper sanitization procedures from poultry growers (Naumovska *et al.*, 2025; Meirhaeghe *et al.*, 2019).

The transmission of *Salmonella* occurs between an infected parent hen and her subsequent offspring during the natural reproduction process (Pande *et al.*, 2016). *Salmonella* can infiltrate the egg through two primary mechanisms: either by penetrating the egg yolk during its development process or by contact of bacteria with the shell while the chicken lays its eggs (Gast *et al.*, 2024). Bacterial entry into an egg results in an infected embryo and newly grown chicken (Rezaee *et al.*, 2021). The transmission through vertical pathways emphasizes an urgent need to control *Salmonella* contamination in breeding birds since this prevents bacterial spread to future generations (Wales & Davies, 2020).

In poultry, *Salmonella* is often spread because of certain external elements. The ability of *Salmonella* to live and be spread depends on several things, such as temperature, humidity, and the presence of vectors (Finn *et al.*, 2013). *Salmonella* is more likely to survive when it is warmer and more humid outside, which also raises the chance that it can be transmitted (Finn *et al.*, 2013; Hellberg & Chu, 2016). When feed is stored inappropriately and the poultry is exposed to dirty water, the presence of bacteria rises in the farming environment (Stanley & Bajagai, 2022). Risky aspects of salmonellosis can be controlled by keeping the area well ventilated, frequently cleaning all areas, and dealing with pests (Zamora-Sanabria & Alvarado, 2017; Ehuwa *et al.*, 2021). The sources and routes of *Salmonella* transmission are illustrated in Figure 3.

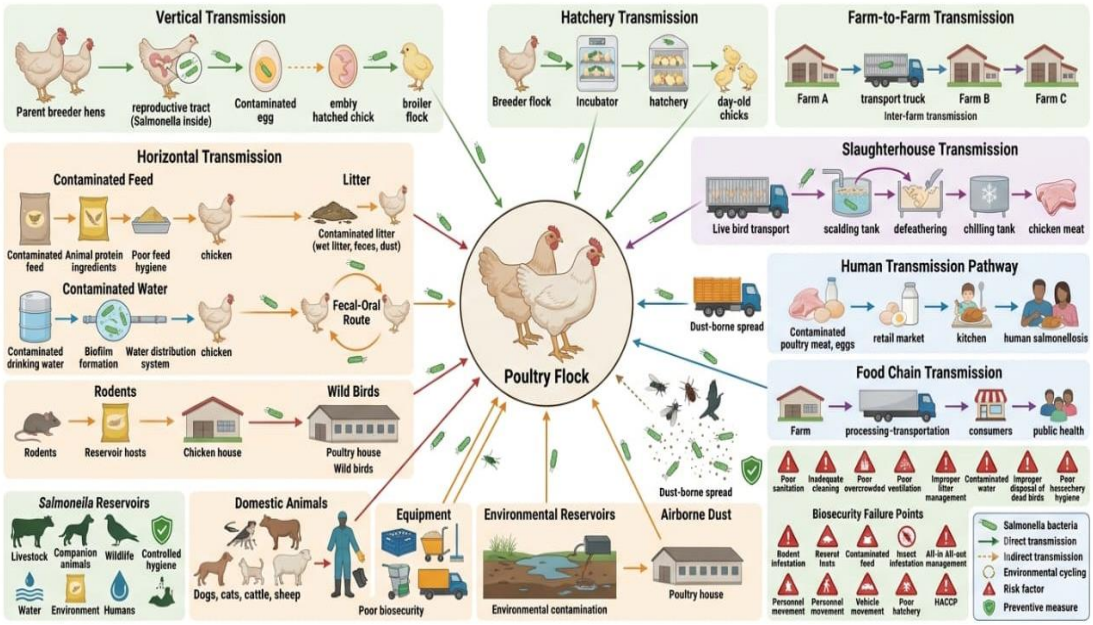


Figure 3. Transmission routes and sources of *Salmonella* bacterium in poultry

Worldwide Prevalence of *Salmonella* Infections in Poultry

Global Trends in Salmonella Prevalence

Salmonella comes after Campylobacter as the most significant foodborne disease affecting poultry farms and human health around the globe (Galán-Relaño et al., 2023). It is evident from prior research that the prevalence of Salmonella in poultry depends greatly on the region, thanks to factors such as how poultry is protected, antibiotic use, and the environment (Abdi et al., 2017). In a meta-analysis, experts found Salmonella may be detected in up to 90% of poultry farms, and this proportion is higher in LMICs because their lack of proper sanitation and regulations allows the bacteria to go unnoticed (Sanni, 2024).

Regional Prevalence and Contributing Factors

a. **Asia:** China has one of the highest incidences of *Salmonella* in poultry, which is largely attributed to scaled production (Liyanagunawardena, 2021). A study published in 2021 reported that 78.8% of Chinese poultry was contaminated with *Salmonella*, and in Nepal, the rate was the lowest at 15% (Subedi *et al.*, 2025).
b. **Europe**: The overall prevalence rate of *Salmonella* reported from Denmark was highest in broiler chickens (up to 60%) and is linked to intensive farming (Hazards *et al.*, 2019). Bosnia and Herzegovina, on the contrary, had the lowest prevalence (≤5%): it is associated with smaller-scale agriculture and strict regulations (Hadžiabdiæ *et al.*, 2015).
c. **Africa:** A high rate of prevalence (40-50%) of *Salmonella* in poultry has been reported from Ethiopia. The basic reason for high prevalence may be associated with insufficient hygienic conditions and overuse of antibiotics in poultry systems. Comparatively, Egypt and Libya have lower prevalence of *Salmonella* in poultry, with estimates ranging from 20 to 30 percent.
d. **North America:** The United States has managed to bring the prevalence of *Salmonella* to a level of 5-10% by introducing improved biosecurity and antibiotic stewardship programs. Nevertheless, there are still cases of outbreaks among industrial farming activities (Siceloff *et al.*, 2022).

Factors Driving Prevalence in Poultry

Biosecurity Gaps

In LMICs, 70–80% of poultry farms lack basic biosecurity measures such as disinfection protocols or controlled feed/water access, facilitating *Salmonella* spread (Hossain *et al.*, 2021; Qiu *et al.*, 2024)

Antibiotic Misuse

Prophylactic antibiotic use in poultry is common in regions like Pakistan and Brazil, accelerating antimicrobial resistance (AMR) in *Salmonella* strains (Hossain *et al.*, 2021). A recent research study (2023) conducted in 2023 reported that 60% of Pakistani poultry farms used antibiotics regularly, often without veterinary oversight (Qiu *et al.*, 2024).

Environmental Reservoirs

Salmonella survives in poultry feces, soil, and water for months, reinfection in flocks (Gast & Porter, 2020). Biofilm formation on farm surfaces further complicates eradication (Butucel *et al.*, 2022).

Treatment of *Salmonella* Infection and Drug-Resistant Strains

Practices of antibiotic use in Pakistani Poultry Farms

Antimicrobial agents are still widely used in different poultry systems for therapeutic, metaphylactic and prophylactic purposes against bacterial infection in Pakistan. Most commonly reported classes of antimicrobials are tetracyclines, fluoroquinolones, β-lactams, aminoglycosides, sulfonamides, polymyxins (colistin), and macrolides. Despite the regulatory efforts to enhance antimicrobial stewardship in recent years, improper prescribing, over-the-counter sales, and irregular application of antimicrobial use policies still encourage selective pressure towards resistant strains of *Salmonella* (Arshed *et al.*, 2025; Fatima *et al.*, 2023; Siddique *et al.*, 2024).

The poultry industry in Pakistan uses about 1,200 tons of antibiotics per year; 80- 90% of farms use the agents either prophylactically or as growth promoters, although non-therapeutic use was banned in 2018. Colistin is the most common (200-500 mg/kg feed, 40% of use), then ciprofloxacin (therapeutic, 25%), tetracyclines (20%), and azithromycin (post-COVID surge, 15%). More than 70% non-adherence can be explained by lax enforcement, cheap generics (about PKR 50 kg^{-1} colistin), and dependency of farmers on it in the presence of 20-30 % losses of flocks to salmonellosis (Arshed *et al.*, 2025; Fatima *et al.*, 2023).

Feed mills are usually designed to include 80-150 g/ton of antibiotics; layers are fed 1.5 times (broilers) to help in egg production. In Punjab and Khyber Pakhtunkhwa (n=150 farms), surveys reveal that 65% of farms administer antibiotics without veterinary supervision, which increases pressure on selection. The critically important drug list of the World Health Organization that encompasses colistin and ciprofloxacin represents 60% of the usage, reflecting the situation in low- and middle-income nations but surpassing the limits in the EU (Arshed *et al.*, 2025; Siddique *et al.*, 2024).

Table 3: Antibiotic usage prevalence in poultry farms (2000-2025)

Province	Colistin (% farms)	Ciprofloxacin (%)	Tetracycline (%)
Punjab	85	32	28
Sindh	72	28	22
KPK	65	20	35
Overall	78	26	26

*Usage by Province (Farm Surveys, n=500) (Arshed *et al.*, 2025; Siddique *et al.*, 2024; Fatima *et al.*, 2023)

The latest national evaluations show that antimicrobial stewardship is improving slowly after the inclusion of the National Action Plan on Antimicrobial Resistance in Pakistan, but there is still a significant regional disparity in the practices of antimicrobial usage, especially in smallholder poultry farms. These results highlight the importance of enhanced surveillance, veterinary care, and antimicrobial stewardship initiatives.

The Antimicrobial Resistance, Prevalence and Profiles

Significant variation in antimicrobial resistance profiles of bacterial isolates was observed among different poultry production systems. Isolates in broilers tended to be more often multidrug-resistant than those in commercial flocks, previously in breeder flocks, which was probably due to differences in antimicrobial use, habitats, flock turnover, and production. Moreover, poultry meat isolates often showed high rates of resistance as compared to those in samples of the environment, showing the significance of hygienic slaughtering and processing facilities.

Poultry *Salmonella* isolates are 85-100% resistant to first-line drugs with multidrug resistance (MDR, at least three drug classes) and extensively drug-resistant (XDR, at least five drug classes) observed in 72% and 18% of isolates, respectively. Combined phenotypic resistance rates in Poultry *Salmonella* isolates are: colistin 94.7%, azithromycin 92.98%, ampicillin 95.3%, tetracycline 87.7% and ciprofloxacin 84.2%. In Lahore poultry meat, 51.35% of the samples were contaminated, 100% of which were erythromycin-resistant, and 93% azithromycin-resistant after 2020 (Fatima *et al.*, 2023; Siddique *et al.*, 2024). The prevalence was reported to be 40% (72/180 samples) in live-bird markets in Islamabad and Rawalpindi, with high tetracycline resistance (71%). Farms in Southern Punjab, based on milk and environmental proxies, had a 27.87% prevalence of Salmonella, with district highs of Dera Ghazi Khan (32%) and Muzaffargarh (31%). Salmonella isolates from meat showed high beta-lactam resistance (30%), reported in Hyderabad broiler farms (Soomro *et al.*, 2010; Pereira *et al.*, 2024; Qamar *et al.*, 2020). Poultry *Salmonella* isolates that were not typhoidal (NTS) were identical to human isolates in 45% of cases, and the baseline prevalence was 25.67% in previous studies. Recent meta-analyses affirm that the AMR burden in Pakistan is higher than in South Asia (colistin resistance 70% vs. 94% in other parts of the world) (Arshed *et al.*, 2025).

Table 4: AMR patterns in poultry *Salmonella* (2000-2025)

Antibiotic Class	Agent	Resistance (%)	n Tested
Polymyxins	Colistin	94.7	1,234
Fluoroquinolones	Ciprofloxacin	84.2	892
Macrolides	Azithromycin	92.98	567
Tetracyclines	Tetracycline	87.7	1,111
Beta-lactams	Ampicillin	95.3	1,456
Phenicols	Chloramphenicol	78.4	789
Sulfonamides	Trimethoprim-SMX	82.1	456

Comprehensive Resistance Profiles (Pooled n=2,345 Isolates) (Fatima *et al.*, 2023; Siddique *et al.*, 2024; Soomro *et al.*, 2010; Pereira *et al.*, 2024; Qamar *et al.*, 2020; Arshed *et al.*, 2025)

Salmonella Enteritidis and *S. Typhimurium* were the reported serovars which show resistance to various antimicrobial classes such as tetracyclines, ampicillin, nalidixic acid, ciprofloxacin and sulfonamides. Several studies have reported multidrug-resistant isolates of *S. Gallinarum* in commercial poultry farms. However, serovar-specific antimicrobial susceptibility information is scarce in Pakistan, which underlines the necessity of nationwide surveillance of antimicrobial susceptibility involving phenotypic susceptibility testing, as well as molecular characterization (Fatima *et al.*, 2023; Siddique *et al.*, 2024).

Phage Therapy: An Emerging Solution against AMR

Principles of Phage Therapy

Bacteriophages (phages) are viruses which infect bacterial cells specifically and are the most common biological entities which exist in nature. Lytic phages, which are the most preferable to be used in treatment, bind to certain receptors on the bacterial surface, enter their genetic material into the host cell, replicate intracellularly and finally lyses the bacterium, releasing newly produced phage particles that can infect other prone bacteria (Abedon *et al.*, 2021; Cha *et al.*, 2024).

Bacteriophages, also known as phages, invade specific bacterial cells (Kasman & Porter, 2022). The Earth contains countless phages since they represent biological entities which outpace all other entities in abundance (Kirksey, 2022). Phages exist throughout natural spaces across the planet where they act as biocontrol agents against bacterial populations. The unique ability of bacteriophages to specifically target bacterial strains and species constitutes their primary characteristic. These viruses selectively eradicate

certain bacteria while leaving other components of the microbial community unaffected. This specificity offers a significant advantage over broad-spectrum antibiotics, as it allows for the preservation of both beneficial and pathogenic microbiota. Tail fibres and adsorption receptors of phages serve as structural proteins to connect with unique molecular patterns that exist on bacterial surfaces (Abedon *et al.*, 2021; Cha *et al.*, 2024). Scientists design techniques to modify lytic phages that specifically detect *Salmonella* spp. pathogens, enabling recognition of poultry-related strains such as *Salmonella enteritidis* and *Salmonella Typhimurium* (Kitchens *et al.*, 2024; Phothaworn *et al.*, 2024). Through its lytic lifecycle, phages bind to bacterial cells, transmit genetic material, use host cell machinery to establish a master-slave relationship, and finally lysis the bacterial cell (Fig 4). The virion then spreads towards neighboring bacteria, and this process creates a perpetual antimicrobial effect that does not require continued medication application (Sahu *et al.*, 2023). The main advantages of phages surpass the benefits of antibiotic medication (Suh *et al.*, 2022). Their specific target mechanism causes minimal destruction of beneficial host bacteria, which decreases the occurrence of dysbiosis and secondary infectious challenges (Zhao *et al.*, 2023; Federici *et al.*, 2021). The activity of phage-encoded enzymes, particularly depolymerases, enables these phages to break down bacterial biofilms, which leads to their disruption (Topka-Bielecka *et al.*, 2021). Phages have a unique characteristic which permits bacterial co-evolution, so they can react to developing resistance mechanisms while representing a long-lasting sustainable treatment option for traditional antimicrobial therapies (Fr, 2024; Lauman & Dennis, 2021).

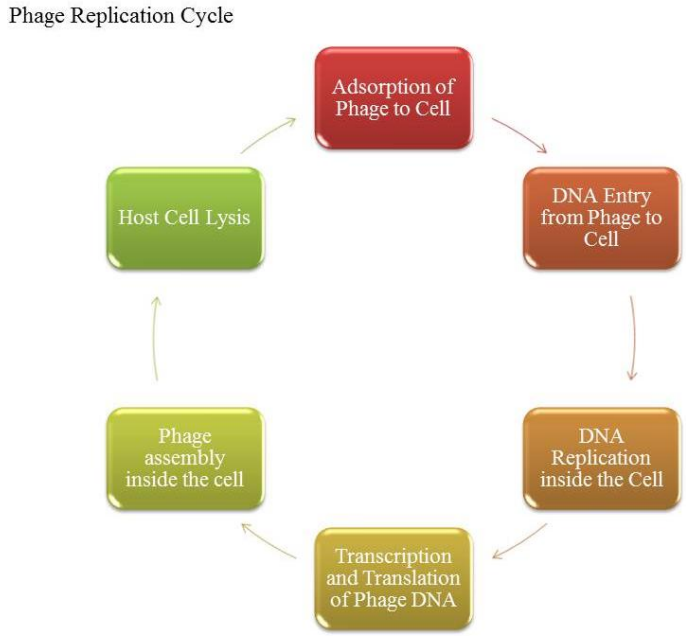


Figure 4. Replication cycle of Phage

Phage therapy offers a targeted approach to reducing *Salmonella* in poultry, as demonstrated in multiple studies. The experimental trial on phage therapy conducted in Brazil showed that phage cocktails reduced *Salmonella* levels in broiler chickens by 90% after 7 days, with no adverse effects on gut microbiota (Vaz *et al.*, 2024). In France, the phage product SalmoFREE® eliminated *Salmonella* in 100% of treated chickens within 33 days, improving feed conversion rates by 15% (Clavijo *et al.*, 2019).

In contrast to general antibiotics, lytic bacteriophages are highly host-specific and thus can be used to target the pathogenic bacteria without significantly disrupting the commensal intestinal microbiota. This particularity will minimize perturbation of the gut microbial ecosystem and could lessen the selection pressure on the development of antimicrobial resistance. Nevertheless, due to a narrow spectrum of bacteria that phages typically infect, therapeutic preparations typically comprise phage cocktails made up of various complementary phages to expand the host range and minimize chances of bacterial escape mutants.

Fundamentals and Mechanisms of Phage Therapy

The process of infection by bacteriophages begins with the attachment of the bacterial cells to their surface receptors, injection of the genetic material, replication in the host, and lysis of the cell, releasing progeny phages. The result of this lytic cycle is the rapid reduction of bacterial populations, with commensal microbiota being maintained and the

deposition of chemical residues, which is common with antibiotic therapy, being avoided. Phages are serovar-specific, and thus, they cause minimal off-target effects and reduce environmental microbiome perturbations (Strathdee *et al.*, 2023; Sahu *et al.*, 2023).

The effectiveness of Phage Therapy in poultry's Salmonellosis

Several experimental research studies have reported that lytic bacteriophages alleviate intestinal colonization, fecal shedding and environmental contamination of *Salmonella* in poultry. Laboratory experiments have reported decreases of about 3-5 log CFU/g of intestinal *Salmonella* counts following phage preparation administration. Equivalent decreases have been found in experimentally contaminated farm environments and poultry meat.

The recent intensive studies that were done in Pakistan have shown that *Salmonella* burdens have been reduced significantly after phage-based interventions. One such study isolated and characterized four lytic phages against *Salmonella gallinarum*, and both encapsulated and unprotected phage preparations were used to experimentally challenge chickens. The concentrations of *Salmonella* in the ceca reduced significantly compared to the untreated controls: unprotected phages produced a reduction of about 4 log CFU/g/day by day 1 and 4 post- challenge, and encapsulated phages produced a similar level of reduction; a combination of the two formulations produced a reduction of up to 2.4 log CFU/g/day by day 7 post-challenge. These results highlight the ability of phages in counteracting colonization and load reduction, a very important response in poultry health and food safety (Rehman *et al.*, 2025).

In another study, a cocktail of four lytic phages targeting *Salmonella pullorum* was evaluated with the use of drinking water and feed orally administered to immunocompromised chicks. The prophylactic use led to a substantial reduction in the bacterial counts (to 1.9 log CFU/g on day 6 after the challenge), and disease severity was delayed; mortality was reduced to 15% relative to untreated controls. Normal saline was less effective. Effectiveness was modulated by the time of phage administration; phage means the greatest effects on bacteria were observed with delivery 72 hours before or 48 hours after challenge, thus phage prophylaxis by oral route is recommended as an effective pre-harvest control approach (Durrani *et al.*, 2022). However, current evidence base has been characterized by laboratory-scale, controlled challenge, and pilot-scale studies. Therefore, it is possible to conclude that, despite current evidence of the biological usefulness of phage therapy, before it can be implemented on a routine commercial basis, more large field trials are needed.

Benefits and Real-Life Implications of Phage Therapy

Phage therapy has several benefits compared to traditional antibiotics. To begin with, phages circumvent plasmid-based resistance through attacking vital bacterial surface structures, and no phage resistance was observed under controlled Pakistani experiments (Rehman *et al.*, 2025; Torkashvand *et al.*, 2024). Second, phages are biologically specific, lysing specific serovars of *Salmonella* such as *S. Enteritidis, S. Typhimurium, S. Gallinarum* and *S. Pullorum* without disrupting the normal gut microbiome. Third, phages are active over a range of variable pH and temperature conditions characteristic of poultry gastrointestinal tracts; encapsulation methods provide additional benefits of delivery protection against gastric acids. Lastly, phage production is economical, and feed or water administration can be adopted at a commercial level, which has economic gains. Phage cocktails should be used preferentially over the single-phage formulations because it offers a wide spectrum of host range and help to curb the development of resistance. The isolates of Pakistani origin used in cocktails have demonstrated extensive lytic effects on local serovars such as a new strain of ST93 (Zaib *et al.*, 2025; Rehman *et al.*, 2025; Ullah *et al.*, 2025).

Poultry Management Intervention

The increased effectiveness is achieved when phage therapy is combined with biosecurity measures, vaccination, and probiotics. Combination forms of these interventions significantly decrease the colonization of the bacteria and, at the same time, increase the growth performance parameters (D'Accolti *et al.*, 2023).

Immune Response to Phage Therapy

Even though bacteriophages are believed to be harmless as they specifically infect bacteria and not eukaryotic cells, when assessing therapeutic efficacy, the interactions between phages and the host immune system should be taken into account (Putra and Lyrawati, 2020).

Repeated administration phages can induce innate and adaptive immune responses such as the production of phage-neutralizing antibodies. These antibodies have the ability to decrease the concentration of phages in circulation and can possibly decrease the level of therapeutic efficacy in treatments that are long-term or repeated (Berkson et al., 2024). On the other hand, a number of studies hypothesize that phages can also indirectly regulate inflammatory reactions by decreasing the bacterial load and curtailing the endotoxin-mediated inflammation (Souza et al., 2023).

Existing research shows that the oral delivery of phages in poultry is both generally well-tolerated and

has no negative effects on intestinal health or other beneficial microbiota (Shaufi et al., 2026). Furthermore, research studies are needed to define the long-term immune response, the best dosing schedule and the effects of repeated exposure to phages in commercial farming.

Applications in Poultry

Successful Case Studies and Laboratory Research

Phage therapy has shown significant results in poultry farming both in laboratory and field studies (Khan & Rahman, 2022; Agapé *et al.*, 2024). For instance, a research study demonstrated that phage cocktails targeting *Salmonella* reduced bacterial loads in experimentally infected chickens by 90% within 48 hours, with no adverse effects on gut microbiota (Martinez-Soto, 2024). In another study, oral administration of phages against *Salmonella enteritidis* in laying hens significantly reduced fecal shedding and egg contamination, highlighting potential for food safety applications (Gast *et al.*, 2024).

Potential for Prophylactic and Therapeutic Use

Prophylactic phage application in poultry feed or water could prevent *Salmonella* colonization in flocks, reducing transmission risks (Jordá *et al.*, 2023). Therapeutically, phages can be administered to infected birds to eliminate established infections (Mosimann *et al.*, 2021). According to an experimental trail broiler chickens infected with *Salmonella* showed that phage therapy restored normal growth rates and reduced *Salmonella* colonization in challenged broiler chickens (Thanki *et al.*, 2023).

Challenges and Considerations

Public Perception and Acceptance

The current public acceptance of phage therapy in poultry production is weak because people misunderstand viral therapies and they lack information about phage therapeutic benefits. The public acceptance of phage therapy depends heavily on transparent communication efforts about phage safety with educational campaigns that clarify their effectiveness (McCammon *et al.*, 2023; Simpson *et al.*, 2023).

Future Directions

Countries like Pakistan could benefit from international partnerships to develop localized phage banks and train farmers in biosecurity practices (Zafar and Sheikh, 2018; Safdar *et al.*, 2023). By integrating phage therapy into poultry health management, global *Salmonella* prevalence could be reduced by 50–70%, significantly lowering AMR risks and improving food safety (Khan & Rahman, 2022; Almutairi *et al.*, 2022).

Potential for Scaling Up Phage Therapy in Pakistan

Scaling up phage therapy requires addressing technical challenges, such as phage stability during storage and immune response mitigation. A study proposed lyophilization (freeze-drying) and encapsulation techniques to enhance phage shelf-life, critical for rural poultry farms with limited refrigeration. Additionally, public awareness campaigns are needed to address misconceptions about phage safety (Rehman *et al.*, 2019; Raza *et al.*, 2021).

Long-Term Impact on AMR and Public Health

Phage therapy could reduce Pakistan's reliance on antibiotics in poultry farming. This would directly address the WHO's warning that AMR could cost Pakistan's economy up to $10 billion annually by 2030. By integrating phage therapy into poultry health management, Pakistan could also reduce *Salmonella*-linked foodborne illnesses, which currently affect an estimated 2.5 million people yearly (Hameed *et al.*, 2024). Long-term success hinges on sustained investment in phage research, regulatory reforms, and international collaborations to position Pakistan as a regional leader in phage-based AMR solutions.

The regulatory framework for approval and commercialization of bacteriophage products in Pakistan

Bacteriophages are specifically referenced as a therapeutic good in the Drug Regulatory Authority of Pakistan (DRAP) Act 2012. DRAP is an autonomous body within the Ministry of National Health Services, Regulations and Coordination of the Federal Government of Pakistan and has the role of ensuring that all drugs, medical devices and health products are of quality, safe and effective. The products of bacteriophages should be registered by DRAP before importation or commercial supply in Pakistan. Recently, the Division of Biological Evaluation and Research (DRAP) revised its regulations on registration of biological products, aligning regulatory requirements with international standards. Applicants are required to file Form-5F (CTD format) to be registered and certain requirements must be met for biological products such as stability, pharmacovigilance, and comparability requirements. Biological products might need further documentation by the BE&R Division. Good Manufacturing Practice (GMP) standards are necessary to guarantee quality, safety and efficacy of bacteriophage therapeutic products to be used in clinical trials and commercial applications. DRAP has a chain of quality control laboratories that conduct approved products testing using the best international practices. DRAP is required to give

written approval to clinical trials using bacteriophage products. Bacteriophages are grouped as investigational biological products, and the process of carrying out clinical trials is regulated to guarantee the safety of the participants and proper design of the experiment. DRAP has also introduced a Lot Release System of biological products, and final products are released by the National Control Lab of Biologicals in Pakistan through the Lot Release System of the World Health Organisation (https://www.dra.gov.pk/wp-content/uploads /2022/ 10/DRAP-2012-_As-Amended-till-Feb-2022.pdf).

Conclusion

This systematic review is a synthesis of the existing evidence on the epidemiology of poultry-associated *Salmonella*, antimicrobial resistance (AMR), and how bacteriophage therapy could help in eradicating Salmonellosis in Pakistan. The analyzed literature proves that poultry production systems are a significant source of *Salmonella enterica*, and broiler flocks, poultry meat, and farm environments are the major sources of bacterial isolations. The most commonly reported serovars nationwide were *Salmonella Enteritidis, S. Typhimurium and S. Gallinarum*, but there was a high degree of regional heterogeneity and methodological heterogeneity in studies. The data show that there is an upward trend in the presence of multidrug-resistant *Salmonella* isolates, especially resistance to the most essential antimicrobials (e.g., fluoroquinolones and colistin).

The national standard surveillance and phenotypic antimicrobial susceptibility testing combined with molecular characterization should be standardized nationwide to enhance the knowledge of the epidemiology and spread of antimicrobial resistance in Pakistan. It can also be seen that the reviewed literature explains that lytic bacteriophages are potentially quite useful as targeted biological agents to control *Salmonella* in poultry. However, the existing data is based mostly on laboratory and pilot-scale studies. Issues such as the creation of standardized phage preparations, tracking phage resistance over time, regulatory acceptance, biosafety assessment, and validation on a commercial scale need to be resolved before routine application in the poultry industry can be suggested. All in all, bacteriophage therapy could be considered an addition to the integrated *Salmonella* control programs as opposed to substitutes to conventional disease-control measures.

Phage therapy combined with vaccination, enhanced biosecurity of farms, antimicrobial stewardship, hygiene control, and constant monitoring will probably be the most sustainable approach to salmonellosis control and curb the spread of antimicrobial resistance in Pakistan. This will need future multicenter field trials, which will be backed up by a suitable regulatory framework and One Health surveillance in order to translate prospective findings of experimental interventions into practical interventions for the poultry industry.

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