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Isolation and Characterization of Lytic Bacteriophages against Antibiotic-Resistant Salmonella enteritidis in Chickens

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Abstract **-** Salmonellosis caused by antibiotic-resistant *Salmonella enteritidis* represents a growing threat to poultry production worldwide, including in Kazakhstan. This study aimed to isolate and characterize *S. enteritidis* strains from poultry and to explore the potential of lytic bacteriophages as an alternative antimicrobial strategy. Bacterial isolation was carried out using MacConkey and bismuth-sulfite agars, followed by biochemical testing on Hiss medium and serological identification using commercial agglutination kits. Confirmation was performed through real-time PCR using VetMAX™ *Salmonella enterica* protocols. Antibiotic susceptibility testing of ten *Salmonella* strains, including eight previously isolated ones, was conducted using the disc diffusion method on Mueller-Hinton agar. The strains demonstrated variable resistance patterns: chloramphenicol and amoxicillin were 100% effective, while bacitracin, virginiamycin, and sulfafurazole showed no inhibition. Statistical analysis using the Friedman test revealed significant differences in antimicrobial efficacy ($p < 0.003$, $\chi^2 = 25.39$). Environmental samples, including poultry litter and soil, were processed to isolate bacteriophages using a host-specific enrichment method with *S. enteritidis* as the indicator strain, followed by centrifugation and membrane filtration. Phage lytic activity was evaluated by spot testing and subculture assays. Three bacteriophage isolates demonstrated effective lysis of multidrug-resistant *S. enteritidis*. These results suggest the potential for bacteriophage-based control as a complementary or alternative approach to antibiotics in managing resistant *Salmonella* infections in poultry farms. Further research will be directed toward optimizing phage formulation, stability, and delivery methods suitable for agricultural application.

Keywords**:** Salmonella enteritidis, antibiotic resistance, poultry farming, bacteriophage therapy, real-time PCR, antimicrobial susceptibility

1. Introduction

Antimicrobial resistance (AMR) has become a critical issue in global poultry farming, with *Salmonella enteritidis* being a major zoonotic pathogen responsible for significant morbidity in both animals and humans. In Kazakhstan and other Central Asian countries, poultry farms face increasing losses due to resistant strains, exacerbated by the widespread use of antibiotics as growth promoters and prophylactics. According to the WHO and recent EFSA data, *Salmonella* spp. remains a leading cause of foodborne outbreaks and economic disruptions in the EU and globally [1]. The limitations of antibiotic use underscore the need for alternative solutions, with bacteriophage therapy emerging as a promising biocontrol method [2], [3]. This study was designed to isolate *S. enteritidis* from poultry, assess their antibiotic resistance profiles, and evaluate bacteriophages as potential therapeutic agents.

2. Materials and Methods

2.1 Bacterial Isolation and Identification Samples were collected from cloacal swabs of broiler chickens on farms in southern Kazakhstan. MacConkey and bismuth-sulfite agars were used for selective cultivation. Colonies showing presumptive morphology were subjected to biochemical analysis in Hiss medium and serotyped using slide agglutination kits (AOOOT Biomed).

2.2 Molecular Confirmation DNA was extracted using a commercial kit (Qiagen). Real-time PCR was performed with VetMAX™ *Salmonella enterica* primers and probes according to the manufacturer's protocol. Ct values <45 were considered

positive.

2.3 Antibiotic Susceptibility Testing Ten *Salmonella* strains, including eight previously isolated strains and two new isolates, were tested on Mueller-Hinton agar using the Kirby-Bauer disc diffusion method. Antibiotics tested included chloramphenicol, amoxicillin, bacitracin, and others. Inhibition zones were measured in millimeters and analyzed statistically.

2.4 Bacteriophage Isolation and Lytic Activity Assay Environmental samples (soil, poultry litter) were enriched with overnight *S. enteritidis* cultures. After incubation, samples were centrifuged and filtered (0.22 μm). Phage activity was detected using the double-layer agar method and confirmed via spot assays.

2.5 Statistical Analysis The Friedman test was applied to evaluate the differences in antibiotic effectiveness across strains using SPSS 25.0. A p-value < 0.05 was considered statistically significant.

3. Results

Two newly isolated *S. enteritidis* strains fermented glucose and mannitol with gas and acid production, while failing to ferment lactose and sucrose. Both showed antigenic profiles consistent with *S. enteritidis* (O9, O12, H-g, H-m). PCR confirmed the presence of *S. enterica* DNA (Ct < 45) (Figure 1). Among tested antibiotics (Table 1), chloramphenicol and amoxicillin exhibited complete inhibition across all strains. Bacitracin, sulfafurazole, and virginiamycin showed no effect (Figure 2). The Friedman test confirmed significant differences in antimicrobial efficacy ($\chi^2 = 25.39$, $p < 0.003$). From environmental samples, three distinct lytic bacteriophages were isolated. They produced clear plaques on lawns of multidrug-resistant *S. enteritidis* and remained active in subcultures, indicating stable host specificity [4].

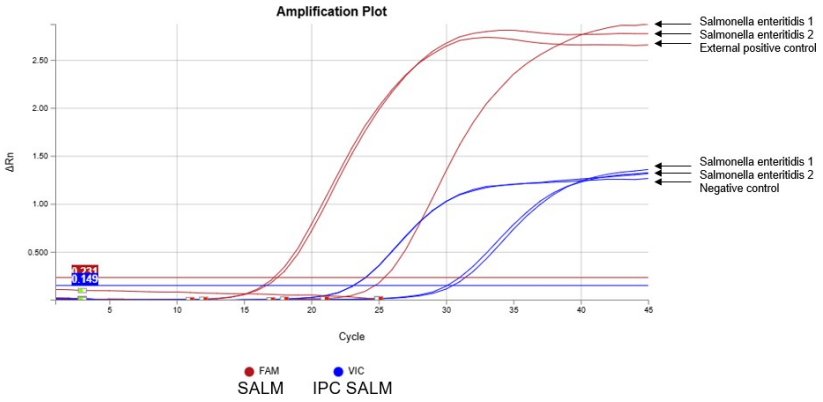

Fig. 1: Amplification plot of isolated strains.

Table 1: Antibiotic susceptibility testing of various salmonella strains

		Strains (mm)									
	Name of antibiotics	S.ent.1 1	S.typh. 2	S.infantis 3	S.ent.2 4	S.typh. m/c	S.ent. 1/1 6	S.ent. 1/3 7	S.ent. 1/5 8	S.typh. 1/7 9	S.typh. 1/8 10
1	Bacitracin B 10	-	-	-	-	-	-	-	-	-	-
2	/ Nalidixic acid 30	17,5	-	-	20	16	15	-	-	-	15
3	Chloramphenicol C 30	19	18	16	22	20	18	20	14	18	18
4	Cefaloride CR 30	-	-	-	-	-	11	-	-	9	9
5	Lincomycin L 2	-	-	-	-	-	-	-	-	-	-
6	Sulphafurazole SF 300	-	-	-	-	-	-	-	-	-	-
7	Co-trinoxazole COT 25	-	-	-	21	-	-	-	-	-	-
8	Vancomicin VA 30 ug	-	-	-	-	-	-	-	-	-	-
9	Chlortetracycline CT 30	10	-	-	13	-	10	-	-	-	-
10	Amoxycillin AMX 10	19	18	17	20	20	20	18	17	16	19
11	Ampicillin AMX 10	14	13	9	10	15	11	11	10	10	11

12	Doxycycline Hydrochloride DO 30	12	15	-	15	12	-	-	-	-	-
13	Cefotaxime CTX 30	21	25	21	23	24	20	23	22	22	24
14	Erytromycin E 15	-	-	-	-	10	-	-	-	-	-
15	Doripenem DOR 10	16	17	17	17	18	17	18	12	20	19

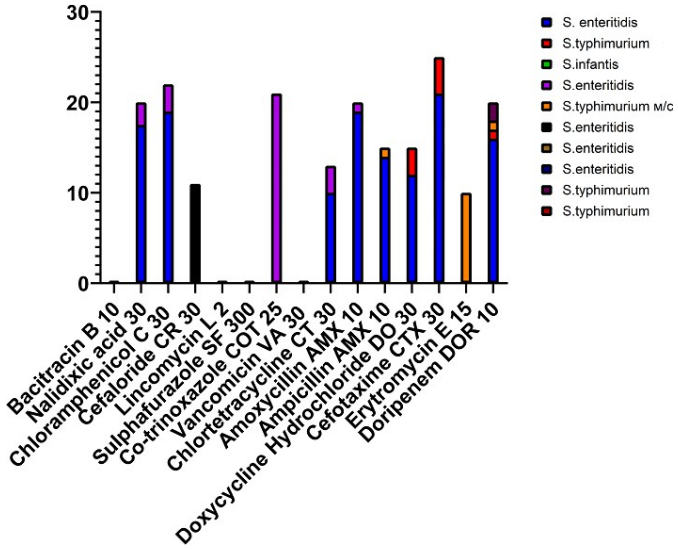

Fig. 2: Sensitivity of 10 Salmonella strains to 15 antibiotics, presented in millimeters of inhibition zone. The chart displays the most sensitive strains for each antibiotic. X-axis: types of antibiotics; Y-axis: sensitivity (mm).

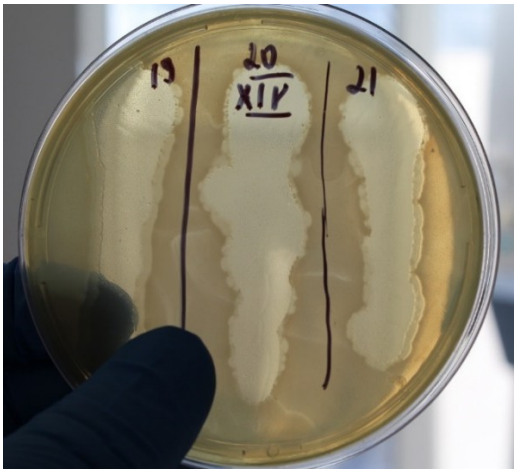

Fig.3: Three isolated phages demonstrating activity against Salmonella strains.

4. Conclusion

This study highlights the increasing resistance of *Salmonella enteritidis* strains to commonly used antibiotics in poultry production. The successful isolation of lytic bacteriophages provides a promising complementary strategy for controlling drug-resistant infections. Phage therapy, combined with biosecurity measures and responsible antibiotic use, could play a pivotal role in sustainable poultry health management in Kazakhstan and similar regions [5].

5. Acknowledgements

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