

Article

Antimicrobial resistance profiling and molecular detection of the *blaTEM-1* gene in *Salmonella* spp. isolated from layer birds in Bangladesh

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Abstract: Antimicrobial resistance in *Salmonella* spp. is a growing concern for poultry production and public health, particularly in settings where antimicrobial use is inadequately regulated. This study investigated the antimicrobial susceptibility profile and occurrence of the *blaTEM-1* gene among *Salmonella* spp. isolated from layer birds in Dinajpur district, Bangladesh. A total of 87 blood samples were collected from 15 layer farms between July 2024 and June 2025. Samples were screened using the rapid serum plate agglutination test (RSPAT), and suspected isolates were characterized by cultural and biochemical methods and confirmed by polymerase chain reaction (PCR) targeting the *invA* gene. Of the 87 samples, 83 (95.4%) were serologically positive, while 53 isolates were confirmed as *Salmonella* spp. by the combined identification procedures. Antimicrobial susceptibility was assessed using the Kirby–Bauer disk diffusion method. All 53 isolates were resistant to amoxicillin, ampicillin, tetracycline, and ciprofloxacin, whereas susceptibility was highest to co-trimoxazole (100%), followed by levofloxacin (71.7%) and ceftriaxone (64.15%). PCR screening of the 53 aminopenicillin-resistant isolates detected the *blaTEM-1* gene in 47 isolates (88.68%), with the expected amplicon size of 643 bp. These findings demonstrate substantial antimicrobial resistance among *Salmonella* isolates from layer farms and a high occurrence of *blaTEM-1* among aminopenicillin-resistant isolates. Practically, the findings support veterinary-supervised antimicrobial use, routine susceptibility testing before treatment, strengthened farm biosecurity, and molecular surveillance of resistance determinants across poultry production chains to guide treatment decisions and reduce the potential transmission of resistant *Salmonella* to humans.

Keywords: antimicrobial resistance; *invA* gene; antimicrobial susceptibility; polymerase chain reaction; poultry

1. Introduction

Salmonella is an important bacterial pathogen belonging to the family Enterobacteriaceae and can cause enteric infections in chickens, resulting in substantial morbidity, mortality, and production losses (Shaji *et al.*, 2023; El-

Shall *et al.*, 2026). Salmonellosis in poultry can occur as acute or chronic infection, with chronic infections potentially affecting flock health and long-term productivity even in the absence of immediate clinical signs (Gast and Porter, 2020). Beyond its effects on poultry health and production, *Salmonella* represents a significant public health concern because contaminated poultry meat, eggs, and other poultry-associated products can serve as vehicles for transmission to humans (Abd El-Ghany, 2020).

Foodborne diarrheal diseases remain a major global health burden, affecting approximately 1.7 billion people annually, including children who are particularly vulnerable to severe outcomes. Salmonellosis is an important contributor to this burden (Thystrup *et al.*, 2024). In poultry production, the rapid serum plate agglutination test (RSPAT) is commonly used to screen birds for antibodies against *Salmonella* and can support flock-level surveillance (Hoseini and Parvandar, 2024; Kabir *et al.*, 2021). However, serological positivity indicates an antibody response and does not, by itself, establish the presence of active bacterial infection. Consequently, combining serological screening with bacterial isolation, biochemical characterization, and molecular confirmation provides a more informative approach to investigating *Salmonella* occurrence in poultry.

Antimicrobials are frequently used by veterinary practitioners to treat and control bacterial infections, including salmonellosis (Ruvalcaba-Gómez *et al.*, 2022). Their effectiveness, however, is compromised when resistant bacterial populations emerge and spread. In Bangladesh and other resource-limited settings, antimicrobial use without appropriate veterinary oversight, together with inadequate awareness of responsible antimicrobial use, may contribute to the selection and dissemination of antimicrobial-resistant bacteria (Sharma *et al.*, 2022). Antimicrobial resistance may develop when bacteria undergo genetic changes or acquire resistance-associated genes from other bacteria through horizontal gene transfer, facilitating the dissemination of resistance traits within and between bacterial populations (Yap *et al.*, 2023). The resulting loss of antimicrobial effectiveness can lead to treatment failure, prolonged infection, increased treatment costs, and greater risks to animal and human health (Huemer *et al.*, 2020; Morrison and Zembower, 2020). Therefore, characterizing antimicrobial susceptibility and identifying the genetic determinants associated with resistance are important for evidence-based treatment decisions and antimicrobial resistance surveillance.

Resistance to different antimicrobial classes may arise through distinct mechanisms. In *Salmonella* spp., the *blaTEM-1* gene encodes a β -lactamase associated with resistance to aminopenicillins, including ampicillin and amoxicillin. Tetracycline resistance may involve determinants such as *tetA* and *tetB* (Abd *et al.*, 2021). The occurrence of *blaTEM-1* in other bacterial genera, including *Escherichia coli* and *Pseudomonas* spp., further illustrates the distribution of antimicrobial resistance determinants across diverse bacterial populations (Saleh and Naji, 2022; McDonagh *et al.*, 2024). Nevertheless, the presence of a resistance gene does not necessarily explain every phenotypically resistant isolate, because resistance may involve additional genes or other mechanisms. Integrating phenotypic antimicrobial susceptibility testing with targeted molecular detection can therefore help clarify the relationship between observed resistance and specific genetic determinants.

Despite the veterinary and public health importance of antimicrobial-resistant *Salmonella*, locally relevant evidence on antimicrobial susceptibility patterns and the occurrence of the *blaTEM-1* gene among *Salmonella* isolates from layer birds in Dinajpur district, Bangladesh, remains limited. In particular, the extent to which aminopenicillin resistance among recovered isolates is associated with the detection of *blaTEM-1* has not been adequately characterized in this study setting. Furthermore, the genetic similarity of the detected *blaTEM-1* sequences to sequences available in public databases requires investigation to place the findings in a broader genetic context. Without such information, local evidence remains insufficient to characterize the resistance patterns of the recovered isolates and support targeted surveillance and responsible antimicrobial use in layer production. Addressing this gap requires the combined application of serological screening, microbiological and molecular identification, antimicrobial susceptibility testing, and sequence-based analysis.

The present study addressed the following research questions: What proportion of blood samples collected from suspected layer birds in selected farms in Dinajpur district, Bangladesh, were serologically positive for *Salmonella* antibodies, and how many isolates were confirmed through cultural, biochemical, and *invA*-targeted molecular methods? What antimicrobial susceptibility and resistance patterns were exhibited by the confirmed *Salmonella* isolates? What proportion of aminopenicillin-resistant isolates carried the *blaTEM-1* gene, and how genetically similar was the detected gene sequence to sequences available in the NCBI GenBank database? The study hypothesized that the *blaTEM-1* gene would be detected in at least some aminopenicillin-resistant *Salmonella* isolates obtained from layer birds in selected farms in Dinajpur district, Bangladesh.

The objective of this study was to investigate *Salmonella* occurrence among layer birds in selected farms in Dinajpur district, Bangladesh, characterize the antimicrobial susceptibility profiles of confirmed isolates, detect the *blaTEM-1* gene among aminopenicillin-resistant isolates, and assess the genetic similarity of the detected sequences to corresponding sequences available in GenBank. The novelty of this study lies in combining

phenotypic antimicrobial susceptibility testing with targeted molecular detection of *blaTEM-1* and sequence-based comparison to characterize aminopenicillin resistance among *Salmonella* isolates from layer birds in this specific geographical setting. The findings provide locally relevant evidence to support veterinary-supervised antimicrobial use, routine antimicrobial susceptibility testing, strengthened poultry-farm biosecurity, and molecular surveillance of antimicrobial resistance, thereby contributing to efforts to reduce the spread of resistant *Salmonella* within poultry production systems and the associated potential risks to public health.

2. Materials and Methods

2.1. Ethical approval

Ethical approval for this study was obtained from the Institutional Ethical Committee (IEC) of Hajee Mohammad Danesh Science and Technology University (HSTU), Bangladesh (Approval No. HSTU/IRT/Ethical/BS/2025/07).

2.2. Study area, periods and sample collection

A total of 87 blood samples were collected from birds on 15 selected poultry farms in Dinajpur district, Bangladesh, between July 2024 and June 2025. The samples were collected from three locations: Biral (n = 21), Dinajpur Sadar (n = 49), and Chirirbandar (n = 17) (Figure 1). Approximately 5 mL of blood was collected aseptically from each suspected bird, and the serum was separated for serological testing. All collected samples were transported to the microbiology laboratory at HSTU, Dinajpur, for further analysis, following the procedure described by Sánchez-Romero *et al.* (2019).

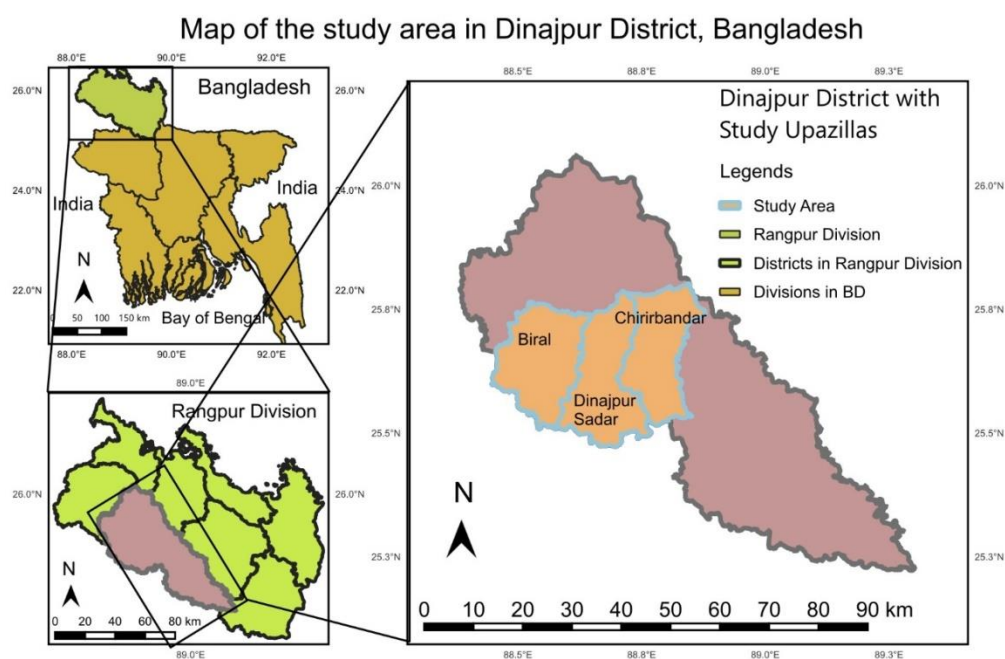


Figure 1. Geographical location of the study sites in Dinajpur district, Bangladesh.

2.3. Separation of serum

Blood was collected into anticoagulant-free tubes and kept at ambient temperature for approximately ten to fifteen minutes to facilitate coagulation and serum release. The tubes were subsequently centrifuged at 3,000 rpm for ten minutes to obtain clear serum. The resulting supernatant was carefully transferred into properly identified containers and preserved under appropriate storage conditions until serological testing was performed.

2.4. Serological examination

The RSPAT was performed to detect antibodies against *Salmonella* spp. Serum samples were brought to room temperature before testing. For each sample, 0.05 mL of serum was placed in the centre of a designated square on the test plate. A drop of positive control serum was placed in the final square. Subsequently, 0.05 mL of rapid serum agglutination antigen was added to each serum sample, and the mixtures were gently agitated by rotating the plate for 2 minutes, following the procedure described by Meher *et al.* (2022). The reactions were

then examined for agglutination. Visible clumping resulting from antigen–antibody reactions was interpreted as a positive result.

2.5. Isolation and identification of *Salmonella* spp.

The collected samples were inoculated onto nutrient agar (HiMedia, HiMedia Laboratories Pvt. Ltd., India) and incubated overnight at 37 °C. Colonies obtained on nutrient agar were subcultured onto MacConkey agar, followed by *Salmonella*–*Shigella* (SS) agar and Brilliant Green agar (HiMedia), using the streak-plate method to examine colony characteristics indicative of *Salmonella* spp. (Shalaby *et al.*, 2022). Suspected colonies were repeatedly subcultured on SS agar to obtain pure cultures. The isolates were then subjected to morphological examination, including assessment of colony size, shape, color, and opacity, and to biochemical tests for further identification, following the procedures described by Cheesbrough (2006). Isolates identified through these procedures were subsequently subjected to molecular confirmation and antimicrobial susceptibility testing.

2.6. DNA extraction

DNA was isolated from the presumptive *Salmonella* strains using a heat-based boiling and centrifugation technique, as outlined by Ramatla *et al.* (2024). The recovered DNA served as the template material for polymerase chain reaction (PCR) assays targeting the *invA* gene for bacterial confirmation and the *blaTEM-1* gene for detection of a resistance-associated determinant.

2.7. PCR amplification of the *invA* and *blaTEM-1* genes

PCR was performed to confirm the identity of presumptive *Salmonella* isolates and detect the *blaTEM-1* gene associated with aminopenicillin resistance. The *invA* gene was targeted for molecular confirmation of *Salmonella* spp. using the forward primer 5'-GTGAAATTATCGCCACGTTTCGGGC AA-3' and reverse primer 5'-TCATCGCACCGTCAAAGGAACC-3'. The *blaTEM-1* gene was screened using the forward primer 5'-CAGCGGTAAGATCCTTGAGA-3' and reverse primer 5'-ACTCCCCGTCGTGTAGATAA-3', following the methods described by Ramtahal *et al.* (2022) and Shen *et al.* (2023). The thermal cycling conditions used for amplification of both target genes are presented in Table 1.

Table 1. Thermal cycling conditions for PCR amplification of the *invA* and *blaTEM-1* genes.

Step	Temperature (°C)		Duration (minutes)		Cycle
	<i>invA</i>	<i>blaTEM</i>	<i>invA</i>	<i>blaTEM</i>	
Initial denaturation	95	95	2	2	1
Denaturation	95	95	1	1	30
Annealing	55	55	1	1	
Extension	72	72	1	1	
Final extension	72	72	5	7	1
Final hold	04	04	Until use	Until use	-

**invA* gene for *Salmonella* confirmation and *blaTEM-1* for aminopenicillin resistance

The PCR reaction mixtures were prepared using GoTaq® Green Master Mix (Promega), gene-specific forward and reverse primers, DNA template, and distilled water. Each reaction had a final volume of 25 µL. The volumes of the individual components used for amplification of the *invA* and *blaTEM-1* genes are detailed in Table 2.

Table 2. Composition of PCR reaction mixtures for amplification of the *invA* and *blaTEM-1* genes.

Component	Volume	
	<i>invA</i> (µL)	<i>blaTEM-1</i> (µL)
GoTaq® Green Master Mix (Promega) 1X	12.50	12.50
Forward primer	1.00	1.00
Reverse primer	1.00	1.00
DNA extract from <i>Salmonella</i> spp.	5.00	5.00
Distilled water	5.50	5.50
Total volume	25.00	25.00
1X= 1-fold concentration, µL= microliter		

Note: 1× indicates the working concentration, and µL denotes microlitres.

2.8. Agarose gel electrophoresis

PCR-amplified DNA fragments were separated and examined using agarose gel electrophoresis with a gel concentration of 1.5%. To prepare the gel, 0.75 g of agarose was mixed with 50 mL of 1× Tris-acetate-EDTA (TAE) buffer and heated in a microwave for four successive 30-second periods until the agarose had completely dissolved. The solution was allowed to cool for five minutes before adding 2.5 µL of ethidium bromide. The prepared mixture was transferred into a gel-casting tray and left undisturbed until polymerization was complete. The solidified gel was subsequently placed in an electrophoresis tank containing enough 1× TAE buffer to immerse the gel. A DNA size marker (6 µL) was introduced into the designated marker well, while 15 µL of each PCR amplicon was pipetted into separate sample wells. Electrophoresis was conducted at 98 V for 45 minutes for the *invA* target and at 100 V for 42 minutes for the *blaTEM-1* target. The run was terminated when the tracking dye had traversed approximately four-fifths of the gel. DNA bands were then detected under ultraviolet illumination using a gel documentation system (UVITEC, United Kingdom).

2.9. Antimicrobial susceptibility testing

Antimicrobial resistance patterns were evaluated in the 53 *Salmonella* isolates molecularly verified through amplification of the *invA* gene. Each isolate was tested against ten antimicrobial agents: ceftriaxone, azithromycin, chloramphenicol, co-trimoxazole, ciprofloxacin, ampicillin, streptomycin, amoxicillin, levofloxacin, and tetracycline. The assessment was conducted using the Kirby–Bauer disc diffusion technique, following the methodology reported by Shalaby *et al.* (2022) and the relevant recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2023). The observed inhibition zones were interpreted using the applicable criteria to classify each isolate as susceptible, intermediate, or resistant to the respective antimicrobial agents.

2.10. Sequencing of the *blaTEM-1* gene

PCR products that tested positive for the *blaTEM-1* gene were selected for sequencing. The PCR products and corresponding *blaTEM-1* primers were submitted to the National Institute of Biotechnology (NIB), Bangladesh, for Sanger sequencing. The sequencing procedure was based on the method described by Sanger *et al.* (1977). The resulting nucleotide sequences were used for subsequent sequence comparison and phylogenetic analysis.

2.11. Phylogenetic analysis of the *blaTEM-1* gene

Chromatogram files generated during sequencing and saved in AB1 format were processed with Chromas software to obtain nucleotide sequences in FASTA format. These sequences were subsequently aligned against comparable entries available in the GenBank repository maintained by the National Center for Biotechnology Information (NCBI), using the Basic Local Alignment Search Tool (BLAST) to assess sequence similarity. Evolutionary relationships among the selected sequences were further examined through the Neighbor-Joining approach, and the inferred clustering pattern was illustrated in a cladogram.

2.12. Statistical analysis

Laboratory data were organized and analyzed using Microsoft Excel. Descriptive statistical methods were used to summarize the serological results, the number of *Salmonella* isolates confirmed by culture, biochemical tests, and PCR, antimicrobial susceptibility patterns, and the detection frequency of the *blaTEM-1* gene. Results were expressed as frequencies and percentages. Antimicrobial susceptibility results were summarized by calculating the proportions of isolates classified as susceptible, intermediate, or resistant to each antimicrobial agent. The nucleotide sequences of the *blaTEM-1* gene were analyzed through sequence comparison using the BLAST against reference sequences available in the NCBI GenBank database. Sequence similarity and phylogenetic relationships were assessed using the Neighbor-Joining method, with the resulting relationships presented in a cladogram. The study-area map was prepared using QGIS software.

3. Results

3.1. Serological investigation using rapid serum plate agglutination test

A total of 87 blood samples collected from suspected diseased birds were examined using the RSPAT. Of these, 83 samples (95.40%) were serologically positive, while the remaining four (4.60%) were negative. Among all samples examined, 30 (34.48%) showed high agglutination, 40 (45.98%) showed moderate agglutination, and 13 (14.94%) showed low agglutination. No samples showed doubtful reactions (Table 3).

Table 3. Distribution of blood samples according to agglutination intensity in the rapid serum plate agglutination test (RSPAT).

Degree of severity	Total number of samples	Number of positive samples	Percentage (%)
High (++++)	87	30	34.48
Moderate (++++)		40	45.98
Low (++)		13	14.94
Doubtful (+)		0	0
Negative (-)		4	4.60

RSPAT= Rapid Serum Plate Agglutination Test; plus (+) and minus (-) signs represent the degree of agglutination observed during the test.

3.2. Cultural and biochemical identification of *Salmonella* spp.

The presumptive *Salmonella* isolates exhibited distinct colony characteristics on the different culture media. On nutrient agar, the colonies appeared moist, smooth, and grayish. On MacConkey agar, they formed pale-colored colonies, whereas on Salmonella–Shigella (SS) agar, the colonies displayed black centers. On Brilliant Green agar (BGA), the colonies appeared reddish-pink. These cultural characteristics, together with the results of biochemical tests, were used for the preliminary identification of the isolates (Figure 2).

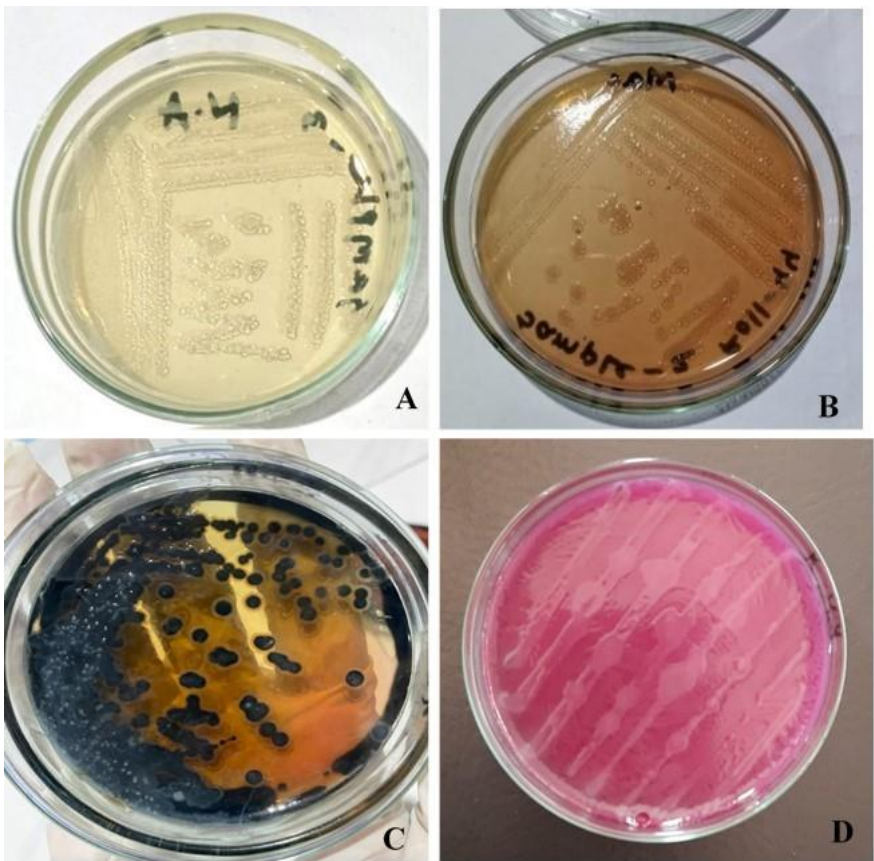


Figure 2. Cultural characteristics of presumptive *Salmonella* spp. on different media: (A) moist, smooth, grayish colonies on nutrient agar; (B) pale-colored colonies on MacConkey agar; (C) black-centered colonies on Salmonella–Shigella (SS) agar; and (D) reddish-pink colonies on Brilliant Green agar (BGA).

The presumptive isolates were further characterized using biochemical tests to support their identification as *Salmonella* spp. The isolates were positive for catalase activity, methyl red (MR), and citrate utilization, but negative for oxidase activity, the Voges–Proskauer (VP) test, indole production, and urease activity. Motility was observed in the motility–indole–urease (MIU) test. In the triple sugar iron (TSI) test, the isolates produced hydrogen sulfide (H₂S), as indicated by blackening of the medium (Figure 3). These biochemical characteristics supported the preliminary identification of the isolates, which were subsequently subjected to molecular confirmation.

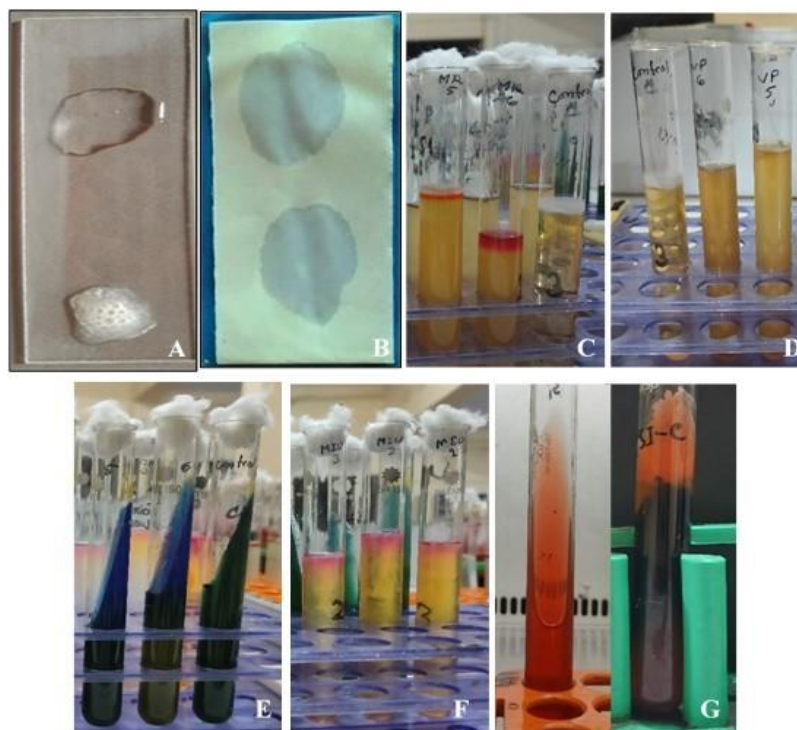


Figure 3. Biochemical characteristics of presumptive *Salmonella* spp.: (A) positive catalase reaction, indicated by bubble formation; (B) negative oxidase reaction, indicated by the absence of colour change; (C) positive methyl red (MR) reaction, indicated by a red colour; (D) negative Voges–Proskauer (VP) reaction; (E) positive citrate utilization, indicated by a colour change to royal blue on Simmons citrate agar; (F) results of the MIU test, showing motility with negative indole and urease reactions; and (G) TSI reaction showing a red slant and H₂S production, indicated by blackening of the medium.

PCR amplification targeting the *invA* gene produced the expected 284 bp amplicon, supporting the molecular confirmation of *Salmonella* spp. (Figure 4). Based on the combined cultural, biochemical, and molecular findings, 53 *Salmonella* isolates were confirmed from the 83 serologically positive samples.

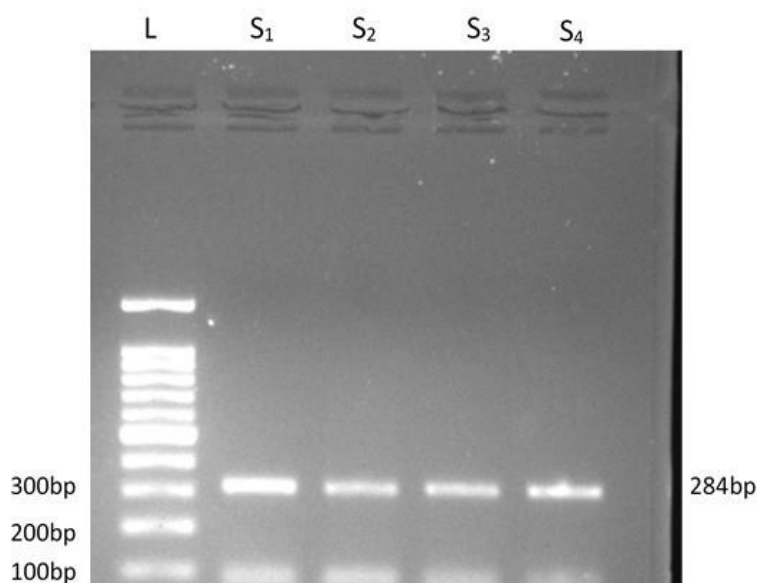


Figure 4. PCR-based molecular confirmation of *Salmonella* spp. through amplification of the *invA* gene, yielding the expected 284 bp product. The PCR products were separated by agarose gel electrophoresis at 98 V for 45 minutes and visualized using a gel documentation system. The annealing temperature used during PCR amplification was 55°C. L: 1,500 bp DNA ladder; S₁–S₄: sample lanes.

3.3. Antimicrobial susceptibility and resistance profile of *Salmonella* isolates

Antimicrobial susceptibility testing was performed on all 53 *Salmonella* isolates confirmed by cultural, biochemical, and molecular methods. All isolates (100%) were resistant to amoxicillin, ampicillin, tetracycline, and ciprofloxacin. In contrast, all isolates (100%) were susceptible to co-trimoxazole. Susceptibility to levofloxacin and ceftriaxone was observed in 71.70% and 64.15% of the isolates, respectively. Resistance to the other antimicrobial agents varied, with the highest resistance observed against streptomycin (66.04%), followed by chloramphenicol (35.85%), azithromycin (30.19%), ceftriaxone (20.75%), and levofloxacin (15.09%) (Figure 5).

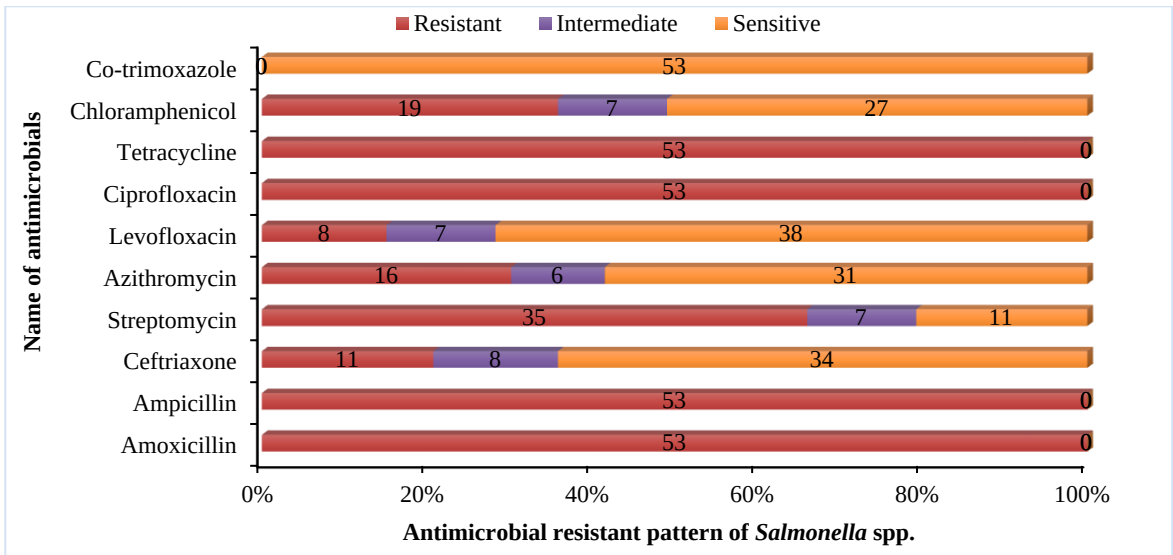


Figure 5. Antimicrobial susceptibility and resistance profiles of *Salmonella* isolates against ten antimicrobial agents.

3.4. Molecular detection of the *blaTEM-1* gene

All 53 amoxicillin-resistant *Salmonella* isolates were screened for the presence of the *blaTEM-1* gene using PCR with the *blaTEM*-F and *blaTEM*-R primers. Amplification produced the expected 643 bp fragment. The *blaTEM-1* gene was detected in 47 of the 53 isolates (88.68%), whereas the remaining six isolates (11.32%) were negative for the target gene (Figure 6).

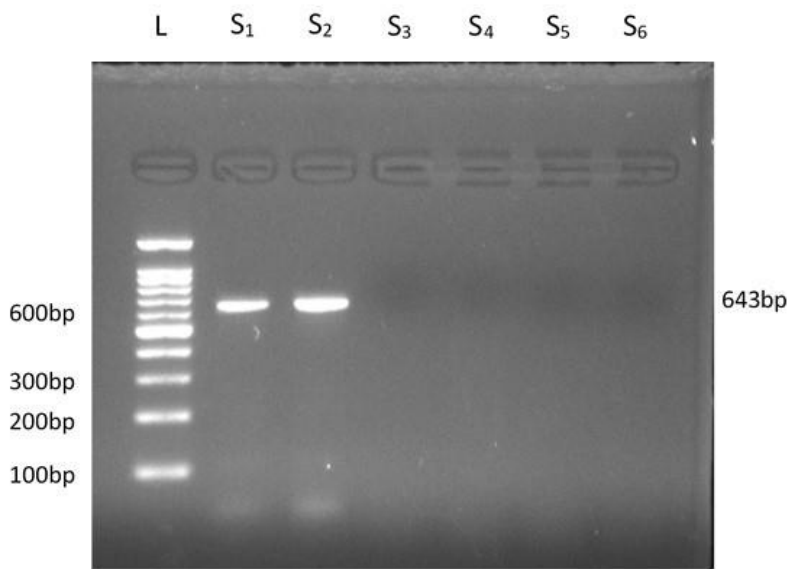


Figure 6. PCR detection of the *blaTEM-1* gene in *Salmonella* isolates, showing the expected 643 bp amplicon. L: 1,500 bp DNA ladder; S₁–S₆: sample lanes.

3.5 Phylogenetic analysis of *blaTEM-1* gene

The nucleotide sequences obtained from the study isolates were analyzed to assess their similarity to reference *blaTEM-1* sequences available in the NCBI GenBank database. Raw sequencing data in AB1 format were processed and converted to FASTA format using Chromas software. The resulting sequences were compared with reference sequences using the BLAST. The sequence comparisons revealed more than 98.5% nucleotide similarity between the study sequences and the selected reference sequences. These included sequences associated with *Klebsiella* sp. strain *Kleb-1*, *Salmonella enterica* subsp. *enterica*, *Streptococcus* sp. strain *GDStrp9*, *Enterococcus* sp. strain *ent10*, and *Pseudomonas* sp. strain *pseudo12*. In the phylogenetic analysis, the study sequence clustered closely with the *blaTEM-1* sequence from *Pseudomonas* sp. strain *pseudo12* (Figure 7).

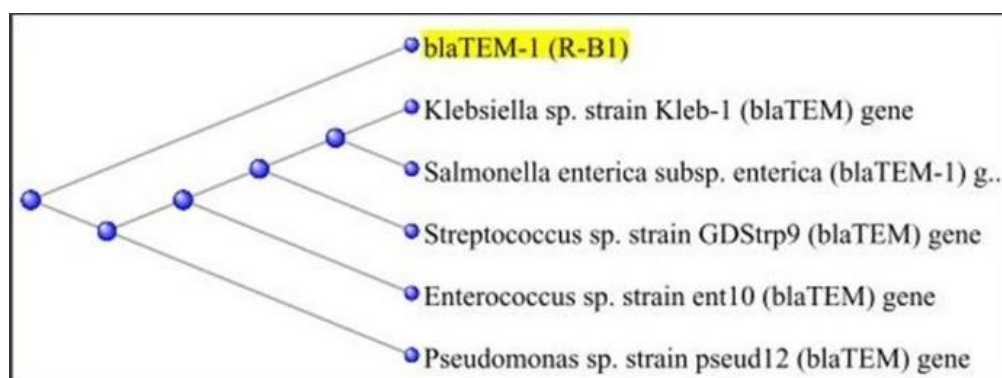


Figure 7. Phylogenetic analysis of the *blaTEM-1* gene sequence obtained from the study isolates and selected reference sequences retrieved from the NCBI GenBank database.

4. Discussion

In the present study, 83 of 87 blood samples (95.40%) were serologically positive by the RSPAT. Among these, 30 samples showed high agglutination, 40 moderate agglutination, and 13 low agglutination. However, only 53 isolates were confirmed as *Salmonella* spp. through cultural, biochemical, and molecular procedures. The seropositivity observed in this study was higher than the 47.8% reported in poultry from the Mymensingh and Gazipur regions of Bangladesh (Meher *et al.*, 2022). This difference may be associated with geographical variation, farm management practices, biosecurity, vaccination history, or differences in sampling and diagnostic methods. Nevertheless, direct comparison should be made cautiously because the studies may have investigated different poultry populations. Importantly, RSPAT detects antibodies and therefore indicates serological reactivity rather than independently confirming active infection. The difference between seropositive samples and confirmed isolates highlights the importance of combining serological screening with microbiological and molecular methods (Jinu *et al.*, 2014; Haider *et al.*, 2023).

The presumptive isolates exhibited characteristic colony appearances on nutrient agar, MacConkey agar, *Salmonella*–*Shigella* (SS) agar, and Brilliant Green agar (BGA). Their biochemical profiles were also consistent with the preliminary identification of *Salmonella*. Similar cultural characteristics have been reported by Mahmood *et al.* (2023) and Sarker *et al.* (2021), while comparable biochemical identification procedures have been described by Karim *et al.* (2020), Pertiwi-Koentjoro *et al.* (2023), and Aktar *et al.* (2023). Furthermore, PCR amplification targeting the *invA* gene produced the expected 284 bp amplicon in the confirmed isolates. These findings demonstrate the value of combining conventional microbiological procedures with molecular confirmation. Nevertheless, cultural and biochemical characteristics alone may overlap with those of other bacteria, making confirmatory testing important for improving identification reliability (Yanestria *et al.*, 2019). The antimicrobial susceptibility results revealed substantial resistance among the 53 confirmed *Salmonella* isolates. All isolates were resistant to amoxicillin, ampicillin, tetracycline, and ciprofloxacin. Resistance was also observed to streptomycin (66.04%), chloramphenicol (35.85%), and azithromycin (30.19%). In contrast, all isolates were susceptible to co-trimoxazole, while susceptibility to levofloxacin and ceftriaxone was 71.70% and 64.15%, respectively. The high resistance to aminopenicillins and tetracycline is consistent with previous studies reporting considerable resistance to beta-lactams and tetracycline among poultry-associated bacteria in Bangladesh (Akond *et al.*, 2012; Sarker *et al.*, 2021). Foysal *et al.* (2024) also reported substantial antimicrobial resistance among *Salmonella* and *Escherichia coli* isolated from commercial poultry farms, highlighting antimicrobial resistance as an important concern in poultry production.

The complete resistance to ciprofloxacin is particularly noteworthy because it contrasts with the high susceptibility reported by Sarker *et al.* (2021). This difference may reflect geographical or temporal variation, differences in farm-level antimicrobial exposure, or variation in the bacterial populations examined. Fluoroquinolone resistance may involve mutations in target genes or other resistance mechanisms, but these mechanisms were not investigated in the present study. Therefore, their contribution to the observed resistance cannot be established (Yang *et al.*, 2025; Ali *et al.*, 2025).

The *blaTEM-1* gene was detected in 47 of the 53 amoxicillin-resistant *Salmonella* isolates (88.68%), while the remaining six isolates (11.32%) were negative for the target gene. This high detection frequency suggests that *blaTEM-1* may be an important contributor to the aminopenicillin resistance observed in the study population. A comparable finding was reported by Alam *et al.* (2020), who detected *blaTEM-1* in 82.85% of phenotypically resistant *Salmonella* isolates from broiler chickens in Bangladesh. The similarity between these findings suggests that the gene warrants further investigation in poultry-associated *Salmonella* populations in the country, although differences in sampling and laboratory procedures should be considered when comparing the reported percentages (Salem *et al.*, 2026).

The detection of *blaTEM-1* is consistent with a potential beta-lactamase-mediated mechanism of resistance to aminopenicillins. However, the presence of this gene does not prove that it was the sole cause of the observed resistance. The six phenotypically resistant but PCR-negative isolates may possess other resistance determinants, or the target gene may not have been detected because of limitations of the assay. Since the present study did not screen for a broader range of beta-lactamase genes or other resistance mechanisms, further molecular characterization is required to explain the complete resistance profile (Castro-Vargas *et al.*, 2020; Salem *et al.*, 2025).

The sequence analysis revealed more than 98.5% nucleotide similarity between the study sequences and selected reference sequences associated with *Klebsiella* sp. strain Kleb-1, *Salmonella enterica* subsp. *enterica*, *Streptococcus* sp. strain GDStrp9, *Enterococcus* sp. strain ent10, and *Pseudomonas* sp. strain pseudo12. The study sequence clustered closely with the *blaTEM-1* sequence associated with *Pseudomonas* sp. strain pseudo12. These results indicate substantial sequence similarity between the analyzed gene and the selected reference sequences (Lee *et al.*, 2022). The occurrence of similar antimicrobial resistance genes in different bacterial taxa is biologically plausible because resistance determinants can be associated with mobile genetic elements, potentially enabling their dissemination between bacteria. However, similarity and clustering based on a resistance-gene sequence alone cannot establish the origin of the gene, direct transmission, or horizontal gene transfer between the study isolates and the reference organisms (Cross *et al.*, 2025). The observed clustering should therefore be interpreted as a relationship between the analyzed sequences rather than proof of an evolutionary or transmission pathway between the bacterial hosts. Further investigation using a broader reference dataset, whole-genome sequencing, and characterization of the genetic context surrounding *blaTEM-1* would help clarify its genomic distribution and potential mobility (Katonge and Ally, 2025; Klümper *et al.*, 2025).

The detection of antimicrobial-resistant *Salmonella* in layer birds is relevant to public health because poultry and poultry products may provide routes for human exposure to foodborne pathogens. The combination of resistance to several tested antimicrobial agents and the frequent detection of *blaTEM-1* highlights the need for continued monitoring of resistant bacteria in poultry production (Hossain *et al.*, 2021). Nevertheless, the present study did not examine eggs, poultry meat, farm environments, or human clinical isolates. Therefore, it cannot establish whether the identified isolates entered the food chain or were transmitted to humans. The findings should instead be considered evidence supporting further investigation through an integrated One Health approach involving animal health, food safety, environmental surveillance, and human health (Osman *et al.*, 2018; Mengistu *et al.*, 2025).

Several limitations should be considered when interpreting these findings. The study involved selected layer farms in a single district, limiting the generalizability of the results to other poultry-producing regions of Bangladesh. Farm-level factors, including biosecurity, litter management, feed and water quality, and antimicrobial-use history, were not assessed. Furthermore, molecular characterization was restricted to the *invA* and *blaTEM-1* targets, limiting the investigation of alternative resistance mechanisms. Future studies should include larger and geographically broader samples, systematic collection of farm-management and antimicrobial-use data, and expanded resistance-gene screening. Whole-genome sequencing and integrated sampling of poultry, poultry products, environmental sources, and human clinical isolates would provide a more comprehensive understanding of the distribution and public health significance of antimicrobial-resistant *Salmonella* in Bangladesh (Álvarez-Espejo *et al.*, 2026).

5. Conclusions

This study revealed substantial antimicrobial resistance among *Salmonella* isolates from layer farms in Dinajpur, Bangladesh, with frequent detection of the *blaTEM*-1 gene among amoxicillin-resistant isolates. The findings highlight the need for responsible veterinary antimicrobial use, routine susceptibility testing, improved farm biosecurity, and continuous antimicrobial resistance surveillance to protect poultry health and public health. However, the underlying resistance mechanisms and potential transmission pathways remain unclear. Future research should investigate a broader range of resistance genes using whole-genome sequencing and include poultry farms across diverse geographical regions to better understand the distribution and transmission of resistant *Salmonella* in Bangladesh.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Generative AI statement

No generative AI was used during preparation of this article.

Authors' contributions

Nazmi Ara Rumi: study design, investigation, manuscript review, and revision; Jakiul Hasan Jihad: investigation, manuscript drafting, review, and revision; Md. Tauhid Morshed: sample collection and investigation; Faiz Arafat Maksud: sample collection and investigation; Md. Khaled Hossain: manuscript writing, editing, and revision; Md. Shajedur Rahman: manuscript writing, editing, and revision; Md. Nurnoby Islam: manuscript editing and revision; Md. Asaduzzaman Nur: manuscript editing and revision. All authors have read and approved the final manuscript.

Conflict of interest

None to declare.

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