Prevalence and Characteristics of Multidrug-Resistant (MDR) Nontyphoidal Salmonella isolates from Poultry in Hazaribagh, along with the associated risks for Food Safety and Public Health

ABSTRACT: As one of the most prevalent infectious agents linked to food and food products of animal origin, Nontyphoidal Salmonella (NTS) is a significant cause of food safety infringement worldwide. Understanding the processes of antibiotic resistance and the genetic determinants of bacterial virulence is essential to comprehending the pathophysiology of NTS. The results show the prevalence of Salmonella in chickens, highlighting a significant public health concern. Effective salmonellosis control strategies require precise carrier identification, comprehensive management systems, and exact medication regimens. By addressing these issues and working to reduce the prevalence of antibiotic-resistant bacteria in chicken meat, stakeholders can safeguard public health and promote food safety. The study highlights the public health risks posed by *Salmonella* in chicken, exacerbated by antibiotic-resistant strains. The widespread use of antibiotics in the chicken industry has led to an increase in antibiotic resistance in chickens, raising concerns about potential future consequences. To combat salmonellosis, effective antimicrobial treatments, proper bird management, and identification of infected flocks are essential. Stakeholders can reduce the prevalence of antibiotic-resistant bacteria in chicken meat by addressing these factors, thereby enhancing food safety and protecting public health. According to the phylogenetic tree analysis in the document, *Salmonella weltevreden* is the most distantly related species, suggesting significant evolutionary divergence. *Salmonella Newport* forms a separate lineage, diverging early from the other species. *Salmonella nchauga* and *Salmonella Schwarzengrund* share a more recent common ancestor, with *Salmonella schwarzengrund* being the most recently evolved species. The study's conclusion—that the isolated Salmonella strains exhibited an alarming rate of antibiotic resistance—further underscores the seriousness of the issue. Controlling the use of antibiotics in the chicken industry and halting the emergence of resistant strains requires immediate action.

Keywords- Antibiotic Resistance, Salmonellosis, Salmonella enterica, Enterobacteriaceae, Multidrug Resistance.

1. Introduction

One of the most prevalent foodborne illnesses, *Salmonella* infections, causes over 93.8 million illnesses and 155,000 fatalities worldwide each year [1]. *Salmonella* is a rod-shaped, facultatively anaerobic, gram-negative bacteria member of the Enterobacteriaceae family. Salmonella enterica and *Salmonella bongori* are the two species of this genus currently known to exist. Six subspecies of *S. enterica* are also found, with the most frequent infections in homeotherm animals caused by *S. enterica subsp enterica* [2]. Their biochemical traits and antigenic structure set them apart from other members [3]. The quantity of *Salmonella* species (spp.) in animal digestive tracts, which act as important reservoirs for the organism, contributes to the species' pervasiveness in the environment [4]. Humans often contract the bacterium through the consumption of contaminated food and water [5]. In the 1800s, *Salmonella* was first discovered. Salmon and Smith cultivated the microbe in 1808. White Kauffmann-Le Minor has

identified nearly 2,600 *Salmonella* serotypes, 1,600 of which are associated with the enterica subspecies [8].

The human and animal digestive tracts appear to be the only home for the genus *Salmonella*. Therefore, fecal contamination explains why *Salmonella* exists in various habitats (food, water, and the natural environment). Specific serovars (serotypes) only live in one host species, such as sheep (serovar Abortusovis), birds (Gallinarum), or humans (serovars Typhi, Paratyphi A) [9].

Salmonella species have emerged as one of the primary foodborne pathogenic bacteria, causing significant rates of illness and mortality globally over the past three decades. Salmonellosis is a human intestinal illness caused by *Salmonella* spp [10]. The disease is prevalent in most underdeveloped nations and can occasionally lead to epidemics in developed countries [14]. In response to this data gap, the World Health Organization (WHO) has initiated a new program to provide more accurate estimates of the global burden of foodborne illness. According to WHO reports, 600 million individuals worldwide—nearly one in ten—have gotten sick after eating tainted food during the past five years. One of India's agricultural industries with the quickest growth rate is poultry. In India and worldwide, poultry meat and its derivatives are highly consumed. The most significant cause of human infection is poultry, a natural *reservoir of the Salmonella species*. *Salmonella* infections can be transmitted from poultry to humans through the food chain. Numerous investigations have demonstrated that chicken and poultry products cause *Salmonella* infection [12]. Most chicken meat produced in developing nations comes from integrated broiler farms. To manage all aspects of the chicken business, including consumer handling, retail distribution, hatchery operations, feed management, broiler slaughter, and breeder flock management [13].

When *S. enterica* ST-contaminated food or water is ingested, infection results. Most individuals who recover from the infection can eliminate the bacteria. Meanwhile, some individuals could remain healthy carriers and consistently excrete *S. enterica* ST in their feces [15].

Cattle infections caused by *Salmonella enterica subsp. enterica serovar Dublin* occurs throughout the world and typically results in severe clinical illness [16]. Controlling serovar Dublin in consistently infected herds is believed to depend on the identification and subsequent culling of carrier animals. According to epidemiological research, the incidence of salmonellosis is rising globally [17]. Typhoid fever is caused by Salmonella enterica serovar Typhi and is estimated to result in approximately 16 million illnesses and 600,000 associated fatalities worldwide [20].

Three distinct clinical syndromes are often linked to the human host-adapted *S. enterica subsp. enterica serovars: Enterica serovar Salmonella enterica subsp*. Nontyphoidal Salmonella (NTS) causes gastroenteritis in immunocompetent individuals but can produce bacteremia in immunocompromised individuals (such as those with advanced HIV illness, severe malaria, and malnutrition in infants), while Typhi causes typhoid fever [21].

1. Material and methods

2.1. Sample collection

For the investigation, samples of chicken intestines were procured from commercial broiler chicken retailers within the Hazaribagh region of Jharkhand, India. These were gathered between December 2023 and September 2024, spanning Hazaribagh's East to West and South to North zones. It includes the locations from each Zone, Nawada, Sindoor, Ichak, and Pelawal in Hazaribagh, Jharkhand, India.

2.2. Culture of Bacteria

A sterile spatula was used to pre-enrich 25 g of each sample in 225 mL of buffered peptone water (BPW) (HiMedia, Mumbai) for 48 hours at 37°C. Following a 48-hour incubation period at 42°C in 10 mL of Rappaport-Vassiliadis broth (RVS), a loopful of the pre-enriched sample was aseptically streaked onto Salmonella-Shigella Agar (SS Agar) and Xylose-Lysine Deoxycholate Agar (XLD). For twenty-four, the plates were incubated at 37°C. Five or more colonies that met the requirements to be presumed. After being cultivated on Salmonella-Shigella agar and XLD agar plates, colonies of Salmonella were selected and subculture on slants of nutritional agar.

2.3. Subculturing

On a MacConkey agar plate, a loop of the infected phosphate-buffered peptone water was streaked and incubated aerobically for 24 hours at 37 °C.

2.4. Isolate Purification and Preservation

Continuous subculturing on nutrient agar was used to purify colonies that did not ferment lactose. Pure isolates were stored on nutrient agar slopes at 4ºC inside the refrigerator. Identification of Bacteria using Gram's staining.

2.5. Identification and Characterization of Bacterial Species using various Biochemical test

Catalase Test: 100 µL of the bacterial sample was placed on a sterile glass slide. A small amount of H2O2 solution was added to it. Bubble formation was then permitted to be seen.

Glucose Utilization Test: The test organism was added to sugar media, which were then incubated overnight at 37 °C. Observations were taken every day for seven consecutive days. The medium becoming pink signified the creation of acid, whereas the air trapped in the Durham tube demonstrated the production of gas.

Motility Test (Hi-Media, Mumbai): In a U-shaped tube, the test organism was inoculated at one end of a semi-solid motility medium, which was then incubated for approximately four days at 37 °C. A positive result was indicated by growth extending to the opposite side of the tube.

Urease Test: After preparation, Urease Broth Base (Hi-Media M111) was autoclaved. The tubes were slanted when the medium was added to them. To observe the media's color shift from yellow to pink, a loop of bacterial culture was inoculated onto it, and the tubes were then placed in an incubator set at 37 °C for two to four days.

Indole Test: The test culture was incubated for 48 hours at 37 °C after inoculation into a peptone water medium. The tube's side was then filled with one milliliter of Kovacs's reagent. Within a minute, the surface developed a pink ring, signifying a favorable reaction hour.

MRVP Test: MRVP medium (glucose-phosphate broth, Hi-medium M070) was prepared and autoclaved. Then, a loop of bacterial culture was placed into each test tube, and they were incubated for 24-48 hours in an incubator. Following incubation, 5 drops of Methyl Red solution were applied to one test tube for the MR test, and the resulting color change was noted. The other test tube was filled with 10 drops of VP-I reagent and three drops of VP-II reagent, respectively, for the VP test. A color shift was seen after 15 to 30 minutes.

Citrate Utilization Test: A test tube was used to produce, autoclave, and solidify Simmons Citrate Agar medium (Hi-medium M099) in the form of a slant. Following that, the tubes were streaked with the sample bacteria, which break down the ammonium ions into ammonia and metabolize the

citrate to ultimately create NaHCO3, raising the pH of the media to an alkaline state and changing its color from green to blue.

Hydrogen Sulfide (H2S) Production Test: Triple sugar iron agar in McCartney bottles was inoculated with the test culture by stabbing into the butt and streaking along the slope. After that, it was incubated at 37 °C for 2 days. The development of a dark hue suggested a positive reaction.

Fermentation of Glucose: After preparation and autoclaving, the glucose medium was divided into two test tubes. After inoculating the medium with a single loop of complete culture, the tubes were incubated in an incubator set at 37 °C for a full day. It was noted that the media's color changed from orange to yellow. Test for Casein Hydrolysis: The test organism was streaked across the surface of skim milk agar plates and incubated at 37°C overnight. The plates were checked for a clean zone encircling the colonies after the incubation time, indicating a successful casein hydrolysis test.

Starch Hydrolysis Test: First, starch agar plates were streaked with the test organism. Next, they were incubated at 37°C for 24 hours. Finally, an iodine solution was applied to the plates. A favorable outcome was suggested by forming a clean zone surrounding the colonies.

2.6. Test for Antimicrobial Sensitivity

The conventional disk diffusion technique was used to assess the sensitivity of *Salmonella* isolates to various antibiotic treatments. Seven distinct antimicrobial drugs used to treat Gram-negative bacteria were evaluated on each isolate. A homogeneous bacterial culture was prepared by emulsifying colonies from each isolate in 2 mL of nutrient broth using vigorous shaking. The resulting suspension was then spread across the entire surface of the agar plates by tilting. After aspirating any extra fluid, the plates were allowed to dry for fifteen minutes. Sterile forceps were used to lay the antimicrobial disks on the agar medium. Following a 24-hour incubation period at 37 °C, the plates were inspected for inhibition zones, which were measured in millimeters. Using the Bauer-Kirby method, the susceptibility of the isolates to various antibiotics was determined, classifying them as susceptible, intermediate, or resistant.

2.7. Genomic DNA Extraction

The genomic DNA of the bacterial isolates was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method, with slight modifications. The steps involved in the extraction process were as follows:

Bacterial cultures were grown overnight in Luria-Bertani (LB) broth at 37°C with shaking. A 1.5 mL aliquot of bacterial culture was centrifuged at 12,000 rpm for 5 minutes, and the supernatant was discarded. The bacterial pellet was resuspended in 1,000 µL CTAB buffer, followed by the addition of 3 µL proteinase K (20 mg/mL). The mixture was incubated at 60°C for 1 hour. An equal volume of chloroform-isoamyl alcohol (24:1) was added and mixed gently. The sample was centrifuged at 12,000 rpm for 10 minutes, and the aqueous phase was transferred to a new tube. DNA was precipitated using an equal volume of cold ethanol and incubated at -20°C for 1 hour. The precipitated DNA was pelleted by centrifugation, washed with 70% ethanol, air-dried, and resuspended in TE buffer. The purity and concentration of the extracted DNA were assessed using a Nanodrop spectrophotometer.

2.8. PCR Amplification of 16S rRNA Gene

The 16S rRNA gene was amplified using universal primers:

Forward primer 8F	5′-GGATCCAGACTTTGATYMTGGCTCAG-3′

Reverse primer 907R	5′-CCGTCAATTCMTTTGAGTTT-3′

The polymerase chain reaction (PCR) mixture contained:
• 50 ng of template DNA
• 2.5 mM MgCl2
• 0.2 mM dNTPs
• 0.5 μM of each primer
• 2.5 units of Taq DNA polymerase
• 1X PCR buffer

2.9. PCR Cycling Conditions

PCR amplification was performed using a thermocycler with the following conditions:
• Initial denaturation at 96°C for 5 minutes.
• 35 cycles of:o Denaturation at 95°C for 30 seconds.
o Annealing at 55°C for 30 seconds.
o Extension at 72°C for 1 minute.
• Final extension at 72°C for 5 minutes.

2.10. Gel Electrophoresis and PCR Product Purification
The amplified PCR products were analyzed by agarose gel electrophoresis using a 1.5% agarose gel stained with ethidium bromide. The gel was visualized under UV illumination to confirm the presence of the expected 16S rRNA amplicons. PCR products were purified using a PCR purification kit to remove unwanted residues.

2.11. Factors determining isolates of Salmonella
2.11.1. Factors affecting the pH variations of isolates of Salmonella
Before sterilization, Experiments were conducted using LB broth with pH values of 5, 6, 7, and 9. Following inoculation with test organisms, optical density readings at 600 nm were taken at 0, 5, and 20 hours to determine bacterial growth.
2.11.2. Factors affecting the temperature at which Salmonella isolates
Salmonella isolates were cultivated in sterile LB broth under varying conditions. Temperature effects were evaluated at 28°C, 38°C, and 43°C, with optical density (OD600) measurements taken at 0, 5, and 20 hours. The influence of NaCl concentration was also assessed using 0.50%, 0.70%, and 0.90% NaCl in the LB broth. After being added to the LB broth, the test organisms were cultured. During the incubation period, the test organism's optical density (OD) value was measured at 600 nm at intervals of 0, 5, and 20 hours.

3. Results and Discussion
3.1. Bacterial isolation
Two hundred samples were examined bacteriologically in total. Out of 111 samples, 55 Gram-negative Enterobacteriaceae were identified. Twenty-two samples did not exhibit any bacterial growth, and thirty-four samples were not further identified because they did not show the characteristic Enterobacteriaceae responses with oxidase, catalase, and glucose fermentation. The

positive isolates were found in 111 chicken samples; sixty-one from the colon tested positive for *Salmonella*.

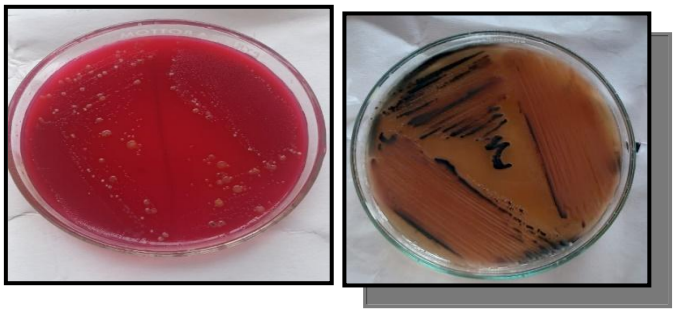


Figure 1. *Salmonella* on SS and XLD agar

Salmonella is found in huge amounts in the Pelawal western region. Samples of retail broiler chicken were gathered from Hazaribagh's zones—fifty-five percent of the 111 samples that were analyzed tested positive for *Salmonella*. The current study showed that the intestine always contains higher levels of *Salmonella*.

Table 1 No. Of *Salmonella* from different Zone

S.No	Chosen region	Positive count
1	East Region	34
2	South Region	20
3	West Region	41
4	North Region	17

In this study, *Salmonella* was isolated from 61 samples, with the following regional distribution: 20% (12) from the East, 55% (33) from the West, 15% (9) from the South, and 10% (6) from the North. The West zone of Hazaribagh showed the highest prevalence.

3.2. Biochemical test results

Table 2 Assay to confirm biochemical characteristics

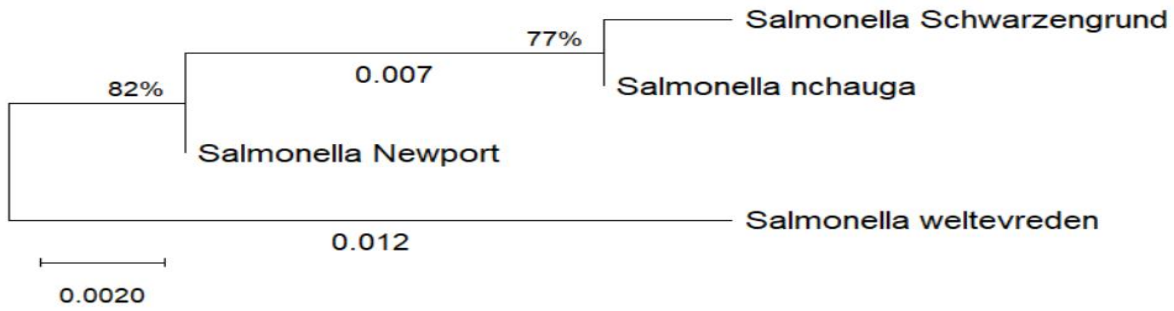
Test	*S. typhi*	*S. enterica*
Gram stain	Negative	Negative
Motility	**Positive**	**Positive**
Catalase	**Positive**	**Positive**
Indole	Negative	Negative
Methyl red	**Positive**	**Positive**
VogesProskauer	Negative	Negative
Citrate	Negative	**Positive**
Nitrate	**Positive**	**Positive**
Urease	Negative	Negative
Casein	**Positive**	**Positive**

Figure 2. Indole Test MP Test VP Test Citrate Test TSI - H2S production Urease Test Casein test Catalase test (left to right)

Indole and VP urease were both negative in the biochemical responses of the *Salmonella* isolates. Methyl red, Citrate Test, TSI - H2S, Casein test, & Catalase test were positive (fig 2). On TSI agar, *Salmonella* colonies exhibited black staining due to the formation of hydrogen sulfide gas, as evidenced by the presence of agar bubbles and a red slant indicating a pH change.

The Catalase test showed a positive. Glucose and mannitol in the carbohydrate fermentation test created gas, whereas lactose and sucrose did not. The isolates generated hydrogen sulfide (Fig 2).

3.3. Evolutionary relationships of 4 taxa of 16S rRNA Gene



Species	Evolutionary Relationship
Salmonella Weltevreden	Most distantly related, greatest divergence
Salmonella Newport	Early diverging, separate lineage
Salmonella Nchauga	Shares recent common ancestor with Schwarzengrund
Salmonella Schwarzengrund	Most recently evolved among the four

Figure 5. Evolutionary relationships of 4 taxa - the evolutionary history was inferred using the Neighbor-Joining method. The optimal tree, with a sum of branch lengths of 0.020, is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. The analytical procedure encompassed 4 nucleotide sequences. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 1,077 positions. Evolutionary analyses were conducted in MEGA12.

3.6. Factors affecting Salmonella isolate growth

This investigation used four extremely drug-resistant *Salmonella* isolates from the intestinal tract. The findings showed that temperature (28˚C, 38˚C, and 43℃), pH levels (5, 6, 7, and 8), and sodium chloride (NaCl) concentrations (0.30%, 0.50%, 0.70%, and 0.90%) these factors significantly affected the survival of *Salmonella* isolates in laboratory media.

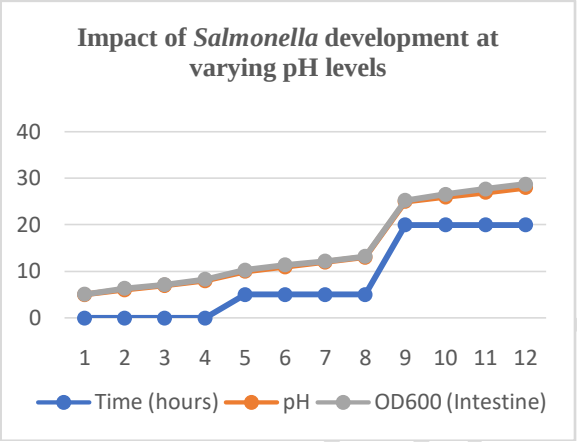
3.6.1. Impact of Salmonella Development at varying pH Levels

As documented in Table 3, the isolates exhibited a wide range of growth at various pH values. *Salmonella* isolates were successfully cultivated at multiple pH levels in the current investigation (Fig 3).

Table 3 The effect of varying pH on *Salmonella* growth patterns growth

S.No	Time (hours)	pH	Intestine

1	0	5	0.060
		6	0.289
		7	0.127
		8	0.233
2	5	5	0.270
		6	0.389
		7	0.200
		8	0.210
3	20	5	0.245
		6	0.605
		7	0.721
		8	0.744



. **Figure 6.** pH effects on *Salmonella*

Table 4 The impact of temperature on *Salmonella* proliferation

S.No	Time (hours)	Temperature (℃)	Intestine
1	0	28	0.038
		38	0.048
		43	0.063
2	5	28	0.489
		38	0.397
		43	0.456
3	20	28	0.473
		38	0.704
		42	0.581

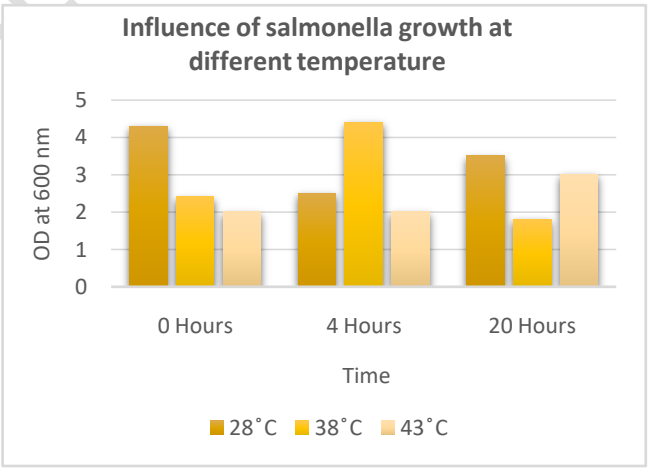


Figure 7. Influence of *Salmonella* growth at different temperature

The Impact of Varying Temperatures on Salmonella Development. The table and Figure demonstrate the broad range of development of Salmonella isolates at various temperatures.

3.6.2. Effects of Salmonella growth at varying NaCl concentrations

As seen in Table 8 and Figure 14, the *Salmonella* isolates grew and tolerated varying concentrations of sodium chloride (NaCl).

3.7. Salmonella antimicrobial resistance pattern (%) in the broiler chicken's gut.

Figure displays the *Salmonella* resistance pattern examined in this investigation. For every drug, there were notable variations in Table 4- *Salmonella* isolates grew and tolerated varying concentrations of sodium chloride (NaCl).

Table 5 *Salmonella* isolates grew and tolerated varying concentrations of sodium chloride (NaCl).

S.No	Time	Concentration	Intestine
1	0	0.30%	0.063
		0.50%	0.073
		0.70%	0.071
		0.90%	0.020
2	5	0.30%	0.30
		0.50%	0.48
		0.70%	0.32
		0.90%	0.25
3	20	0.30%	0.12
		0.50%	0.29
		0.70%	0.25
		0.90%	0.35

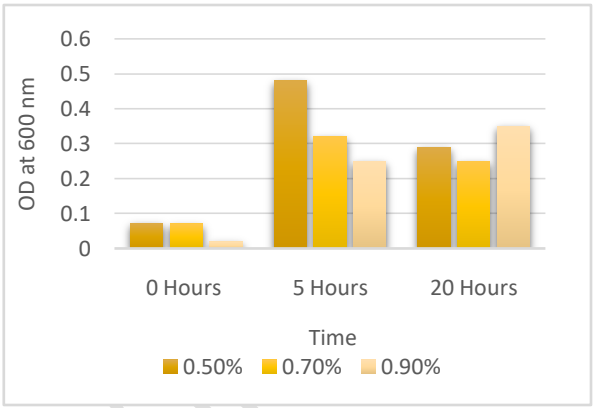


Figure 8. Influence of *Salmonella* growth at different temp

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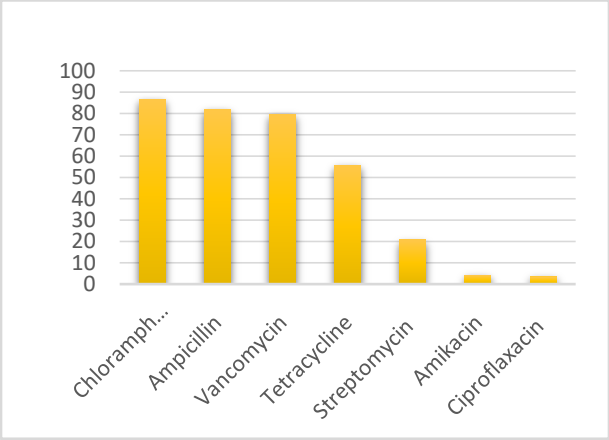

Figure 9. Pattern of antibiotic resistance in the intestines

Figure displays the *Salmonella* resistance pattern examined in this investigation. For every drug, notable variations were observed in the diffusion zone diameters. Tetracycline resistance was found in all samples of Salmonella.

The antimicrobial resistance of *Salmonella* isolates from the intestinal samples is shown in Table 5. The following exhibit a high resistance level to the sensitive antibiotic resistance patterns of intestinal samples. *Salmonella* isolates are highly resistant to Chloramphenicol (86.61%).

Salmonella is also resistant to Ampicillin (81.59%), Vancomycin (79.32%), Tetracycline (55.55%), Streptomycin (47.57%), Amikacin and Ciproflaxacin (4.20%).

3.7.1. Antimicrobial resistance pattern average

Resistance to Chloramphenicol (86.61%), Ampicillin (81.59%), Vancomycin (79.32%), Tetracycline (55.55%), Streptomycin (20.57%), Amikacin (4.42%) and Ciprofloxacin (3.30%) was found in *Salmonella* isolates from the intestine

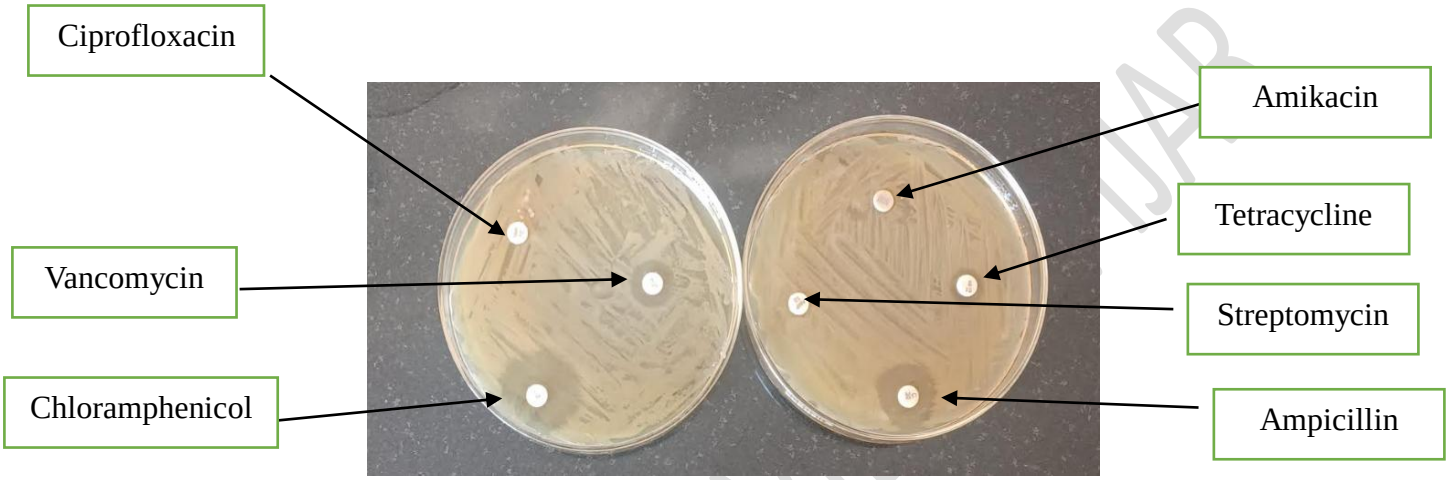

Figure 10. Antibiotic Resistance pattern

S/N	Antibiotics	Conc. (µg)	Zone of inhibition (mm)	Interference
1.	Chloramphenicol	30	≥11	Resistance
2.	Ampicillin	30	≤13	Resistance
3.	Vancomycin	30	≤14	Resistance
4.	Tetracycline	30	≤14	Susceptible
5.	Ciprofloxacin	30	≥21	Susceptible
6.	Streptomycin	30	≤11	Susceptible
7.	Amikacin	30	≤14	Susceptible

Salmonella isolates are highly resistant to Chloramphenicol (86.61%). Ampicillin, Vancomycin, tetracycline and Streptomycin are also resistant to *Salmonella* isolates, whereas Amikacin and Ciprofloxacin showed very low resistance.

4. Discussion

Salmonellosis has become one of the most significant bacterial illnesses affecting chickens due to its widespread incidence and the poultry industry's considerable growth. In addition to being an important public health issue, salmonellosis continues to have a substantial financial impact on the poultry industry worldwide [8]. This study aimed to determine the prevalence of Salmonella in some areas of Hazaribag City. *Salmonella* was the most common isolate in this investigation, with 55% of the samples testing positive. This current study showed a significant

finding of Salmonella in the gut of broiler chickens. The rate of *Salmonella* isolation was similar to that reported in previous investigations.

After analyzing 1488 samples, Yagoub and Mohammed found 58 *Salmonella* isolates [9]. In a separate investigation, Ezdihar recovered 45 Salmonella isolates, or 7.4% of the total, from 610 chicken samples in Sudan. Hazaribag City's west Zone has the highest isolation rate [82%] of any area. This can be the result of this farm's inadequate cleanliness. The intestinal sample had the highest isolation rate of all the analyzed samples. This discovery suggests a significant amount of *Salmonella* shedding from the birds on this farm's digestive systems. The most considerable serovar in chicken flocks is S. enteritidis, which has lately become highly prevalent worldwide [10, 11]

According to Phillips and Optiz, S. enteritidis infects the ovum during ovulation through initial attachment to granulosa cells [12]. However, S. enteritidis was able to infiltrate eggs through the holes in the shell and contaminate them. From a public health perspective, S. enteritidis has been linked to an upsurge in human salmonellosis in France and the United States of America [23].

Although its ideal development temperature is 37°C, *Salmonella* can quickly adapt to harsh environments. There have been reports of Salmonella growth temperatures as low as 5.9°C and as high as 54°C under specific experimental conditions. According to the anti-biogram of the *Salmonella* isolates found in this investigation, most organisms had developed resistance to more than four or five antibiotics. Numerous reports of *Salmonella* as antibiotic-resistant in fish and poultry from Indian markets have already been made [17, 18]. The use of tetracycline to treat day-old chicks may have contributed to the emergence of tetracycline resistance in Salmonella in broiler flocks and layers [19]. Our study findings showed that the isolates of Salmonella from commercial and non-commercial layer hen eggs had comparatively higher levels of tetracycline resistance.

The subtherapeutic use of antibiotics in the industrial production of pigs, eggs, and poultry has encouraged the growth and persistence of MAR-harmful bacteria in these animals' habitats, according to a substantial body of research evaluated by Novick [19]. In contrast to the patterns of antibiotic resistance found in industrialized nations, the level of antibiotic resistance was significantly greater. A significant reason for this issue is the widespread and uncontrolled use of antibiotics in our country. Antibiotics are easily obtained without a prescription, and there is insufficient oversight to guarantee patients finish their prescribed regimen.

The emergence of enhanced selection pressure may facilitate the evolution of resistant strains. Additionally, a significant factor in the rise of antibiotic resistance among pathogenic bacteria of animal origin is the widespread use of antibiotics in animal production systems. Compared to the level reported by Hatha and Lakshmana Perumalsamy, the resistance level was significantly higher [18, 19]. Foodborne illness has been documented in cases of eating undercooked egg dishes (Barrow et al., 2003). The impact of environmental variables, such as temperature [24], pH, and sodium chloride [25], on microbial development has been assessed in several studies. The *Salmonella* strains identified in this investigation exhibit lower levels of ampicillin resistance than those reported by Suresh et al. [21]. There is a growing trend of using Ciprofloxacin, a fluoroquinolone antibiotic, to treat human septicemia effectively. *Salmonella* isolates from humans and animals have occasionally been reported to be resistant to Ciprofloxacin. Similar resistance patterns and levels in *Salmonella* suggest that they come from the same source.

The diversity of resistance patterns suggests that these bacteria possess a substantial reservoir of resistance plasmids, which could be hazardous if released into the environment. Additionally, treating infections brought on by these MAR types is challenging. *Salmonella* with multidrug resistance has been shown to be highly prevalent in chicken waste, frequently exhibiting resistance to colistin, streptomycin, and tetracycline [22]. In the current investigation, nearly every strain from various origins exhibited resistance to vancomycin. Consistent with our findings, another investigation documented similar vancomycin resistance in *Salmonella* strains from multiple sources.

All *Salmonella* strains tested were found to be susceptible to Ciprofloxacin, gentamicin, co-trimoxazole, nitrofurantoin, amikacin, and trimethoprim. In their report, 10% of 105 Salmonella isolates were resistant to gentamycin [6]. Additionally, quinolone resistance was building up globally. *Salmonella* decreased susceptibility to Ciprofloxacin is now known to cause treatment failure [7]. Based on a logical interpretation of the MAR index data, the Salmonella strains found in this study likely originated from high-risk contamination sources. It is known that one of the primary sources of *Salmonella* species is poultry.

Salmonellosis control or eradication initiatives that are successful rely on reliable management systems, correct medication, and the identification of carrier birds. The multidrug resistance of the majority of these *Salmonella* strains exacerbates the situation, and the incidence of *Salmonella* in chicken was comparatively high when compared to other environmental factors. We reiterate the necessity of regulating the use of antibiotics in the poultry industry in light of the findings. Globally, *Salmonella* is the most significant agent associated with foodborne illness outbreaks [11].

5. Conclusion

The primary goal of this study was to examine the prevalence, distribution, growth-influencing factors, and antimicrobial activity of multidrug-resistant (MDR) Salmonella isolates from chicken samples in the East, West, North, and South regions of Hazaribagh city, Jharkhand, India, between December 2023 and September 2024. Fifty-five percent of the 200 samples analyzed were positive for *Salmonella*. The current study revealed that 84% of the intestinal tract was infected with *Salmonella*. *Salmonella* was found in significantly higher quantities in the Hazaribagh West Zone. Additionally, the results show that the variety and presence of *Salmonella* species fluctuate over time and are significantly impacted by pH, turbidity, seasonal precipitation, and heterotrophic bacteria. Salmonellosis cases exhibit a distinct seasonal pattern, steadily increasing over the winter. Although peak yearly temperatures are associated with higher case rates, it remains unknown what variables influence this seasonality on a regional level or how this pattern may be related to the pathogen's environmental presence. Infected humans and animals are treated with antimicrobial medications, shielding them against infectious illnesses and promoting quicker growth. However, the majority of infections are now resistant to widely used drugs. Salmonellosis control or eradication initiatives that are successful rely on reliable management systems, correct medication, and the identification of carrier birds. The multidrug resistance of the majority of these *Salmonella* strains exacerbates the situation, and the incidence of *Salmonella* in chickens was comparatively high when compared to other environmental factors. We reiterate the necessity of regulating the use of antibiotics in the poultry industry in light of the findings. Globally, *Salmonella* is the most significant agent associated with foodborne illness outbreaks. The Phylogenetic tree shows that *Salmonella weltevreden* is the most distantly related species, suggesting significant evolutionary divergence. *Salmonella*

newport forms a separate lineage, diverging early from the other species. *Salmonella Nchauga* and *Salmonella schwarzengrund* share a more recent common ancestor, with *Salmonella schwarzengrund* being the most recently evolved species. This analysis provides valuable insights into the evolutionary divergence of these *Salmonella* strains. The distinct clustering suggests possible differences in adaptation, virulence, or host specificity, which could have implications for understanding transmission dynamics and developing control strategies for nontyphoidal *Salmonella* infections in poultry.

Salmonellosis control or eradication initiatives that are successful rely on reliable management systems, correct medication, and the identification of carrier birds. The multidrug resistance of the majority of these *Salmonella* strains exacerbates the situation, and the incidence of Salmonella in chickens was comparatively high when compared to other environmental factors. We reiterate the necessity of regulating the use of antibiotics in the poultry industry in light of the findings. Globally, Salmonella is the most significant agent associated with foodborne illness outbreaks (Lacey et al., 1993).

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CONFLICT OF INTEREST

The authors have no conflicting or competing interests.

AUTHOR CONTRIBUTIONS

Anubha Kumari, Conceptualization, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review and editing | Avinash Kumar, Supervision, Visualization, Writing – review and editing