



# Occurrence, Virulence and Drug Resistance Characterization of Non-typhoidal Salmonella in Poultry and Environmental Samples of Rewa City, India

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## ABSTRACT

The Experiment was conducted during June–December, 2022 at Department of Veterinary Public Health & Epidemiology, College of Veterinary Science & Animal Husbandry, Rewa, NDVSU, Jabalpur, M.P. India. to attribute the possible risk of poultry and its product as an increasing cause for food borne Salmonellosis, a cross sectional study was carried out in poultry samples from Rewa city of Madhya Pradesh. A total of 300 sample (Poultry and environment) were collected and processed for isolation and identification of *Salmonellae* by culture, biochemical methods and molecular methods. Only poultry samples were found positive while all the environmental sample were negative. A total of 5.5% occurrence of *Salmonella* was found in poultry samples with 5% positivity in meat sample and 6% in the caecum. Two serotype *Salmonella* Typhimurium (4%) and *Salmonella*. Infantis (1.5%) were detected. Drug resistance pattern of isolated *Salmonella* indicated a very high resistance towards many of antibiotics which are critical for clinical use for animal, birds and humans as well. All the isolates were positive for Virulence marker *invA*, *spiA*, and drug resistance gene, *bla*<sub>TEM</sub> while *ampC* and *tetA* were detected only in *S. Typhimurium* with 23 and 50% positivity and *spvR* and *spvC* virulence genes were not detected in any isolated salmonella. As Non typhoidal *Salmonella* are of zoonotic importance, their presence in poultry meat and fecal samples, indicating the need to implement effective prevention and control measures throughout food chain that is from farm to fork level.

**KEYWORDS:** Antibiotics, non-typhoidal, poultry, salmonella

**Citation (VANCOUVER):** Sharma et al., Occurrence, Virulence and Drug Resistance Characterization of Non-typhoidal Salmonella in Poultry and Environmental Samples of Rewa City, India. *International Journal of Bio-resource and Stress Management*, 2024; 15(5), 01-09. [HTTPS://DOI.ORG/10.23910/1.2024.5305a](https://doi.org/10.23910/1.2024.5305a).

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**Conflict of interests:** The authors have declared that no conflict of interest exists.

RECEIVED on 18<sup>th</sup> March 2024

RECEIVED in revised form on 30<sup>th</sup> April 2024

ACCEPTED in final form on 14<sup>th</sup> May 2024

PUBLISHED on 19<sup>th</sup> May 2024

## 1. INTRODUCTION

*Salmonella* spp. is one of the most reported pathogen of zoonotic potential, as well as development of antimicrobial-resistant (AMR) strains of *Salmonellae* are becoming a major public health threat (Espunyes et al., 2022). According to the White–Kauffmann–Le Minor scheme, single species *S. enterica* (have six subspecies) composed of over 2600 serotypes, which have where most (approximately 1600 serotypes sharing high sequence similarity) of the them belong to the subspecies *enterica* (Elnekave et al., 2020, Yang et al., 2014). Non-typhoidal serovars (NTS) normally manifested as self-limited gastroenteritis causing intestinal inflammation and diarrhea. NTS has been reported as a major cause of enteritis of bacterial origin in humans, responsible for 93.8 million cases of gastroenteritis with 155,000 deaths each year, all over the world (Ngogo et al., 2020, Gong et al., 2022). NTS may cause extra-intestinal, invasive disease resulting bacteremia and focal systemic infections mediated by the Type 3 secretory system (T3SS) particularly reported in developed countries (Hu et al., 2021, Das et al., 2022). Moreover, invasive NTS is a major cause of systemic infection primarily in immune-competent individuals. *Salmonella* has a wide host range including reptiles, avian species, and mammals along with humans (Lou et al., 2019). The most common animal reservoirs are chickens, swine, turkeys, and cows (Coburn et al., 2007, Paclibare et al., 2017). More than 200 serotypes have been identified which may cause pathogenicity in humans, *Salmonella* Enteritidis (*S. Enteritidis*) and *Salmonella* Typhimurium (*S. Typhimurium*) are the two most common serotypes causing human salmonellosis (Xue et al., 2021; Xu et al., 2021, Patrick et al., 2004, Ceyssens et al., 2015).

The pathogenicity of *Salmonella* is mediated by the interaction of numerous virulence genes, located in the *Salmonella* pathogenicity islands (SPIs), plasmids, lipopolysaccharides, and enterotoxins (Farahani et al., 2018, Foley et al., 2013, van and van, 2005). Antimicrobial resistance (AMR) is the silent pandemic the world is facing and causing the treatment failure to causes which were originally effectively treatable infections (Domenech et al., 2015). Antimicrobials resistance may be due to Intrinsic resistance or externally acquired by various mechanisms such as selective pressure and horizontal gene transfer (HGT) from resistant strains can also facilitate the emergence and dissemination of AMR bacteria (Miller and Gilligan, 2012, Elnekave et al., 2022). High phenotypic resistances along with multidrug resistance (MDR) or non-susceptibility to any antibiotic from three or more group of antibiotics in *Salmonella* isolated from animals is a matter of high concern (Cho et al., 2020, Van et al., 2015). The presence

of plasmids, transposons, integrons, and insertion sequences can contribute to the development of antibiotic resistance (Ramatla et al., 2020, Jaja et al., 2019). However poultry that has never been exposed to antibiotic growth promoters has meat contaminated with antibiotic-resistant *Salmonella* (Novoa et al., 2022, Rama et al., 2022).

Poultry is one of the common source of NTS in human via direct transmission or consumption of its food products. *Salmonella* may enter in meats, at any stage of slaughtering process and cross-contaminated occur during further processing, distribution, handling and marketing (Jiang et al., 2021).

The detection of *Salmonella* in retail meats is an integral part of food microbial quality assurance (Wang et al., 2021, Zhu et al., 2019). Thus, *Salmonella* infections represent a major concern to public health, animals, and food industry worldwide. Food borne Salmonellosis associated with Non-typhoidal *Salmonellae* is highly neglected and its prevalence is increasing at an alarming rates throughout India. In India, NTS infections pose a significant threat to public health, often overlooked due to inadequate surveillance. Therefore, the present cross sectional study was carried out to detect the occurrence and characterization of virulence and drug resistance pattern of *Salmonella* spp in poultry in Rewa city of Madhya Pradesh.

## 2. MATERIALS METHODS

### 2.1. Sample collection

The study was carried out in the time period of June–December 2022, in the Rewa city (latitude–24.530727, longitude–81.299110) of Madhya Pradesh, India. Two stage stratified sampling was adopted to collect total of 300 sample including 200 poultry sample (including 100 cecum and 100 meat sample) and 100 environmental samples (knife and slaughter slab swabs) randomly from five zones north east west, south, east and central zones. These areas were considered a first strata and from each zone 10 raw chicken outlets as a second strata were selected randomly using a table method. All samples were collected aseptically into sterile containers, labelled clearly, placed into cooler boxes, and then transported immediately to the laboratory. Samples were processed at earliest preferably within four to six hours of collection. Standard protocol of isolation and identification of *Salmonella* sp. described in Bacteriological Analytical Manual (BAM), U.S. Food and Drug Administration (USFDA) was followed with necessary modifications. (Wallace et al., 2023)

### 2.2. Processing of samples and primary isolation

Samples were triturated using 1 ml distilled water, to mix it properly using sterilized pestle and mortar and the

homogenate was inoculated in the ratio of 1:10 of pre-enrichment media. All the swab samples were inoculated in to 10 ml of the pre-enrichment broth that was medium Buffered peptone water (BPW) and incubated for 16 to 20 hours at 37°C. Later for enrichment the samples were inoculated to the selective enrichment broth that was Rapaport Vasiliadis medium and incubated at 42°C for 18-24 hr and later a loop full of inoculum was streaked on XLD, HEA and BGA agar plates and incubated at 37°C for 24 hr. Culture plates were examined for the presence of typical colonies based on morphological characteristics.

### 2.3. Phenotypic confirmation of salmonella

All presumptive Salmonella isolates were subcultured and pure colonies on nutrient agar and further tested for biochemical identification. Catalase test, oxidase test, Iodole production, methyl red, Vogues Procures, Citrate utilization, urease production and triple sugar iron test (Popoff and Le Minor, 2005). Single colonies were sub-cultured onto nutrient agar and subsequently stored in TSB supplemented with 10% glycerol at -60°C until further analysis.

### 2.4. Molecular confirmation of salmonella

DNA was extracted using the boiling and snap chill method. Colonies from an overnight culture grown on nutrient agar were briefly suspended into 200 µl of distilled water and lysed at 100°C for 15 minutes, followed by centrifugation at 13000 rpm for 5 minutes. The product was further purified by ethanol precipitation and thereafter, 120 µl of supernatant was transferred to a clean microcentrifuge tube and stored at 4°C until further analysis.

DNA concentration and purity was checked by Nanodrop 200C, the DNA concentrations were determined and corrected to 50 ng/ul for further molecular research. DNA samples (with an optical density ratio of 1.8 to 2 at 260/280 nm), were used for PCR. All the Salmonella isolates were first screened for the genus confirmation by genus specific primer for *invA* gene that is also a virulence marker for invasiveness of bacteria. The PCR was carried out for the *invA* gene primers product size 284 as described by Rhan et al., 1992 (Table 1). The PCR was carried out in the thermal cycler (Veriti) with pre-heated lid (Lid temp. 105°C). A 12.5 µl reaction mixture containing 6.25 µl master mix, 1.0 µl of each of the forward and reverse primers (20 µM), 3.25 µl of nuclease-free water, and 1 µl of template DNA was prepared. Cycling conditions included as an initial activation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds (s), annealing at 60°C (30s), extension at 72°C (30 s), and a final extension at 72°C for 1 min. *Salmonella enterica* subsp. *enterica* serovar Choleraesuis ATCC 10708 was used as a positive control reference strain and in place of DNA template nuclease-free water was added for negative control.

Table 1: Primer (sequences and relevant information) used in the current study

Genes	Primer sequence (5' to 3')	Size (bp)	Tm (°C)	Reference
<i>invA</i>	F: GTG AAA TTA TCG CCACGT TCG GGC AA  R: TCA TCG CAC CGT CAAAGG AAC C	284	62	Rhan et al., 1992
<i>Spv</i>	F: TTG TTC ACT TTT TAC CCC TGA A  R: CCC TGA CAG CCG TTA GAT ATT	401 bp	57	De Freitas et al. (2010)
<i>SdfI</i>	F: TGT GTT TTA TCT GAT GCA AGA GG  R: TGA ACT ACG TTC GTT CTT CTG G	304 bp	57	De Freitas et al. (2010)
<i>spiA</i>	F: CCAGGGGTC- GTTAGTGTATT- GCGTGAGATG  R: CGCGTAA- CAAAGAACCCG- TAGTGATGGATT	550	55	Ochman et al. (1996)
<i>spvC</i>	F: AAGGTC- GTTCAACAAGCC  R: CATTTCACCAC- CATCACG	252	54	(Hai et al., 2020)
<i>spvR</i>	ATGGATTTTCAT- TAATAAAAAATTA  TCAGAAGGTG- GACTGTTTCAG TTT	894	55	(Huang et al., 2005)
<i>ampC</i>	AAC ATG GGG TAT CAG GGA GAT G  CAA AGC GCG TAA CCG GAT TGG	380	58	P é r e z - P é r e z and H a n s o n 2002
<i>tetA</i>	F: GCTACATCCT- GCTTGCCTTC  R: CATAGATCGC- CGTGAAGAGG	210	58	(Carlson et al., 1999)
<i>b l a - TEM</i>	F: GGTCTCTCC- GATCGTTGTCAG  R: TTCATC- CATAGTTGCCT- GACT	310	68	(Carlson et al., 1999)

### 2.5. Serotyping of isolate

To identify the serotype of the isolated *Salmonella*, a multiplex PCR was carried out for the most commonly occurring *Salmonella* i.e., Enteritidis and *Salmonella* Typhimurium. The oligonucleotide primer *Salmonella* Typhimurium Spy (401 bp) *Salmonella* Enteritidis Sdf (304 bp) used (De Freitas et al., 2010) (Table 2). Multiplex PCR assay was carried out following cycling mixture contain 6.25 µl PCR mixture containing dNTP, MgCl<sub>2</sub>, Taq polymerase), Forward primer 1 µl, Reverse Primer 1µl, template DNA 1 µl, Nuclease free Water 3.25 µl. With the total reaction mixture 12.5 µl. Cycling condition included as initial activation at 94°C for 5 min, followed by 35 cycles of denaturation 94°C for 30 sec, annealing at 57°C (1 min), Extension at 72°C (1 min), and final extension of 30 sec at 72°C, with holding at 4°C for 10 min.

The isolates which could not be serotyped were sent to National Salmonella and Escherichia Centre (NSEC), Central Research Institute (CRI), Kasauli, Himachal Pradesh, India.

### 2.6. Detection of virulence gene in salmonella isolates

All the confirmed *Salmonella* isolates from different sources were screened for the presence of virulence *spiA*, *spvR* and *spvC* gene which are the marker for virulence and biofilm formation. Optimum PCR reaction mixture and thermal cycling conditions for *Salmonella spiA* virulence genes followed Ochman et al. (1996) and for the *spvR* and *spvC* gene the reaction mixture of 12.5 volume was optimized containing 6.25 µl 2X PCR master mix, 1 µl of each forward and reverse primer, 3.25 µl nuclease free water and 1 µl of

DNA template with the initial denaturation at 94°C for 5 min, followed by 30 cycles of the denaturation 94°C for 30 sec, annealing at 48°C for 30s (*spvR*), and 52°C for 30 (*spvC*) Extension at 72°C (1 min), and final extension of 30 sec at 72°C, with holding at 4°C for 10 min. The amplified products were run in agarose gel electrophoresis through 1% agarose gel (Hai et al., 2020, Huang et al., 2005) (Table 1).

### 2.7. Antibiotic susceptibility test

All the *Salmonella* isolates were subjected to antibiotic susceptibility test as by agar disc diffusion method described by Bauer et al., 1966. A total of 12 discs consisting of to 7 groups were tested results were recorded following criteria of Clinical Laboratory Standards Institute 2020 (Supplementary Table 1). The isolates were further tested for presence of *bla*<sub>TEM</sub> gene for 800 bp PCR product using specific primers as described by Dallenne et al. (2010). The AmpC and tetA genes were tested using Perez-Perez and Hanson, 2002 and Carlson et al., 1999 methodology (Table 2).

## 3. RESULTS AND DISCUSSION

**A**mong 200 samples, 5% (5/100) from chicken ceca, 6% (6/100) from chicken thigh, with the total 5.5% (11/200) positive for *Salmonella* (Table 2). The phenotypic confirmation was done by assessing the specific colony characteristics on XLD, BGA, and HEA agar plates, along with biochemical characterization by IMViC, (– – + +) reactions on TSI agar slants were typical of *Salmonella* (alkaline slant and acidic butt and produce H<sub>2</sub>S) and negative urease production. All *Salmonella* isolates exhibited swimming and swarming motility. The molecular

Table 2: Positivity of *Salmonella* in test samples

S1. No.	Sample source	No of samples (n)	Positive samples (%)	Serovars isolated	No of serovars out of positive samples
1.	Poultry				
	Poultry Cecum	100	6	<i>S. Typhimurium</i>	4
				<i>S. Infantis</i>	2
2.	Poultry meat (Neck)	100	5	<i>S. Typhimurium</i>	4
				<i>S. Infantis</i>	1
3.	Environment	100	0	-	-

confirmation by PCR for *invA* gene given 284 bp specific band on agarose gel electrophoresis (Figure 1). Serotypic PCR Multiplex PCR was done to identify serotype, and 8 out of 11 isolates were *Salmonella enterica* serovars Typhimurium (8/200) 4% (Figure 2). While other three were sent to CRI Kasauli, Himachal Pradesh, India, for serotyping and were confirmed as the *Salmonella* Infantis

1.5% (3/200) (Table 2). Only poultry samples were found positive for *Salmonella* while all the environmental sample were found negative. In poultry cecum the prevalence of both isolate was *S. Typhimurium* and *S. Infantis* was 4% and 2%, while the in meat sample their prevalence was 4 % and 1%. Respectively.

Non-typhoidal *Salmonella* gastrointestinal infections is

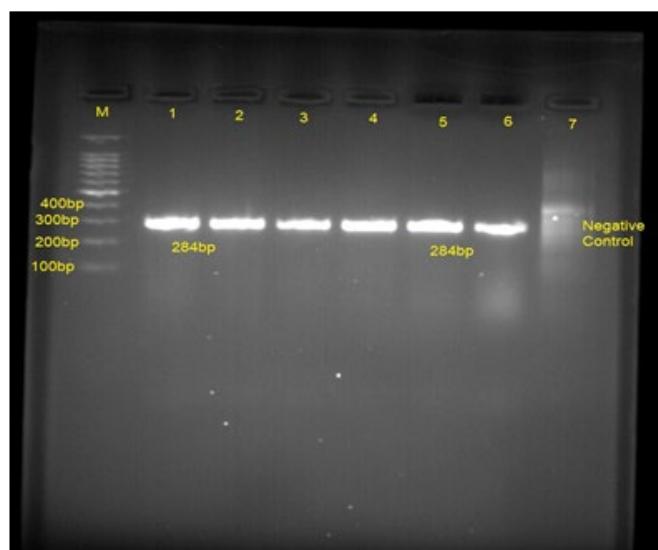


Figure 1: Agarose gel electrophoresis showing amplification products of genus specific (InvA gene) PCR, LANE M: Molecular weight marker (100bp), Lane 1: Positive control of *Salmonella*, L 2: Lane 6: Isolates of *Salmonella*, L 7: Negative control



Figure 2: Agarose Gel electrophoresis showing amplification products (*S. Typhimurium*- spy gene, 401bp) of serotype specific multiplex PCR. Lane M: Molecular weight marker 100 bp, Lane 1: Negative control, Lane 2: Positive control of *S. Typhimurium*, L3-6: *Salmonella* Isolates samples of *S. Typhimurium* from different source, L7: Blank

a major public health concern in both developed and developing countries. Consumption of undercooked/semi-cooked or cross contaminated poultry products is a major source of *Salmonella* infection to humans. The study revealed the overall 5.5% occurrences of *Salmonella* in

collected samples. There was strong correlation among the occurrence of salmonella in cecum and meat of chicken. This study revealed the 6% occurrences of *Salmonella* in chicken ceca was in agreement with the 6.9% prevalence reported by Amini et al. (2010), 5.8% by Jafari et al. (2007) and 4% by Gracia et al. (2011). In contrast, very high prevalence of upto 84% was reported by Ramya et al., 2012. Variable prevalence of *Salmonella* in poultry and its products has been reported like from reported a prevalence rate of 1.73 % to 33.3% (Saravanan et al., 2015, Balakrishnan et al., 2018). Different studies conducted in India and China reported higher prevalence of 23.7% and 33.33% as per Kaushik et al. (2014) and Balakrishnan et al. (2018) respectively and lower prevalence of 1%, 3.2% and 3.7% were reported by many authors like Wang et al. (2009), Issa et al. (2017) and Yoon et al. (2014), respectively. In many studies the most commonly occurring serotype is *S. Enteritidis* followed by *S. Typhimurium*, however in study the *S. Enteritidis* was not detected while *Salmonella* Infantis, a less common serotype was observed. Poultry product have always topped the incidence of salmonellosis in many of the developing countries including India. Contamination of *Salmonella* in poultry products can occur at multiple steps along the food chain including distribution, retail marketing, and handling of the product like washing of the meat with contaminated water at retail shops.

We had collected 100 environmental sample from the different source like from knife swab, slaughter slab swab, from poultry butcher shop and water samples. None of the environmental sample found to be positive for *Salmonella*. As per our findings environmental samples seem not to be the sources of *Salmonella*. Absence of *Salmonella* in poultry farm water samples may be due to the water being given to the birds was supplied from Nagar Nigam water filter plant.

Antimicrobial resistance was assessed using phenotypic (disc diffusion method) and genotypic (PCR for drug resistance gene) approaches, and there was strong correspondence between resistances and the presence of tested AMR determinants. Drug resistance pattern of isolated *Salmonella* indicated a very high resistance towards many of antibiotics which are critical for clinical use for animal, birds and humans as well. The study depicted that all the isolates were 100% susceptible to Co-trimoxazole while towards others the susceptibility was Imipenem 82%, Trimethoprim 82%, Azithromycin 82%, Streptomycin 64%, Gentamicin 64%, Tetracycline 55%, Ceftriaxone 55%, Cefixime 18%, Ceftazidime 9%, Cefotaxime 18, Ciprofloxacin 9%. Intermediate sensitivity was immediately observed towards Ciprofloxacin 27%, Streptomycin 27, Imipenem 18, Cefoperazone 27, Cefotaxime 18, Ceftazidime 18 and for Ceftriaxone 9, Cefixime 9, Gentamicin 9, Trimethoprim 9 intermediate sensitivity. Highest resistant was observed



among Ampicillin 100%, followed by Cefoperazone 73%, Cefotaxime 64%, Ceftazidime 73%, Cefixime 73%, Ceftriaxone 36, Ciprofloxacin 64%, Tetracycline 45%, Gentamicin 27, Azithromycin 18%, Streptomycin 9%, Trimethoprim 9%.

Out of 11 positive isolates we detected Five isolates were of *S. Infantis* and six *S. Typhimurium* serotype and both the isolates were 100% sensitive for towards Co-Trimoxazole. While for Imipenem the sensitivity was 100% and 67 % for *S. Infantis* and *S. Typhimurium* respectively. There much difference was found in the both the isolates for Tetracyclines as *S. Infantis* was 100% sensitive but *S. Typhimurium* was only 17 %. Likewise, the *S. Infantis* was 100% sensitive for Trimethoprim but *S. Typhimurium* was only 67% sensitive. Among the Cephalosporin group we found that 100% resistance of *S. Infantis* for Cefotaxime and Cefoperazone while for *S. Typhimurium* 100% resistance for Cefixime and Cefotaxime (33%) and Cefoperazone (50%) resistance detected. The slight variation was found in antibiotics like Streptomycin where 80% and 50% sensitivity were for *S. Infantis* and *S. Typhimurium* respectively. In a similar way Azithromycin was 80% sensitive for *S. Infantis* and 83% for *S. Typhimurium*. Resistant for gentamycin was 100% and 50% for *S. Infantis* and *S. Typhimurium* was detected (Figure 3 and 4). All the 11(92%) isolates obtained from different sources showed multidrug resistance these were further proceed for detection of  $\beta$ -lactamase gene. PCR were standardized for the detection of  $\beta$ -lactamase genes in *Salmonella* isolates. PCR was targeted to detect 800 bp, fragments of *bla*<sub>TEM</sub> and all the 11 isolates were found to have

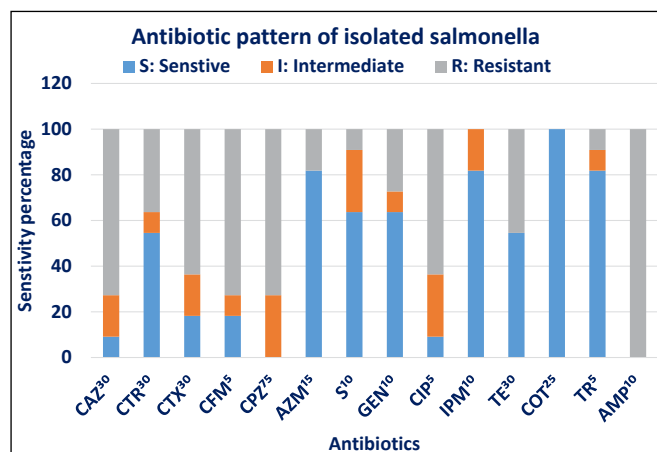


Figure 3: Antibiotic resistance pattern of *Salmonella* spp; Ceftazidime (CAZ<sup>30</sup>), Ceftriaxone (CTR<sup>30</sup>), Cefotaxime (CTX<sup>30</sup>), Cefixime (CFM<sup>5</sup>), Cefoperazone (CPZ<sup>75</sup>), Azithromycin (AZM<sup>15</sup>), Streptomycin (S<sup>10</sup>), Gentamicin (GEN<sup>10</sup>), Ciprofloxacin (CIP<sup>5</sup>), Imipenem (IPM<sup>10</sup>), Tetracycline (TE<sup>30</sup>), Co-Trimoxazole (COT<sup>25</sup>), Trimethoprim (TR<sup>5</sup>), Ampicillin (AMP<sup>10</sup>)

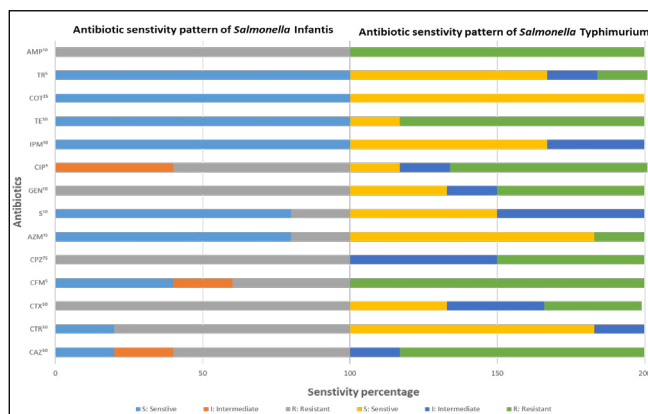


Figure 4: Antibiotic resistance pattern of *Salmonella* Infantis and *S. Typhimurium*; Ceftazidime (CAZ<sup>30</sup>), Ceftriaxone (CTR<sup>30</sup>), Cefotaxime (CTX<sup>30</sup>), Cefixime (CFM<sup>5</sup>), Cefoperazone (CPZ<sup>75</sup>), Azithromycin (AZM<sup>15</sup>), Streptomycin (S<sup>10</sup>), Gentamicin (GEN<sup>10</sup>), Ciprofloxacin (CIP<sup>5</sup>), Imipenem (IPM<sup>10</sup>), Tetracycline (TE<sup>30</sup>), Co-Trimoxazole (COT<sup>25</sup>), Trimethoprim (TR<sup>5</sup>), Ampicillin (AMP<sup>10</sup>)

*bla*<sub>TEM</sub> gene. However *AmpC* and *tetA* gene was detected only in *S. Typhimurium* with 23% and 50% positivity.

Emergence of MDR *Salmonella* is one of the major concern and in this study the AMR pattern of isolated *Salmonella* indicated that the 92% of isolated *Salmonella* were Multi Drug Resistant and showed resistant to one or more antibiotic of more than three group of antibiotics. In last few decades the resistance of most *Salmonella* species to first-line antibiotics in clinical cases was predominant, so antibiotics of critical important such as fluoroquinolones, third and fourth generation cephalosporins, macrolide, etc. have become a therapeutic choice for the of invasive *Salmonella* infection (Collignon et al., 2016). However in our study that all the isolates were resistant to ampicillin and very high resistance was reported for cephalosporin group indicating the severity of situation if these salmonellae cross the species barrier and infect the human being.

All the confirmed *Salmonella* isolates from different sources were screened for the presence of virulence genes such as *spiA* which involve both biofilm formation and virulence. All the isolates were found to carry the *spiA* gene, with the 500bp band as shown in (Figure 5). However other virulence genes (*spvR* and *spvC*) were not detected in the isolated *Salmonellae*. All the isolates were found to carry the *invA* gene and *spiA* gene suggesting their pathogenic potential, but none possessed *spvR* and *spvC* virulence genes similar to Rance and Windell, 2023. The *invA* gene from *Salmonella* contains unique DNA sequences and is proven to be a PCR target gene suitable for *Salmonella* detection. The *invA* gene encodes proteins in bacterial cell membranes that are needed for invasion into host epithelial cells. This

gene is located in Pathogenicity Island I or referred to as *Salmonella* Pathogenicity Island (SPI) in the DNA region is related to the pathogenicity of *Salmonella enterica* and is owned by all serotypes. There are four *Salmonella* invasion proteins (Sips), namely, Sips A–D. These Sips are exported and translocated into the plasma membrane of host cell or cytoplasm and play essential and complex roles in the secretion and translocation of SPI-1 effectors. *SipA* is an actin-binding protein and enhances the efficiency of the entry process of *Salmonella* into host cells by influencing different stages in the formation of membrane ruffles and rearrangement of the actin cytoskeleton (Lou et al., 2019).

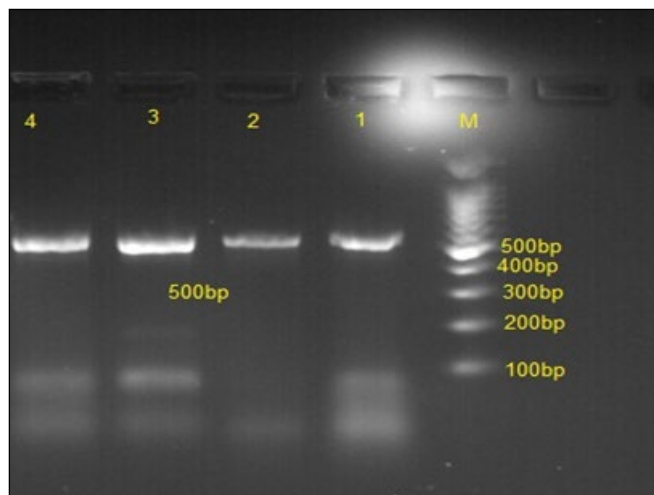


Figure 5: Agarose Gel electrophoresis showing amplification products of virulence *spiA* gene (500bp) specific PCR in isolated *Salmonella*, Lane M: Molecular weight marker (100bp), Lane 1 positive control L2–L3: isolated *Salmonella* samples

*SipA* regulates the concentration, polymerization and stability of the actin molecules at the site of bacterial entry and increases the bundling activity of host cell fimbrin.

#### 4. CONCLUSION

Two non typhoidal serotypes of *Salmonella* including *S. Typhimurium* and *S. Infantis* with the overall occurrence of 5.5%, were detected in the poultry samples of the Rewa city. The detected *Salmonellae* showed very high resistance (phenotypically as well as genotypically) towards antibiotics of critical clinical importance. Presence of virulence markers, indicating the possibility of invasiveness and causation of severe illness. These serotypes are known to cause infection in humans emphasizing the need to make people aware about food safety issues.

#### 5. FURTHER RESEARCH

Further epidemiological risk attribution of the poultry food products towards zoonotic transmission of

nontyphoidal salmonella needed to done so that proper prevention and control measure can be developed and implemented.

#### 6. ACKNOWLEDGEMENT

All the authors would like to thank the Director Research, Dr. S.S. Tomar, Nanaji Deshmukh, Veterinary Science University, Jabalpur, M.P.

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