

# Occurrence, Characterization and Antimicrobial Resistance Pattern of Pathogenic *Salmonella* Isolated from Chicken and Chicken Meat Products

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## ABSTRACT

The objective of this investigation was to study the occurrence, characterization, and antimicrobial resistance patterns of pathogenic *Salmonella* isolated from samples (n=400) comprising faecal and cloacal samples, environmental samples, chicken meat, and restaurant meat products collected at Udgir, Maharashtra. Following primary isolation and biochemical tests, two isolates were found to be positive for *Salmonella* spp. and were subjected to *in vitro* pathogenicity tests. *Salmonella* genus identification was accomplished by targeting the genus-specific *invA* gene, and serotype identification was done by targeting the *spy* and *sdfI* genes by Polymerase Chain Reaction (PCR). The results showed that both isolates were positive for the *Salmonella* Typhimurium *spy* gene, indicating a 0.50% overall prevalence of pathogenic *S. Typhimurium*. Similarly, Both *S. Typhimurium* isolates tested were positive on virulent marker (*invA*) based PCR, as well as pathogenic potential on Congo red dye binding assay and haemolysis on 5% sheep blood agar, indicating the efficient use of a targeted gene, *invA*, for early determination of pathogenic potential of *Salmonella* isolates. An antibiogram study using 15 antibiotics revealed remarkably high resistance to tetracycline, sulphadiazine, nalidixic acid, and ampicillin, suggesting a spike in multidrug resistance in food-borne pathogens to the most commonly used drugs in animal and human medicine.

**Key words:** Antibiotic resistance, Chicken meat products, *invA*, *S. Typhimurium*

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## INTRODUCTION

*Salmonella* is a major pathogen that causes food-borne infections. Salmonellosis becomes more dangerous when antibiotic-resistant strains are discovered in food, particularly chicken, which is one of the pathogen's primary vehicle for human transmission (da Cunha-Neto *et al.*, 2018). Contaminated poultry and poultry-derived products, such as meat and eggs, are a major source of Salmonellosis in the food (Barua *et al.*, 2013). Typhimurium, Enteritidis, Heidelberg, and Newport are the epidemiologically significant nontyphoidal *Salmonella* (NTS) serovars linked to a high burden of foodborne *Salmonella* outbreaks in humans worldwide (Jajere, 2019). *Salmonella* is a Gram-negative, flagellated, motile, rod-shaped aerobic and facultative anaerobic organism of the Enterobacteriaceae family. The *Salmonella* genus comprised of two species, *Salmonella enterica* and *Salmonella bongori* (Popoff *et al.*, 2004). *Salmonella enterica* has 2637 serovars, while *Salmonella bongori* has 22 (Issenhuth-Jeanjean *et al.*, 2014). *Salmonella enterica* is the most pathogenic species of *Salmonella* that can be transmitted to humans through food contamination in the farm-to-fork chain, most commonly through infected animal-based foods such as poultry and poultry-related products (Jajere, 2019). *Salmonella* Typhimurium, *Salmonella* Gallinarum, and *Salmonella* Enteritidis are the most common serovars in India, contributing for 96.2% of isolates (Kumar *et al.*, 2019).

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The indiscriminate use of antibiotics as a growth promoter in food animal feed and veterinary medicine primarily results in the emergence of *Salmonella* with antimicrobial resistance (Hyeon *et al.*, 2011). This study was designed to assess the prevalence of the vital foodborne pathogen *Salmonella* in faecal samples of broiler birds, environmental samples, chicken meat samples, and different poultry products as well as phenotypic and molecular characterization, pathogenicity testing, and multidrug resistance profiling of recovered isolates.

## MATERIALS AND METHODS

### Sample Collection

A total of 400 samples, including faecal and cloacal samples (100) of broiler poultry birds, environmental samples (150) from commercial broiler poultry sheds, chicken meat samples (100) from retail market chicken shops, and chicken meat product samples (50) from restaurants were collected at Udgir city of Maharashtra. Poultry shed samples, including cloacal swabs and faecal swabs, were collected using Hi-media sterile cotton swabs collecting tubes. While the retailer's shop samples, such as raw chicken meat samples, were placed in sterile zip lock bags, chopping board/knife swabs were collected in Hi-media sterile cotton swab collecting tubes. All samples were properly labelled, including the type of sample, location, date, and time. These samples were immediately transported to the laboratory while maintaining a cold chain for cultural isolation and *Salmonella* identification using standard protocol.

### Isolation and Identification of *Salmonella* species

In accordance with the ISO 6579:2002 methodology (ISO, 2002), samples were collected aseptically and processed immediately for the isolation of *Salmonella* species. A battery of biochemical and sugar fermentation tests were used to characterize isolates phenotypically as described in Bergey's Manual of Systematic Bacteriology (1984). The pathogenic potential of recovered *Salmonella* isolates was evaluated phenotypically using *in-vitro* pathogenicity tests (Agarwal *et al.*, 2003; Kalambe *et al.*, 2016).

### Confirmation of *Salmonella* Isolates by Genus and Serotype-Specific PCR

The recovered *Salmonella* isolates characterized by biochemical tests and sugar fermentation tests were further confirmed by PCR, targeting genus-specific gene (*invA*), and serotype-specific genes (*sdfI* and *spy*), adopting the protocol suggested by Nair *et al.* (2015) and De Freitas Neto *et al.* (2010) with certain modifications. The DNA was extracted as per the protocol given in HiPurA™ Multi sample DNA Purification kit (MB 554). The PCR assay for the detection of gene *invA* in a final volume of 20 µL was done using the following components: 2X Go Taq master mix, 10 pM of each Primer and extracted DNA template. Further, similar components were used for *spy* and *sdfI* genes in separate PCR reactions and standard strain of *Salmonella* Typhimurium (ATCC 14028) and characterized field *Salmonella* Enteritidis strain available in laboratory were used as a positive control. The PCR conditions and primers used are presented in Tables 1 and 2. PCR reactions carried out in two different thermal cyclers were Master Cycler® Vapo Protect (Eppendorf, Germany) for *invA* gene reaction and Master Cycler Prima 96 (Himedia Pvt. Ltd. Mumbai) for *sdfI* and *spy* reactions. The amplified PCR products were subjected to electrophoresis in a 1% agarose

gel (in TBE buffer), stained with ethidium bromide (10 mg/mL) solution, and finally visualized with a UV transilluminator coupled with a digital gel documentation system (Gel-Pro Analyzer, Syngene, USA).

**Table 1:** Primers and amplicon size of targeted genes for PCR

Target gene	Primer sequence (5'-3')	Amplicon (bp)	Reference
<i>invA</i>	F: TCG TGA CTC TAA ATG GCG ATA	423	Nair <i>et al.</i> (2015)
	R: GCA GGC GCA CGC CAT AAT CAA TAA		
<i>Spy</i>	F: TTG TTC ACT TTT TAC CCC TGA A	401	De Freitas Neto <i>et al.</i> (2010)
	R: CCC TGA CAG CCG TTA GAT ATT		
<i>sdfI</i>	F: CCC TGA CAG CCG TTA GAT ATT	304	De Freitas Neto <i>et al.</i> (2010)
	R: TGA ACT ACG TTC GTT CTT CTG G		

**Table 2:** Amplification conditions for the PCR assays

Target gene	Amplification conditions
<i>invA</i>	94°C (5 min), 94°C (30 sec), 56°C (1 min), 72°C (1 min 30 sec), 72°C (10 min), 30 cycles
<i>sdfI</i> , <i>spy</i>	94°C (5 min), 95°C (30 sec), 57°C (1 min), 72°C (1 min 30 sec), 72°C (7 min), 30 cycles

### Antibiotic Sensitivity Test

Antimicrobial resistance profiling of recovered isolates was carried out using the disc diffusion method as described by Bauer (1966). The isolates were evaluated against a panel of 15 antimicrobial drugs from various classes that are commonly used in both livestock and human treatment. Antimicrobial discs were placed on agar plates at the appropriate points and incubated at 37°C. Using an antibiotic zone scale, zones of inhibition were analyzed after 18 h and again after 48 h of incubation. The antibiotic susceptibility of recovered *Salmonella* isolates was determined employing CLSI data (2017).

## RESULTS AND DISCUSSION

### Prevalence of Pathogenic *Salmonella* species

In present investigation, on microbiological screening of 400 samples from different sources, two presumptive *Salmonella* isolates were recovered and confirmed as *Salmonella* Typhimurium based on results of array of biochemical tests and molecular detection by PCR, giving an overall occurrence of *Salmonella* Typhimurium to the tune of 0.50%. The presence of pathogenic *S.* Typhimurium in faecal and cloacal samples, as well as samples collected from the retailer's shop, was found to be 1% in each group, while no positivity was found in any other remaining group of samples for *Salmonella*. These findings are consistent with those of Nair



*et al.* (2015), Kalambhe *et al.* (2016), Tarabees *et al.* (2017), and Dagnew *et al.* (2020), who reported prevalence rates of 1.73 to 5.50%. The low occurrence of *Salmonella* spp. observed in this study may be attributed to regular vaccination practiced against Avian Salmonellosis on the farms being used for sample collection. Vaccines, such as the Nuobilis 9R, have been shown to be effective in controlling Salmonellosis. Vaccination, according to Pavic *et al.* (2012) is the best way to manage Salmonellosis in poultry flocks.

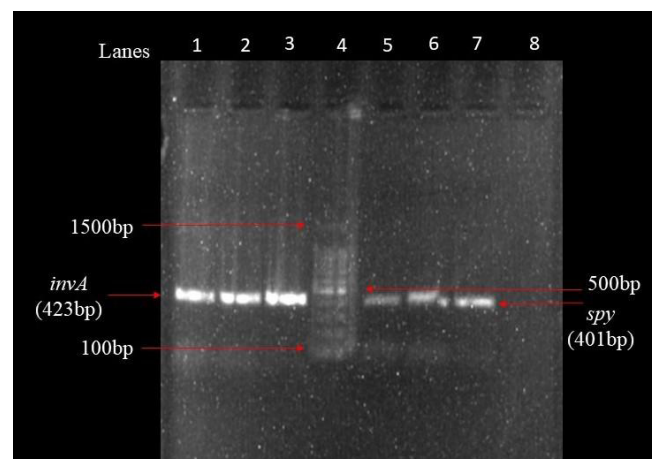
### In-Vitro Pathogenicity Testing

In the current study, both recovered *Salmonella* isolates developed well-defined brick red colonies on Congo red agar, and both isolates of *Salmonella* spp. showed strong hemolysis on 5% sheep blood agar, indicating that the isolated organism was highly pathogenic in nature. Pathogenicity testing was also carried out by Pande *et al.* (2016), who reported the use of the Congo red agar assay for testing the pathogenic potential of *Salmonella* isolates. Kalambhe *et al.* (2016) performed a similar assessment of 5% sheep blood agar for pathogenicity testing of *Salmonella*, with 66% of isolates positive for hemolysis.

### PCR Confirmation of *Salmonella* Isolates

Sareyyupoglu *et al.* (2008) suggested combining sensitive PCR methods with traditional culture methods to improve *Salmonella* detection and allow for more accurate assessments of *Salmonella* spp. prevalence. In this study, both the isolates showed positivity for *Salmonella* genus-specific *invA* gene and were positive for *S. Typhimurium* (*i.e.*, for *spy* gene) and showed negativity for *S. Enteritidis* - specific *sdfI* gene as shown in Figure 1. Shi *et al.* (2012) used a similar marker tool, where nearly 97% of isolates of *Salmonella* showed positivity for the *invA* gene, which is also defined as a virulent factor in *Salmonella* along with the *invE* gene. The positivity shown by both recovered isolates of *Salmonella* species for the *S. Typhimurium* serovar in the present study resembles a similar study conducted by Thung *et al.* (2016), who obtained 2.5% positivity for *S. Typhimurium*. However, the current findings contradict those of Afshari *et al.* (2018), who found a higher percentage of *S. Enteritidis* (43%) than *S. Typhimurium* (36%). Tarabees *et al.* (2017) screened chicken meat samples and discovered a slightly higher side of *S. Enteritidis* (3%) than *S. Typhimurium* (2%). This difference in percent positivity can be attributed to the ingestion of water by poultry birds that may have been contaminated with human faecal material, as *S. Enteritidis* serovar is commonly found in human cases. This assumption is supported by Galanis *et al.* (2006), who screened *Salmonella* isolates of human origin and discovered that *S. Enteritidis* accounted for 65% of the isolates, 12% of the isolates were positive for *S. Typhimurium*, and 4% of the isolates were positive for *S. Newport*. While, amongst non-human isolates, a total of 17% of isolates were *S. Typhimurium*, 11% of isolates were belonging to *S. Heidelberg* and 9% of

isolates were *S. Enteritidis*. It suggests that *S. Typhimurium* is most prevalent in non-human isolates and *S. Enteritidis* is the most common in human isolates.



**Fig. 1:** PCR profile of *Salmonella* isolates for *invA* and *spy* genes. Lane 1 and 2 - Positive isolates for *Salmonella* species; Lane 3 - Positive control template for species specific *invA* gene; Lane 4 - Takara 100 bp DNA ladder (Dye Plus), Lane 5 and 6 - Positive isolates for *Salmonella Typhimurium*, Lane 7 - Positive control template for *S. Typhimurium* specific *spy* gene, and Lane 8 - NTC (Negative control template)

### Antibiotic Sensitivity Test

Antibiogram analysis of isolates revealed multiple drug resistance patterns. A set of 15 different antibiotics was used, along with their various therapeutic concentrations, and all isolates demonstrated complete resistance (100%) to four antibiotics from different groups: tetracycline, sulphadiazine, nalidixic acid, and ampicillin. The highest sensitivity was revealed by *S. Typhimurium* isolates against cefotaxime, streptomycin and ciprofloxacin (100% each). Based on the antimicrobial classes, both isolates were found to be extremely resistant to broad-spectrum antibiotics such as tetracyclines, sulphonamides, quinolones, and  $\beta$ -lactams. These findings are consistent with the observations made by Kaushik *et al.* (2014) regarding isolates showing resistance to nalidixic acid and ampicillin (100%). Islam *et al.* (2016), Waghmare *et al.* (2018), and Castro-Vargas *et al.* (2020) also found that most of the *Salmonella* isolates were resistant to nalidixic acid and ampicillin. The complete resistance observed in this study to tetracyclines and sulphadiazine is similar to the results reported by Dagnew *et al.* (2020). The higher sensitivity observed in the current study against cefotaxime, streptomycin, and ciprofloxacin is similar to that reported in previous studies by Kalambhe *et al.* (2016), Waghmare *et al.* (2018), and Castro-Vargas *et al.* (2020). The resistance observed in the current study against important antimicrobials may be attributed to the uncontrolled use of antibiotics in poultry feed and water, which may lead to the development of multidrug resistance to drugs such as ampicillin, an acid-resistant penicillin derivative. Trimethoprim is mostly used in poultry health management in conjunction with sulphonamides to control diarrheal

conditions, and it is only administered orally via drinking water and feed. Besides, the multidrug resistance observed in this research work may also be attributed to other factors like the under-dosing of antimicrobials and their unnecessarily indiscriminate utilization without any proper prescription.

## CONCLUSIONS

The recovery of pathogenic *Salmonella* Typhimurium from faecal samples collected at poultry farms and raw chicken meat samples from retailer's shops is suggestive of vulnerability of *Salmonella* infection and/or contamination occurring at initial points of poultry chain. Both the isolates *S. Typhimurium* and *S. Enteritidis* were found very resistant to broad spectrum and extended spectrum antibiotics, belonging to group tetracycline, sulphonamides,  $\beta$ -lactams and quinolones signifying the advancement of multi-drug resistance in well-known food pathogens, against the most commonly used and competent drugs of choices in animal and human medicine which is enlightening the noteworthy hazard posed to public health. Hence, it can be suggested that *Salmonella* illness can be wisely controlled by choosing suitable sensitive antimicrobial drugs preparations to treat the illness.

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