

**STUDY ON DISTRIBUTION OF *Salmonella enterica* SEROTYPES IN  
POULTRY FARMS OF JAMMU**

**By**

**ARVINDERPAL SINGH  
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**Thesis submitted to Faculty of Veterinary Sciences and Animal Husbandry  
in partial fulfillment of the requirements  
for the degree of**

**MASTER OF VETERINARY SCIENCE**

**IN**

**VETERINARY PUBLIC HEALTH AND EPIDEMIOLOGY**



**Division of Veterinary Public Health and Epidemiology**

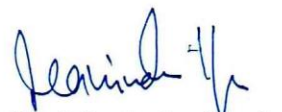
**Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu  
Main Campus, Chatha, Jammu-180009**

**2021**

## CERTIFICATE-I

This is to certify that the thesis entitled “**Study on distribution of *Salmonella enterica* serotypes in poultry farms of Jammu**” submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Science in Veterinary Public Health and Epidemiology to Faculty of Veterinary Sciences and Animal Husbandry**, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further the level of minor similarities has been declared after checking the manuscript with URKUND software provided by the University.

The work has been carried out by **Mr. Arvinderpal Singh** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.




**Dr. Maninder Singh**  
(Major Advisor)

**Place:** R. S Pura

**Date:** 10-9-2021



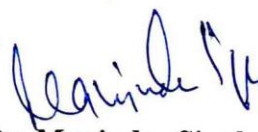
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## CERTIFICATE-II

We, the members of Advisory committee of **Mr. Arvinderpal Singh** Registration No.**J-19-MV-601**, a candidate for the degree of **Master of Veterinary Science in Veterinary Public Health and Epidemiology**, have gone through the manuscript of the thesis entitled “**Study on distribution of *Salmonella enterica* serotypes in poultry farms of Jammu**” and recommend that it may be submitted by the student in partial fulfillment of the requirements for the degree.



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## CERTIFICATE-III

This is to certify that the thesis entitled “**Study on distribution of *Salmonella enterica* serotypes in poultry farms of Jammu**” submitted by **Mr. Arvinderpal Singh**, Reg. No. **J-19-MV-601**, to the Faculty of Veterinary Science, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, in partial fulfillment of the requirements for the degree of **Master of Veterinary Science in Veterinary Public Health and Epidemiology**, was examined and approved by the advisory committee and external examiner(s) on 17-09-2021



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*For mostly with folded hands, I bow my head with reverence and dedicatedly award my gratitude to the GOD, most powerful, beneficent, gracious, merciful and compassionate whose glory and blessings made me do my work smoothly. May peace, mercy and blessings of Almighty be upon the last Apostle.*

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*Needless to say, all omissions and errors are mine.*

*Dated: 17.09.2021*

*Place: R.S. Pura, Jammu*

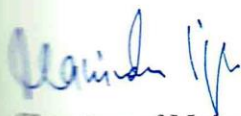
  
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## ABSTRACT

**Title of the Thesis** : Study on distribution of *Salmonella enterica* serotypes in poultry farms of Jammu.  
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Salmonellosis is a major public health concern worldwide and poultry is one of the largest reservoirs of *Salmonella*. In this regard, the present study identified the serotypes of *Salmonella* circulating in the poultry farms of Jammu region, Jammu and Kashmir. A total of 101 samples of poultry droppings from 30 poultry farms were processed for *Salmonella* isolation. Out of 30 poultry farms, 5 poultry farms (16.67%) were positive for *Salmonella*. From 5 positive farms, 19 *Salmonella* isolates were obtained. 19 *Salmonella* isolates belonged to four serotypes viz., *S. Kentucky*, *S. Infantis*, *S. Agona* and *S. Typhimurium*. All positive farms were having independent operations and none of farms on contract with private company was positive. In antibiotic sensitivity assay, all isolates were resistant to ampicillin and tetracycline (100%). For other antibiotics, resistance pattern in descending order was doxycycline (94.73%) followed by nalidixic acid (73.64%), sulphadiazine (68.42%), gentamicin (26.31%), chloramphenicol (26.31%), ciprofloxacin (36.84%), cefotaxime (15.78%), ceftriaxone (15.78%) and cefoperazone (10.53). However, all 19 isolates were sensitive to amoxycylav. Serotypes differ in their antibiotic sensitivity pattern. *S. Kentucky* and *S. Agona* isolates have higher resistance for cephalosporins and fluoroquinolones than *S. Infantis* and *S. Typhimurium*. None of the poultry farms sampled in the study was using foot bath at the entry while 13 (43.3%) farms were using quick lime foot rub at the entrance. Majority of farms were using antibiotics for prevention and control of diseases on the farm. The study found the poultry being a reservoir of *Salmonella* in the region emphasizing the need to use intervention strategies at farm and from farm to fork to prevent non-typhoidal *Salmonella* infections in humans.

**Key words:** *Salmonella*, Kentucky, Agona, Infantis, Typhimurium, poultry, Jammu



Signature of Major Advisor



Signature of the Student

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## ABBREVIATIONS AND SYMBOLS USED

|                   |   |  |
|-------------------|---|--|
| >                 | : | Greater than                                   |
| <                 | : | Lesser than                                    |
| ≤                 | : | Lesser than or equal to                        |
| ≥                 | : | Greater than or equal to                       |
| °C                | : | Degrees Celsius                                |
| μg                | : | Microgram                                      |
| BAM               | : | Bacteriological Analytical Manual              |
| bp                | : | Base pairs                                     |
| cm                | : | Centimeter                                     |
| DNA               | : | Deoxyribonucleic acid                          |
| dNTPs             | : | Deoxyribonucleotide triphosphates              |
| <i>et al.</i> ,   | : | Any other people                               |
| fig.              | : | Figure   |
| g                 | : | Gram   |
| HE                | : | Hektoen Enteric Agar                           |
| Hrs               | : | Hours  |
| ISO               | : | International Organisation for Standardization |
| IVRI              | : | Indian Veterinary Research Institute           |
| LIA               | : | Lysine iron agar                               |
| MgCl <sub>2</sub> | : | Magnesium Chloride                             |
| ml                | : | Millilitre                                     |
| NTS               | : | Non typhoidal <i>Salmonella</i>                |

|      |   |                                 |
|------|---|---------------------------------|
| PCR  | : | Polymerase Chain Reaction       |
| RV   | : | Rappaport Vassiliadis Medium    |
| TSI  | : | Triple Sugar Iron Agar          |
| TT   | : | Tetrathionate broth             |
| UV   | : | Ultraviolet                     |
| V    | : | Volts                           |
| v/cm | : | Volt/centimeter                 |
| XLD  | : | Xylose Lysine Deoxycholate Agar |

# Chapter-1

## *Introduction*

## CHAPTER–1

## INTRODUCTION

---

*Salmonella* are rod-shaped Gram-negative, facultative anaerobic and non-sporulating bacterium of family Enterobacteriaceae (Fàbrega and Vila, 2013). On the basis of 16S rRNA sequence analysis, genus *Salmonella* is divided into two species namely *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is further divided into six sub-species on the basis of their biochemical properties and genome (Reeves *et al.*, 1989) namely *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*. Among these, *S. enterica* subsp. *enterica* is mainly found in mammals and is the main cause of infection in humans and warm blooded animals (Brenner *et al.*, 2000). Apart from this classification, Kauffman and White have classified *Salmonella* into more than 2600 serotypes based on their antigenic determinants *viz.*, flagellar (H), somatic (O) and capsular (K) antigens (Brenner *et al.*, 2000) with many serotypes being able to adapt to a wide range of animal hosts including humans (Allerberger *et al.*, 2003).

*Salmonella* species are facultative intracellular pathogens (Jantsch *et al.*, 2011) mainly invading gastrointestinal tract (Ryan *et al.*, 2004). The infection of *Salmonella* is called salmonellosis which is one of the most important bacterial diseases affecting diverse number of hosts worldwide (Bäumler *et al.*, 1998). *Salmonella* is a major cause of gastroenteritis in humans globally with about 93.8 million cases, out of which 80.3 million cases are of foodborne origin (Majowicz *et al.*, 2010). In USA, salmonellosis has been the second most important infection in humans (incidence 18.3 per 100000 human population) transmitted via contaminated food, just after campylobacteriosis (incidence 19.5 per 100000 human population) (Tack *et al.*, 2019).

Poultry is the reservoir of many *Salmonella* serotypes (EFSA, 2012) responsible for clinical infection in poultry and humans. *S. Gallinarum* and *S. Pullorum* are the two host-adapted *Salmonella* serotypes which infect poultry birds exclusively resulting in huge economic losses in terms of morbidity and mortality (Rahn *et al.*, 1992; Dobhal *et al.*, 2014). *S. Gallinarum* in poultry is called fowl typhoid and is more common in adult birds while *S. Pullorum* is responsible for clinical disease in young birds (Shivaprasad and Barrow, 2008). The infection of these serotypes in poultry are common in Central

and South America, Africa and Asia while these infections has been eradicated in many developed countries including USA, Canada, New Zealand, Australia, Japan and many European countries (Spickler and Rovid., 2019). Fowl typhoid in domestic poultry is reported in India (Arora *et al.*, 2013; Pal *et al.*, 2017; Kumar *et al.*, 2019). In Jammu and Kashmir, Mir *et al.* (2010) has reported the presence of *S. Gallinarum* in government poultry farms with the prevalence of 5.83%. In another study, Shah *et al.* (2012) detected 8.8% prevalence of *S. Gallinarum* in commercial poultry farms of Srinagar.

Apart from poultry-adapted *Salmonella* serotypes, poultry may also harbor zoonotic serotypes in their intestines which are responsible for non-typhoidal salmonellosis (NTS) in humans. The most notably of these serotypes are *S. Enteritidis*, *S. Typhimurium*, *S. Virchow* and *S. Newport*. However, recent studies are indicating about the shift in pattern being *S. Kentucky* as the emerging serotype in the poultry (Barua *et al.*, 2014; CDC, 2011). It is reported that poultry is the reservoir of 10% of non-typhoidal zoonotic *Salmonella* (Gast, 2007). Poultry being reservoir of zoonotic *Salmonella* have been reported from various parts of world including India (Mir *et al.*, 2010; Mir *et al.*, 2015; Malik, 2013; Sharma *et al.*, 2019; Waghmare *et al.*, 2018 Kumar *et al.*, 2019). Kumar *et al.* (2019) at National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh have identified various serotypes from poultry samples including *S. Enteritidis* (30.65%) and *S. Typhimurium* (21.9%) which are of great zoonotic potential. However, recent studies in India have identified many other NTS serotypes circulating in poultry of country; the most notably are *S. Infantis*, *S. Virchow* and *S. Newport* (Sharma *et al.*, 2019; Bangera *et al.*, 2019). The transmission of NTS serotypes from poultry to human occurs via ingestion of undercooked contaminated poultry and poultry products (Behraves *et al.*, 2014).

In the past decades, antibiotic resistance among bacterial pathogens is emerging (Davies and Davies *et al.*, 2010) posing a threat to public health. Similar phenomenon has been observed in *Salmonella* serotypes and contaminated poultry products may transmit these pathogens to humans. Antibiotic resistance in *Salmonella* serotypes isolated from poultry is reported in various parts of world including India (Singh *et al.*, 2013; Kumar *et al.*, 2015; Waghmare *et al.*, 2018). *S. Typhimurium* DT104 has long been known for its antibiotic resistance and this strain has been reported in almost every part of world (Helms *et al.*, 2005). Similar instances are emerging in other *Salmonella* serotypes also. The recent example is *S. Kentucky* ST198 which is a fluoroquinolone resistant strain and

has been reported in human diarrheic cases in North India (Sharma *et al.*, 2020). Similarly, Anbazhagan *et al.* (2019) has reported the antibiotic resistance towards cephalosporins in *S. Typhimurium*.

In India, more than 53 serotypes of *Salmonella* have been isolated (Singh *et al.*, 2004). The studies on poultry being reservoir of *Salmonella* are reported in many areas of country including Jammu and Kashmir, Punjab, Maharashtra, Rajasthan, Uttarakhand and Uttar Pradesh (Mir *et al.*, 2015; Mir *et al.*, 2010; Malik, 2013; Sharma *et al.*, 2019; Waghmare *et al.*, 2018). Further, these studies have identified the diversity of *Salmonella* serotypes being circulating in their study areas.

In Jammu and Kashmir, few studies have been conducted on this aspect with majority from Kashmir region. The studies from Kashmir region have reported *S. Gallinarum*, *S. Enteritidis* and *S. Typhimurium* being present in the poultry flocks of Kashmir region (Mir *et al.* , 2010; Shah *et al.*, 2012). Jammu region of Jammu and Kashmir is comprised of 10 districts and has 2128 poultry farms [2018-19 data received from Deputy Director Research (Poultry), Jammu]. Till date, only one study (Malik, 2013) has been conducted to identify *Salmonella* serotypes circulating in the poultry of region and data on this aspect is largely not available. In this regard, the present study was conducted with the following objectives:

1. To study the distribution and serotype diversity of *Salmonella enterica* in poultry farms of Jammu.
2. To determine the antibiotic sensitivity pattern of *Salmonella enterica* isolates.

Chapter-2

*Review of Literature*

## CHAPTER-2

### REVIEW OF LITERATURE

---

*Salmonella* was first discovered and isolated from the intestines of pig by Theobald Smith in 1855 while the bacterium was named after Dr Daniel Elmer Salmon, an American pathologist who worked with Smith. The genus *Salmonella* is classified into two species, *Salmonella enterica* and *Salmonella bongori* based on difference in their 16Sr RNA sequence analysis. *S. enterica* is further classified into six subspecies based on their genome and biochemical properties (Reeves *et al.*, 1989). The subspecies are denoted by roman numerals: I. *S. enterica* subsp. *enterica*; II. *S. enterica* subsp. *salamae*; IIIa. *S. enterica* subsp. *arizonae*; IIIb. *S. enterica* subsp. *diarizonae*; IV. *S. enterica* subsp. *houtenae*; and VI. *S. enterica* subsp. *indica*. Among these, *S. enterica* subsp. *enterica* is found primarily in mammals and contribute to about 99 percent of *Salmonella* infections in humans and warm blooded animals whereas rest of the five subspecies and *S. bongori* are found mainly in the environment and in cold-blooded animals, and hence are rare in humans (Brenner *et al.*, 2000). Apart from this classification, Kauffman and White also developed a scheme to classify *Salmonella* into serotypes based on three major antigenic determinants *viz.*, somatic (O), capsular (K) and flagellar (H) antigens (Brenner *et al.*, 2000). Within the genus *Salmonella*, around 2600 serotypes have been identified with more than 50% of serotypes belonging to *S. enterica* subsp. *enterica*. Majority of *S. enterica* subsp. *enterica* serotypes have the ability to adapt to variety of hosts including humans (Allerberger *et al.*, 2003) (Majowicz *et al.*, 2010). In USA, salmonellosis has been the second most important infection in humans (incidence

*Salmonella* is a major cause of gastroenteritis in humans globally with about 93.8 million cases, out of which 80.3 million are food (18.3 per 100000 human population) transmitted via contaminated food, just behind campylobacteriosis (incidence 19.5 per 100000 human population) (Tack *et al.*, 2019). The data on India is not available, however data of South-East Asian region. As per World Health Organization foodborne disease burden epidemiology reference group, in South East Asia region-D, which is comprised of India, Bangladesh, Bhutan, Democratic People's Republic of Korea, Maldives, Myanmar, Nepal and Timor-Leste, *S. Typhi* is

responsible for 113 disability adjusted life years (DALYs) per 100000 human population while non-typhoidal salmonellosis is responsible for 58 DALYs per 100000 human population. Non-typhoidal salmonellosis (NTS) is a foodborne problem worldwide (Anonymous, 2019). As per 2015 report of Foodbone Disease Active Surveillance Network (FoodNet), USA, among *Salmonella* species isolated from human foodborne cases, the maximum isolates were of *S. Enteritidis* (19.2%) followed by *S. Newport* (11.4%) and *S. Typhimurium* (10.4%) (Anonymous, 2017). Further, many other NTS are emerging in these cases including *Ketunky*, *Virchow*, *Infantis* and *Agona*. The latter serotypes have been reported in number of outbreaks (many References). In India also, *S. Enteritidis* and *S. Typhimurium* are reported in NTS human cases and there has been surge in cases of other less known serotypes such as *S. Ketunky* ST398.

*Salmonella* species are facultative intracellular pathogens (Jantsch *et al.*, 2011) primarily invading gastrointestinal tract. Food animals such as swine, poultry and cattle are the main sources of *Salmonella* infections. Salmonellosis is one of the most important bacterial diseases affecting diverse number of host worldwide (Bäulmer *et al.*, 1998). *Salmonella* is the most frequently isolated foodborne pathogen and is predominantly reported in poultry, eggs and dairy products (Silva *et al.* 2011).

Poultry are the main reservoir of many *Salmonella* serotypes including poultry specific serotypes such as, *S. Gallinarum* and *S. Pullorum*, and zoonotic serotypes such as *S. Enteritidis* and *S. Typhimurium*, among others. Thus salmonellosis in poultry is an important area of study from public health point of view (Mir *et al.*, 2015). Poultry specific serotypes (*S. Gallinarum* and *S. Pullorum*) are the two host-adapted *Salmonella* serotypes which infect poultry birds exclusively resulting in huge economic losses in terms of morbidity and mortality (Rahn *et al.*, 1992; Dobhal *et al.*, 2014). Further, the zoonotic serotypes can be transmitted to human through contaminated poultry and poultry products. Earlier *S. Enteritidis* and *S. Typhimurium* were considered as the most common poultry serotypes, capable of causing infection in humans (Akinola *et al.*, 2019). However, recent reports have emphasized on the change in this pattern and *S. Kentucky* is emerging as the common serotype in poultry (Barua *et al.*, 2012; CDC, 2011). Apart from *S. Ketunky*, *S. Virchow* and *S. Infantis* have also been reported in poultry worldwide (Le Hello *et al.*, 2013; Magdy *et al.*, 2019).

The gold standard method of detection of salmonellosis is isolation of the

bacterium. Isolation of *Salmonella* can be done by non-selective pre-enrichment followed by a selective enrichment, plating onto selective agars and biochemical tests for confirmation. It is followed by agglutination test for serotype detection. The current ISO horizontal method (ISO 6579: 2002) consists of a pre-enrichment of samples in buffered peptone water followed by selective enrichment in Rappaport Vassiliadis (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn). Rappaport Vassiliadis (RV) medium and tetrathionate (TT) broth are the official *Salmonella* enrichment media in the methods of FDA Bacteriological Analytical Manual (BAM) and FERN. The selective enrichment media containing *Salmonella* at the level of above  $10^4$  cells/ml are streaked on solid media to isolate presumptive positive *Salmonella* colonies (Carrique-Mas and Davies, 2008). Commonly used plating media include *Salmonella*-Shigella agar (SS), brilliant green agar (BGA), bismuth-sulfite agar (BSA), Hektoen enteric (HE), and xylose-lysine-deoxycholate agar (XLD). The *Salmonella* colonies identified from these media are tested by triple sugar iron (TSI) test and lysine iron agar (LIA) test followed by urease test and other biochemical tests. Genus *Salmonella* is done using *invA* gene whereas *S. Typhimurium* is detected using *typh* gene (Alvarez *et al.*, 2004). A multiplex PCR protocol to detect *invA* and *sefA* virulence genes can be employed (Cortez *et al.*, 2006). Similarly, *stn*, *sopB* and *spvC* genes can be detected with PCR protocols with primer sequences described by Rahman (1999 and 2006), and Chiu and Ou (1996), respectively. These *Salmonella* isolates are further subjected to agglutination tests for serotype identification (Hoorfar *et al.*, 1999). It takes about 4 to 7 days to culture and identify *Salmonella* serotypes by this protocol.

Polymerase chain reaction (PCR) can be being used for rapid and reliable detection of *Salmonella* by using genus specific 16S rRNA primers along with laboratory procedures for poultry *Salmonella* (Lin and Tsen, 1996). Further serotype specific PCR are also used for detection of *S. Enteritidis* and *S. Typhimurium* (Alvarez *et al.*, 2004) while *S. Gallinarum* can be detected by using allele-specific PCR (Shah *et al.*, 2005). There are some specific target genes used in identification of serotypes by PCR such as *sefA* for *S. Enteritidis*, Dublin and Gallinarum. *sdf* for *S. Enteritidis* only and *fliC* for serotypes Typhimurium and Kentucky (O'Regan *et al.*, 2008).

The emergence of antibiotic-resistant foodborne pathogens has raised the concern of the public as these pathogens are more virulent, causing an increase in the mortality rate of infected patients (Chiu *et al.* 2002). Antimicrobial agents such as

ampicillin, chloramphenicol and trimethoprim–sulfamethoxazole are used as the traditional first line treatments for *Salmonella* infections. Resistance in *Salmonella* spp. towards these agents is referred to as multi-drug resistant (MDR). Most of the *Salmonella* isolates have developed resistance against multiple drugs due to their indiscriminate and repeated use. Therefore monitoring drug resistant pattern among *Salmonella* isolates is very important to devise a proper prophylactic and chemotherapeutic schedule (Murugkar *et al.*, 2005).

## **WORK DONE ON SALMONELLOSIS GLOBALLY**

Limawongprance *et al.* (1999) conducted a study to find out the presence of *Salmonella* in 2345 cecal contents of broiler chickens. During the study, a total of 336 (14.3%) *Salmonella* species were isolated comprising of 242 (72.0%) *S. Blockley*, 60 (17.9%) *S. Hadar*, 15 (4.5%) *S. Bredeney*, 9 (2.7%) *S. Schwarzengrund*, 4 (1.2%) *S. Anatum*, 3 (0.9%) *S. Enteritidis*, 2 (0.6%) *S. Ohio* and 1 (0.3%) *S. Livingstone*.

Tavechio *et al.* (2002) carried out the serotyping of *Salmonella* from nonhuman sources at Saõ Paulo, Brazil. The studied showed that *S. Enteritidis* was the most prevalent (32.7%) followed by *S. Senftenberg* (10.3%), *S. Hadar* (6.8%), *S. Agona* (5.1%) and *S. Typhimurium* (2.4%). Some rough strains of *S. enterica* subsp. *enterica* (4.8%), *S. enterica* subsp. *arizonae* (1%), *S. enterica* subsp. *diarizonae* (1%) and *S. enterica* subsp. *houtenae* (1%) were also seen.

A total of 60 samples of poultry products from two local butcher shops and canteen were examined by Antunes *et al.* (2003) in the city of Porto. After isolation and serotyping, 60% of samples showed presence of *Salmonella* comprising of 10 serotypes. *S. Enteritidis* and *S. Hadar* were the most prevalent serotypes. These isolates were resistant to many antibiotics and eight distinct resistant patterns were recorded with enrofloxacin and Nalidixic acid showing resistance to almost half of the isolates recovered.

Rodriguez *et al.* (2006) analyzed different environmental farm samples for the prevalence of *Salmonella*. A total prevalence of 4.7% was recorded. Maximum isolates were recovered from swine farms (57.3) and lower occurrence was observed in dairy farms (17.9%), poultry farms (16.2%), and beef cattle farms (8.5%). The most commonly isolated serotypes was *S. Anatum* (48.4%) followed by *Salmonella* serotypes *Arizonae* (12.1%) and *Javiana* (8.8%).

Parveen *et al.* (2007) studied the prevalence of *Salmonella* in processed poultry carcasses in the mid-Atlantic region. Around 480 pre and post chill whole carcasses were examined for presence of *Salmonella*. Results showed that about 88.4% and 84.1% pre and post chill samples were positive for *Salmonella* respectively. Serotyping identification showed presence of 13 different serotypes and the most common were *S. Kentucky* (59.5%) and *S. Typhimurium* (17.8%). Around 339 (79.8%) of the isolates were resistant to at least one antimicrobial and 53.4% were resistant to three or more antimicrobials. Resistance was observed to tetracycline (73.4% of isolates), ampicillin (52.9%), amoxicillin-clavulanic acid (52%), ceftiofur (51.7%), streptomycin (35.2%) and sulfisoxazole (21.8%).

Al-Zenki *et al.* (2007) collected 2882 samples from poultry farm and 360 samples from processing plant environment for the presence of *Salmonella*. Results at farm showed the prevalence rates of 10%, 1.5%, 0.7%, 0.2%, 13.5%, and 12.6% from hatching eggs, litter, feed, drinkers, bird rinse and ceca respectively whereas at processing plant the prevalence rates were 6.1% and 3.3% from carcass rinse and ceca samples, respectively. Paper liner, water, or air samples were all negative for *Salmonella*. Most prevalent serotype was *S. Enteritidis*. All of the isolates were resistant to more than one antibiotic and 10 different resistance profiles were found in the isolates of *S. Enteritidis*. Resistance to ampicillin, nalidixic acid and tetracycline were the most common.

Aragaw *et al.* (2011) conducted a study to determine the prevalence of *S. Gallinarum* and *S. Pullorum* in apparently healthy chickens and to assess the proportional morbidity/mortality due to these two serotypes in sick/dead chickens. The prevalence of *Salmonella* infection in healthy chickens was 0.8% (3/380), while isolation was 16.1% (5/31) in sick/dead chickens. The study concluded that fowl typhoid and pullorum disease have significant role in morbidity and mortality of intensively managed chickens

Alali *et al.* (2010) carried out a study to investigate and compare the prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. The results showed that in fecal samples the prevalence of *Salmonella* was 5.6% (10/180) in organic and 38.8% (93/240) in conventional farms. Among feed samples, prevalence was 5.0% (3/60) and 27.5% (22/80) in organic and conventional farms, respectively. None of the water samples was positive for *Salmonella*. Seventy isolates were



isolated from Ecuadorian Broilers at slaughter age. Out of 1094 *Salmonella* isolates, 78 serotypes were identified. The top five most common *Salmonella* serotypes on raw chicken carcasses were Enteritidis (19.2%), Indiana (15.2%), Typhimurium (14.6%), Agona (7.1%), and Thompson (6.6%). These isolates (779) were most commonly resistant to sulfisoxazole (74.1%) and tetracycline (71.1%) and least resistant to ceftriaxone (22.5%) and cefoxitin (19%). Antibiogram of these isolates led to the conclusion that only 4% of the isolates were susceptible to 15 antimicrobial agents used where as 45% were resistant to 1 to 5 agents, 29% to 6 to 10 agents and 22% to 11 to 15 agents.

Nidaullah *et al.* (2017) determined the *Salmonella* prevalence and serotype identification in chickens from carcass contact and environmental samples. The results showed that all samples of floor, bench, wash water, whole chicken carcass and chicken cuts, transport crate, wash water, cage, drum, knife, chopping board, display table and drain water were positive for *Salmonella*. A total of 17 serotypes were isolated with *S. Albany* (57/161), *S. Corvallis* (42/161) and *S. Brancaster* (37/161) being the most predominant.

Ahmed *et al.* (2019) conducted a study to determine the prevalence of *Salmonella* on poultry farms. From January to August 2007 a total of 900 samples from poultry environment, health and dead birds were collected. 58 (6.4%) *Salmonella* isolates were recovered and the most predominant serotypes were *Salmonella enterica* ser. 6.7: d (29.0%), *S. Agama* (28.0%) and *S. Typhimurium* (16.0%).

Magdy *et al.* (2019) analysed fecal droppings, litter and dropping swabs to identify *Salmonella* serotypes from broiler breeder chicken farms. Results showed the presence of 20 serotypes including *S. Kentucky* (11), *S. Enteritidis* (4), *S. Typhimurium* (3), *S. Breanderp* (1), and *S. Newport* (1). Antimicrobial sensitivity testing analysis revealed that about 95% isolates were resistant to penicillin, eighty five to norfloxacin and colistin sulfate, 75% to gentamicin, 70% to nalidixic acid and 60% to flumequine.

The prevalence of *Salmonella* in laying hens and broiler chicken farms was analysed by Tabo *et al.* (2013). Samples of fresh fecal droppings, food and water were collected. A total of 84 *Salmonella* isolates were recovered comprising of 27 different serotypes. The most common serotypes were *S. Colindale* (19%), *S. Minnesota* (18%), *S. Havana* (6%), *S. Riggi* (6%), *S. Kottbus* and *S. Amager* (4.7%), *S. Idikan*, *S.*

Mississippi, and *S. Muenchen* (3.6%). Among 84 *Salmonella* isolates tested for antibiotic sensitivity, *S. Colindale* showed decreased susceptibility to fluoroquinolones whereas one *S. Limete* was resistant to three and fifteen *S. Minnesota* isolates were resistant to five different antimicrobials.

Orji *et al.* (2005) conducted a study to isolate *Salmonella* from poultry droppings and other environmental sources. Samples of 120 poultry droppings, 96 fresh beef, 96 fresh beef retail table and 96 beef retailers' aprons were collected. Isolation rate of serotype Paratyphi was 12.5%, 4.2%, 2.1%, 4.2% from poultry droppings, fresh beef, meat retail aprons and tables samples, respectively. In addition to Paratyphi, other serotypes namely *S. Typhimurium*, *S. Enteritidis*, *S. Gallinarum*, *S. Pullorum*, *S. Typhi* and *S. Agama* were also identified

Velasquez *et al.* (2018) studied the antimicrobial resistance patterns of *Salmonella* isolates from poultry farms in southeastern United States. Four poultry farms were sampled by collecting cloacal swabs, drag swabs and litter samples prior to recommended biosecurity changes (March-April) and post recommendations (October-November). Sixteen drag swab samples, 16 litter samples and 48 cloacal swab samples from each farm were collected. Prevalence of *Salmonella* was 3 to 4% during pre-recommendations, while the prevalence was higher ranging from 5 to 14% during post recommendations. Higher *Salmonella* prevalence was observed for pre- and post-recommendation phases by sample type in cloacal and drag samples – 5% for farm 1, drag swab – 6% on farm 2, cloacal swab – 6% for farm3, and drag swab –17% on farm 4. Six serotypes of *Salmonella* were identified with *S. Enteritidis* (52%) being the most prevalent followed by *S. Berta* (38%), *S. Mbandaka* (7%), *S. Typhimurium*(2%), *S. Kentucky* (0.4%) and *S. Tennessee*(0.4%). A total of 7% isolates exhibited resistant to at least one of the 8 antimicrobials. Higher resistance was observed for tetracycline, streptomycin and nalidixic acid.

Manning *et al.* (2015) conducted screening for *Salmonella* in backyard poultry in South Australia. Three serotypes viz., *S. Agona*, *S. Wandsbek* and *S. Bovismorbificans* were isolated. All *S. Wandsbe* isolates were resistant to ampicillin, cephalothin while intermediate resistance towards florphenicol. However, *S. Agona* isolates showed intermediate resistance towards ampicillin whereas *S. Bovismorbificans* isolates were susceptible to all antibiotics tested.

Antimicrobial Resistance of *S. Enteritidis* from retail chicken products in Shanghai, China was analysed by Zhou *et al.* (2018). Approximately 42% (61/146) of the isolates were susceptible to all 13 antimicrobials tested. More than half of the isolates (50.70%) were resistant to ampicillin, 49.32% to sulfisoxazole, 17.12% to tetracycline, and 15.75% to doxycycline.

Moe *et al.* (2017) conducted a cross-sectional investigation concerning prevalence, antimicrobial resistance, multidrug resistance patterns and serotype diversity of *Salmonella* in chicken meat sold at retail in Yangon, Myanmar. The 141 chicken meat samples were collected at 141 retail markets in the Yangon Region, Myanmar from 1 November 2014 to 31 March 2015. *Salmonella* was recovered from 138 (97.9%) samples. The isolates were most frequently resistant to trimethoprim-sulfamethoxazole (70.3%), tetracycline (54.3%), streptomycin (49.3%), and ampicillin (47.1%). Resistance was also found to chloramphenicol (29.7%), amoxicillin-clavulanic acid (17.4%), ciprofloxacin (9.4%), tobramycin (8.7%), gentamicin (8%), cefazolin (7.2%), lincomycin-spectinomycin (5.8%) and norfloxacin (0.7%). Among the 138 *Salmonella* isolates, 72(52.2%) were resistant to three or more antimicrobial agents. Twenty-four serotypes were identified among the 138 *Salmonella* positive samples. *S. Albany*, *S. Kentucky*, *S. Braenderup* and *S. Indiana* were found in 38, 11, 10, and 8% of samples respectively.

Yang *et al.* (1998) assessed the trends in antimicrobial resistance among clinical *Salmonella* infections from 1989 to 1996 in southern Taiwan. The rates of resistance to ampicillin, chloramphenicol, and tetracycline were 65, 67, and 78%, respectively. Resistance to trimethoprim-sulfamethoxazole (TMP-SMX) increased from 25% in 1989–1992 to 35% in 1993–1996. For new quinolones and extended-spectrum cephalosporins, no resistant strains were encountered. As a result in Taiwan, ampicillin, chloramphenicol and even TMP-SMX are no longer the drugs of choice for treatment of serious nontyphoidal *Salmonella* infections. The extended-spectrum cephalosporins are now the preferred drugs for treatment of invasive *Salmonella* infections in children.

Lestari *et al.* (2009) isolated and characterized 126 *Salmonella* isolates from conventionally raised ( $n = 141$ ) and organically raised ( $n = 53$ ) chicken carcasses obtained from 27 retail stores in Baton Rouge, Louisiana. *Salmonella* was isolated from 22% of conventional and 20.8% of organic chicken samples. They reported that all

*Salmonella* isolates were susceptible to amikacin, ceftriaxone, and ciprofloxacin; however, decreased susceptibility to quinolones (7.1%) or extended-spectrum cephalosporins (45.2%) was observed. Antimicrobial resistance profiles differed greatly among *Salmonella* serotypes and also depended on the type of chicken from which they were recovered. *S. Kentucky* isolates from organic chicken samples were susceptible to 11 of the antimicrobials tested, whereas those from conventional chickens were only susceptible to 4 antimicrobials.

*Salmonella* isolation from poultry products, poultry, poultry environment was carried out by Roy *et al.* (2002) from the Pacific northwest. Five hundred sixty-nine (11.99%) *Salmonella* were isolated out of 4745 samples. A total of 16 serotypes were identified. The serotypes were *S. Heidelberg* (25.77%), *S. Kentucky* (21.64%), *S. Montevideo* (11.34%), *S. Hadar* and *S. Enteritidis* (5.15% each), *S. Infantis*, *S. Typhimurium*, *S. Ohio*, and *S. Thompson* (4.12% each); *S. Mbandaka* and *S. Cerro* (3.09% each); *S. Senftenberg* (2.06%); *S. Berta*, *S. Istanbul*, *S. Indiana* and *S. Saintpaul* (1.03% each); and an unnamed monomorphic *Salmonella* (2.06%). Ninety two *Salmonella* were tested for drug sensitivity with nine different antimicrobials. All 92 *Salmonella* were resistant to erythromycin, lincomycin, and penicillin except one sample (*S. berta*), which was moderately sensitive to penicillin. All of the tested *Salmonella* were susceptible to sarafloxacin and ceftiofur. The percentages of *Salmonella* susceptible to sulfamethoxazole-trimethoprim, gentamicin, triple sulfa, and tetracycline were 97.83%, 92.39%, 86.96% and 82.61%, respectively.

Haley *et al.* (2019) studied *S. Kentucky* isolates obtained from human clinical cases in the State of Maryland from 2011 to 2015 (n=12). Isolates were sequenced and compared with previously available genomes. Out of 12 human isolates, 9 were ST198, 2 were ST152 and 1 was ST314. Forty one percent of these isolates were recovered from patients having a history of recent international travel and 58% of isolates had genome characteristics similar to those obtained outside the United States.

Myoujin *et al.* (2003) studied *S. Agona* infection in a pheasant flock in 1995 and 2000. Nine hundred and seventy three (56.2%) birds died at 4 to 5 days of age in 1995, whereas 80 (4%) died at 15 to 25 days of age in 2000. It indicated that susceptibility of pheasants to *S. Agona* is age dependent.

## WORK DONE ON SALMONELLOSIS IN INDIA

Sharma *et al.* (2020) conducted a study to estimate the prevalence of NTS among hospitalized diarrhoeal cases in and around Delhi, India. During their study, 81 NTS isolates were recovered. The most common serotypes were *S. Kentucky* (48) and *S. Virchow* (22). Antimicrobial sensitivity pattern revealed that the isolates were resistant to nalidixic acid (73%), ciprofloxacin (48%), ampicillin and norfloxacin (36% each), and gentamicin (31%). The isolates were resistant to fluoroquinolones, which is the current choice of drug in treatment of diarrheal cases.

Bangera *et al.* (2019) studied NTS infection in poultry and isolated 58 NTS serotypes from 396 poultry meat, intestinal and faecal samples. Prevalence of 14.64% was observed. The most common serotype isolated was *S. Infantis* (43.1%). Other serotypes reported were Kentucky, Worthington, Typhimurium, Poona, Weltevreden, Agona Takoradi and Kouka. Antibiotic sensitivity assay of *Salmonella* isolates showed resistance towards ciprofloxacin (72.41%), ampicillin (32.8%), gentamicin (17.24%), cotrimoxazole (29.31%) and amoxicillin-clavulanic acid (6.9%) was observed.

Mahindroo *et al.* (2019) studied fluoroquinolone resistant *S. Kentucky* ST198 in Northern India. 1912 human and 906 animal samples (419 meat and 487 stool from sheep, goat, pig and chicken) were processed for isolation. A total of 117 NTS isolates were recovered and the most common serotype was Kentucky (n=23) including 6, 15 and 2 originated from human, poultry and goat samples, respectively. Fifteen isolates of *S. Kentucky* of poultry origin were from from 3 poultry farms of Punjab state. All *S. Kentucky* isolates were resistant to ciprofloxacin, but susceptible to third-generation cephalosporins (ceftazidime, cefepime), carbapenems (meropenem, imipenem), azithromycin and colistin

Kumar *et al.* (2014) studied the prevalence of *Salmonella* in pigs and broilers. The total prevalence of *Salmonella* in pigs was 8.6% (intensive production system + free-range system Most common serotype isolated was *S. Typhimurium* (62.5%) followed by *S. Bredney* (25%) and *S. Infantis* (12.5%) whereas prevalence of *Salmonella* in poultry was 12.28% (8.45 in cloacal samples and 22% in tissue samples). Most common isolated serotype was of *S. Typhimurium* (n=20; 46.5%) followed by *S. Enteritidis* (n=16; 37.2%) and *S. Gallinarum* (n=7; 16.2%).

Sharma *et al.* (2019) conducted a study to determine the prevalence and antimicrobial resistance of NTS isolated from retail chicken meat shops. Prevalence of 9.43% (40% in chicken meat samples and 20% in poultry faeces) was observed. Three serotypes of *Salmonella* viz., *S. Kentucky* (74.29%), *S. Virchow* (17.14%) and *S. Typhimurium* (7.14%) were identified. All isolates were multidrug resistant (MDR) showing resistance to three or more classes of antibiotics. Complete resistance was observed to tetracycline (100%), erythromycin (100%), followed by nalidixic acid (98.57%), ampicillin (95.71%) and ciprofloxacin (82.86%).

Suresh *et al.* (2006) conducted a study to determine the incidence of *Salmonella* Enteritidis and other serotypes among eggs shells, egg contents and on egg-storing trays. 492 eggs and 82 egg storing trays were examined and *Salmonella* was confirmed in 7.7% eggs (29 in egg shell and 9 in egg content) and 7.5% egg storing trays. All isolates from egg content were *S. Enteritidis*. Other serotypes identified were *S. Cerro*, *S. Molade* and *S. Mbandaka* from eggshell and *S. Cerro* from egg-storing trays. Prevalence pattern showed marked seasonal fluctuation with a higher prevalence during monsoon months followed by post-monsoon and premonsoon. The isolates from eggs and egg storing trays were resistant to ampicillin, neomycin, polymyxin-B and tetracycline. However none of the isolates was resistant against gentamicin and chloramphenicol. Some variations in results were noticed for ciprofloxacin as 8.9% of egg isolates were resistant whereas all isolates from egg storing were sensitive.

Molecular epidemiology and antimicrobial susceptibility of *Salmonella* isolated from poultry in Kashmir was studied by Mir *et al.* (2010). They took 429 fecal and 51 tissue samples from dead birds in their study. Results revealed that existence of three main serotypes namely *S. Gallinarum* (28), *S. Enteritidis* (3) and *S. Typhimurium* (2). Antibiotic sensitivity pattern showed that most of them were resistant to cefpodoxime, nalidixic acid and sulphadiazine while sensitive to chloramphenicol, cefotaxime and tetracycline.

Kumar *et al.* (2012) studied the prevalence of *Salmonella* serotypes from poultry in different parts of Haryana. A total of 88 *Salmonella* isolates were recovered. Serotypes identified were *S. Gallinarum* (n=53), *S. Pullorum* (n=16), *S. Enteritidis* (n=13) and *S. Typhimurium* (n=6). Antibiotics namely chloramphenicol, amikacin, gentamicin, cephalexin and cephadroxil were subjected to sensitivity test and results demonstrated that *S. Gallinarum* and *S. Enteritidis* isolates were sensitive to

chloramphenicol and Gentamicin came out as second most effective antibiotic. However multiple drug resistant patterns was also observed.

Singh *et al.* (2013) collected 720 samples from poultry and poultry environmental (egg, feed, water, cloaca, and faeces) for *Salmonella* detection. *S. Typhimurium* was the most prevalent serotype. All isolates were resistant to clindamycin, oxacillin, penicillin and vancomycin. However were sensitive to ampicillin, enrofloxacin and colistin.

Samanta *et al.* (2014) conducted a study to determine the prevalence of *Salmonella* in birds kept under the backyard system. *Salmonella* were recovered from cloacal swabs, feed samples, drinking water samples and eggs. All isolates were found to be resistant to chloramphenicol, ciprofloxacin, gentamicin, levofloxacin, norfloxacin and oxytetracycline

Mir *et al.* (2015) studied the serotype diversity and resistance pattern of *Salmonella* isolates from poultry. 202 caecal content and 305 fecal samples were studied. *S. Enteritidis*, *S. Typhimurium* and *S. Gallinarum* were identified by serotype specific PCR. Further all isolates were analyzed for 20 most commonly used antibiotics in the region. Antibiotic sensitivity pattern showed that resistance was maximum (100%) to oxacillin, penicillin and clindamycin followed by ampicillin, tetracycline, nalidixic acid and colistin whereas chloramphenicol and meropenem were highly sensitive.

Kumar *et al.* (2015) analysed 240 swab samples from forty flocks. *Salmonella* was isolated from 10 samples. Seven *S. Gallinarum* and three *S. Pullorum* isolates were identified. All isolates were resistant to doxycycline hydrochloride and erythromycin while showed marked sensitivity towards gatifloxacin, amikacin, and levofloxacin

Arora *et al.* (2015) investigated the prevalence *Salmonella* in poultry. Two hundred fifty three isolates comprising of *S. Gallinarum* (183), *S. Enteritidis* (41) and *S. Typhimurium* (29) were recovered. The antimicrobial sensitivity pattern showed that *S. Gallinarum* isolates were sensitive to gentamicin (76%), amikacin (72%), kanamycin (71%), chloramphenicol (71%) and streptomycin (70%).

Waghmare *et al.* (2018) studied the antibiotic sensitivity pattern of *Salmonella* serotypes in poultry farming and processing environment. A total of 956 samples were analyzed. Out of 71 positive isolates, 42 subjected to antibiotic susceptibility testing.

Maximum resistance was seen against doxycycline (100%) followed by oxytetracycline (97.62%), neomycin (88.10%), erythromycin (83.33%), tetracycline (78.57%) and ceftizoxime (35.71%). Results also demonstrated that serotype *S. Virchow* dominated all other serotypes.

Suresh *et al.* (2019) conducted a study to characterize *Salmonella* species of animal origin. A total of 516 samples comprising poultry cloacal swabs (249), raw foods of animal origin (118 chicken samples, 65 mutton and 30 pork), poultry liver swabs (17) and poultry farm water samples (37) were examined. Overall prevalence of *Salmonella* isolates was found to be 4.06% (21/516) with highest prevalence in chicken samples (6/118, 5.08%) followed by cloacal swabs of poultry (12/249, 4.81%), mutton (2/65, 3.07%) and pork (1/30, 3.33%).

Sharma *et al.* (2019) determined the prevalence of *Salmonella* in 39 retail chicken meat shops. *Salmonella* prevalence was 9.43% (70/742). Highest prevalence was seen in Chicken meat samples (40%) followed by poultry feces (20%). Three serotypes of *Salmonella* were identified namely *S. Kentucky* (74.29%), *S. Virchow* (17.14%) and *S. Typhimurium* (7.14%). All the isolates were multidrug resistant. Highest resistance was seen against tetracycline (100%), erythromycin (100%), nalidixic acid (98.57%), ampicillin (95.71%) and ciprofloxacin (82.86%).

Bangera *et al.* (2019) analyzed 396 samples of poultry meat, faecal matter and intestinal contents. Prevalence of 14.64% (58 isolates) was obtained. *S. Infantis* was the most common serotype isolated. Antimicrobial resistance pattern was in order of ciprofloxacin (72.41%) followed by ampicillin (32.8%), gentamicin (17.24%), co-trimoxazole (29.31%) and amoxicillin-clavulanic acid (6.9%) were resistant.

Kumar *et al.* (2019) analyzed *Salmonella* isolates received at National *Salmonella* and *Escherichia* Centre from year 2011 to 2016 to study the distribution of *Salmonella* serotypes among poultry in India. *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum* were the most prevalent serotypes accounting for 96.2 % of isolates. In addition, *S. Infantis* (2.7%), *S. Montevideo* (0.64%), *S. Newport* (0.26%) and *S. Pullorum* (0.13%) were also identified.

Chapter-3

*Materials and Methods*

## CHAPTER-3

### MATERIALS AND METHODS

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#### 3.1 Location of work

The work was conducted at the Division of Veterinary Public Health and Epidemiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST –J, R.S. Pura, Jammu.

#### 3.2 Sample Collection for *Salmonella* isolation

The study was aimed to identify *Salmonella enterica* serotypes circulating in the poultry farms of Jammu region of Jammu and Kashmir Union Territory of India. To achieve this objective, poultry droppings from poultry farms from Jammu region were collected and processed for isolation of *Salmonella*. Jammu region of Jammu and Kashmir has 10 districts. For the sampling, four districts viz., Jammu, Samba, Kathua and Udhampur were randomly selected. From these 4 districts, 30 poultry farms were sampled. The sampling from selected districts was conducted from June 2020 to July 2021. Sample details and their geographical information are given in the Table 1.

A total of 101 poultry dropping samples from 30 poultry farms were collected. From each farm, samples were collected according to the number of sheds on the farm. The droppings were collected in sterilized sampling containers and transported to laboratory on ice. The samples were processed immediately further for isolation of *Salmonella*.

**Table 1: Details of poultry farms (n=30) sampled in the study**

| <b>District</b> | <b>Block</b> | <b>Poultry Farm Location</b> | <b>Latitude</b> | <b>Longitude</b> |
|-----------------|--------------|------------------------------|-----------------|------------------|
| Jammu           | Satwari      | Balicharana                  | 32.707653       | 74.840394        |
|                 | Bhalwal      | Barnai                       | 32.768310       | 74.807735        |
|                 | Akhnoor      | Mandrian 1                   | 32.958673       | 74.731532        |
|                 |              | Mandrian 2                   | 32.950894       | 74.731080        |
|                 |              | Baruie                       | 32.969704       | 74.738196        |
|                 | R. S Pura    | Kadyal                       | 32.595650       | 74.746157        |
|                 |              | Gazipur 1                    | 32.590787       | 74.730717        |
|                 |              | Gazipur 2                    | 32.589029       | 74.729246        |
|                 |              | Rangpur Malanian 1           | 32.609210       | 74.694541        |
|                 |              | Rangpur Malanian 2           | 32.608797       | 74.694832        |
|                 |              | Chohala                      | 32.603931       | 74.760225        |
|                 |              | Satrayan Kalan               | 32.546301       | 74.674037        |
|                 |              | Chak Roi                     | 32.512464       | 74.684710        |
|                 |              | Darsopur                     | 32.599030       | 74.765715        |
| Magowali        |              | 32.516584                    | 74.711824       |                  |
| Chak Roi        | 32.513695    | 74.683784                    |                 |                  |
| Samba           | Samba        | Sangwal 1                    | 32.561015       | 75.140034        |
|                 |              | Sangwal 2                    | 32.558233       | 75.136819        |
| Kathua          | Kathua       | Muthi khurd                  | 32.458915       | 75.133848        |
|                 |              | Janglote                     | 32.432935       | 75.525835        |
|                 |              | Mahi chak                    | 32.403183       | 75.419814        |
|                 |              | Jandore 1                    | 32.415099       | 75.483951        |
|                 |              | Jandore 2                    | 32.412228       | 75.488930        |
|                 |              | Barwal                       | 32.446033       | 75.479735        |
|                 |              | Kalspur                      | 32.401459       | 75.588198        |
|                 |              | Palli morh                   | 32.405267       | 75.449971        |
|                 |              | Nangal                       | 32.372395       | 75.408743        |
| Udhampur        | Ramnagar     | Ramnagar 1                   | 32.808385       | 75.312876        |
|                 |              | Ramnagar 2                   | 32.807156       | 75.314079        |
|                 |              | Thaplal                      | 31.798127       | 75.319535        |



(a)



(b)

**Plate 1: Collection of droppings from poultry farm**

**Table 2: Details of poultry dropping samples (n=101) collected in the study**

| <b>Farm No</b> | <b>Farm Location</b>    | <b>Age of birds (Days)</b> | <b>Flock Size</b> | <b>No. of shed/farm</b> | <b>Samples collected</b> |
|----------------|-------------------------|----------------------------|-------------------|-------------------------|--------------------------|
| 1              | Balicharana             | 60                         | 2500              | 4                       | 4                        |
| 2              | Barnai                  | 22                         | 2005              | 3                       | 3                        |
| 3              | Mandrian 1              | 20                         | 5500              | 4                       | 4                        |
| 4              | Mandrian 2              | 20                         | 4500              | 2                       | 2                        |
| 5              | Barui                   | 13                         | 2000              | 5                       | 5                        |
| 6              | Kadyal                  | 15                         | 1500              | 3                       | 3                        |
| 7              | Gazipur 1               | 7                          | 2000              | 2                       | 2                        |
| 8              | Gazipur 2               | 7                          | 2500              | 4                       | 4                        |
| 9              | RangpurMalanian1        | 14                         | 3000              | 3                       | 3                        |
| 10             | RangpurMalanian2        | 14                         | 3110              | 5                       | 5                        |
| 11             | Chohala                 | 20                         | 2050              | 3                       | 3                        |
| 12             | Satrayan Kalan          | 6                          | 1500              | 2                       | 2                        |
| 13             | Chak Roi                | 20                         | 5050              | 5                       | 5                        |
| 14             | Darsopur                | 25                         | 2020              | 3                       | 3                        |
| 15             | Magowali                | 20                         | 1950              | 3                       | 3                        |
| 16             | Chak Roi                | 21                         | 2200              | 2                       | 2                        |
| 17             | Sangwal 1               | 18                         | 2000              | 2                       | 2                        |
| 18             | Sangwal 2               | 18                         | 1730              | 6                       | 6                        |
| 19             | Muthi khurd             | 33                         | 5410              | 3                       | 3                        |
| 20             | Virpur                  | 15                         | 1500              | 5                       | 5                        |
| 21             | Mahi chak               | 25                         | 5526              | 2                       | 2                        |
| 22             | Jandore 1               | 14                         | 4284              | 7                       | 7                        |
| 23             | Jandore 2               | 25                         | 4000              | 3                       | 3                        |
| 24             | Barwal                  | 14                         | 3318              | 4                       | 4                        |
| 25             | Lachipur                | 14                         | 2045              | 4                       | 4                        |
| 26             | Palli morh              | 3                          | 2550              | 2                       | 2                        |
| 27             | Nangal                  | 22                         | 2000              | 3                       | 3                        |
| 28             | Ramnagar 1              | 25                         | 2120              | 4                       | 4                        |
| 29             | Ramnagar 2              | 25                         | 2570              | 3                       | 3                        |
| 30             | Thaplal                 | 19                         | 2800              | 2                       | 2                        |
|                | <b>Total Farms = 30</b> |                            |                   |                         | <b>101</b>               |

### 3.3 Attributes of poultry farms (n=30) sampled in the study

The data on various attributes of poultry farms including their antimicrobial usage was collected using a questionnaire. Face to face interview was conducted. The information collected is depicted in Table 3.

**Table 3: Attributes of poultry farms (n=30) sampled in the study**

| <b>S No.</b> | <b>Farm attribute</b>                                      | <b>Category</b>                 |
|--------------|--|---------------------------------|
| 1.           | Type of farm   | Layer/Broiler                   |
| 2.           | Facility type  | Independent                     |
|              |  | Contracted                      |
|              |  | Government                      |
| 3.           | Age of birds at farm                                       | <1 week                         |
|              |  | 1-3 week                        |
|              |  | 3-6 week                        |
|              |  | >6 week                         |
| 4.           | Antimicrobial usage (disease treatment/disease prevention) | Yes                             |
|              |  | No                              |
| 5.           | Usage of specific antimicrobials                           | Tetracyclines                   |
|              |  | Fluoroquinolones                |
|              |  | Aminoglycosides                 |
|              |  | Sulphadiazine                   |
|              |  | Tylosin                         |
| 6.           | Precautions used at the entry of farm                      | Foot dip                        |
|              |  | Foot Rub(quick lime/burnt lime) |
|              |  | Gloves and mask                 |
| 7.           | Isolation of sick birds                                    | Yes                             |
|              |  | No                              |
| 8.           | Education level of respondent                              | Illiterate                      |
|              |  | ≤ matriculation                 |
|              |  | 10+2                            |
|              |  | Graduation                      |

### 3.4 MEDIA, REAGENTS AND BIOLOGICALS

#### 3.4.1 Buffered Peptone Water (HiMedia) (Catalogue No: M614)

##### Ingredients per liter of media

|                               |         |
|-------------------------------|---------|
| Proteose peptone              | 10.000  |
| Sodium Chloride               | 5.000   |
| Disodium phosphate, anhydrous | 3.500   |
| Potassium Hydrogen Phosphate  | 1.500   |
| Final pH                      | 7.2±0.2 |

20 g of media was added in 1000 ml of distilled water and heated to dissolve the medium completely. The contents were autoclaved at 15lbs pressure (121) for 15 minutes.

#### 3.4.2 Tetrathionate broth (HiMedia) (Catalogue No: MM032)

##### Ingredients per liter of media

|                     |        |
|---------------------|--------|
| Meat extract        | 0.900  |
| Peptone             | 4.500  |
| Yeast extract       | 1.800  |
| Sodium chloride     | 4.500  |
| Calcium carbonate   | 25.000 |
| Sodium thiosulphate | 40.700 |

77.40 g of media was suspended in 980 ml of distilled water and heated to boil. Cool the contents of media below 45-55°C and then 20ml iodine solution (6 grams of iodine and 5 grams of potassium iodide in 20 ml distilled water) were added to it. Mixed properly and transferred 10 ml quantities in sterile tubes.

#### 3.4.3 Rappaport Vassiliadis Medium (HiMedia) (Catalogue No: M883)

##### Ingredients per liter of media

|                                |         |
|--------------------------------|---------|
| Soya peptone                   | 4.500   |
| Sodium chloride                | 7.200   |
| Potassium dihydrogen phosphate | 1.440   |
| Magnesium chloride             | 36.000  |
| Malachite green                | 0.036   |
| Final pH ( at 25°C)            | 5.2±0.2 |

49.17 grams of media was added in 1000 ml distilled water and heated to dissolve completely. Autoclaved at 10 lbs pressure (115°C) and then transferred into sterilized tubes..

#### **3.4.4 Xylose-Lysine Deoxycholate Agar (XLD Agar) (HiMedia) (Catalogue No: M031)**

##### **Ingredients per liter of media**

|                         |         |
|-------------------------|---------|
| Yeast extract           | 3.000   |
| L-Lysine                | 5.000   |
| Lactose                 | 7.500   |
| Sucrose                 | 7.500   |
| Xylose                  | 3.500   |
| Sodium chloride         | 5.000   |
| Sodium deoxycholate     | 2.500   |
| Sodium thiosulphate     | 6.800   |
| Ferric ammonium citrate | 0.800   |
| Phenol red              | 0.080   |
| Agar                    | 15.000  |
| Final pH ( at 25°C)     | 7.4±0.2 |

56.68 g of media was mixed in 1000 ml of distilled water and heated the contents to boil with frequent agitation. Transferred to a water bath at 50°C and allowed to cool before pouring into sterile Petri plates.

#### **3.4.5 Hektoen Enteric Agar (HiMedia) (Catalogue No: M467)**

##### **Ingredients per liter of media**

|                         |        |
|-------------------------|--------|
| Proteose peptone        | 12.000 |
| Yeast extract           | 3.000  |
| Lactose                 | 12.000 |
| Sucrose                 | 12.000 |
| Salicin                 | 2.000  |
| Bile salts mixture      | 9.000  |
| Sodium chloride         | 5.000  |
| Sodium thiosulphate     | 5.000  |
| Ferric ammonium citrate | 1.500  |

|                  |         |
|------------------|---------|
| Acid fuchsin     | 0.100   |
| Bromothymol blue | 0.065   |
| Agar             | 15.000  |
| Final pH (25°C)  | 7.5±0.2 |

76.67 g of media was added in 1000 ml of distilled water and heated to dissolve. Allowed the contents to cool at 45-50°C. Mixed properly and poured into sterile Petri plates.

### **3.4.6 Triple Sugar Iron Agar (HiMedia) (Catalogue No: M021)**

#### **Ingredients per liter of media**

|                     |        |
|---------------------|--------|
| Peptone             | 10.000 |
| Tryptone            | 10.000 |
| Yeast extract       | 3.000  |
| HM Peptone          | 3.000  |
| Lactose             | 10.000 |
| Sucrose             | 10.000 |
| Dextrose (Glucose)  | 1.000  |
| Sodium chloride     | 5.000  |
| Ferrous sulphate    | 0.200  |
| Sodium thiosulphate | 0.300  |
| Phenol red          | 0.024  |
| Agar                | 12.000 |

64.52 g of media was added in 1000 ml of distilled water and heated to dissolve. Autoclaved the contents at 15 lbs pressure (121°C) for 15 minutes and transferred into sterilized tubes. Allowed the medium to rest in sloped form with a butt about 1 inch long.

### 3.4.7 Lysine Iron Agar (HiMedia) (Catalogue No: M377)

#### Ingredients per liter of media

|                         |        |
|-------------------------|--------|
| Peptone                 | 5.000  |
| Yeast extract           | 3.000  |
| Dextrose (Glucose)      | 1.000  |
| L-Lysine                | 10.000 |
| Ferric ammonium citrate | 0.500  |
| Sodium thiosulphate     | 0.040  |
| Bromocresol purple      | 0.020  |
| Agar                    | 15.000 |

34.56 g of media was added in 1000 ml distilled water and heated to boil for proper mixing of contents. It is then autoclaved at 15lbs pressure (121°C) for 15 minutes and transferred into sterile tubes. Keep the tubes in slanted position to form slants with deep butts.

### 3.4.8 Urea Agar Base (Christensen) (HiMedia) (Catalogue No: M377)

#### Ingredients per liter of media

|                                |        |
|--------------------------------|--------|
| Peptone                        | 1.000  |
| Dextrose (Glucose)             | 1.000  |
| Sodium chloride                | 5.000  |
| Disodium hydrogen phosphate    | 1.200  |
| Potassium dihydrogen phosphate | 0.800  |
| Phenol red                     | 0.012  |
| Agar                           | 15.000 |

Added 24.01 g of media in 950 ml of distilled water and heated to boil for complete mixing. Sterilized the contents at 10 lbs pressure (115°C) for 20 minutes. Allowed to cool below 45-50°C and added 50 ml of sterile 40% urea solution and mix properly. Transferred into sterile tubes and kept in slanting position.

### 3.5 Isolation of *Salmonella*

The samples were processed as per the Bacteriological Analytical Manual (BAM) (2020). Poultry droppings (25g) were added in 225 ml sterile buffered peptone water, mixed well and incubated at 35°C for 24 hours. From the mixture, 0.1 ml inoculum was transferred to 10 ml Rappaport–Vassiliadis broth and incubated at 42°C for 24 hours while 1 ml inoculum was transferred to 10 ml Tetrathionate broth and incubated at 43°C for 24 hours. After incubation, the inoculum from each broth was streaked on Hektoen enteric agar (HE) and xylose lysine deoxycholate agar (XLD) plates followed by incubation at 35°C for 24 hours. Blue green colonies with black center and pink colonies with black center on HE and XLD, respectively, were suspected for *Salmonella*. The suspected colonies were further streaked on nutrient agar for purification. The purified colonies were subjected to phenotypic identification and biochemical tests to identify *Salmonella* isolates.

### **3.6 Phenotypic and Biochemical identification of *Salmonella* isolates**

The suspected isolates of *Salmonella* were purified on nutrient agar. The purified colonies were subjected to Gram staining and biochemical tests for identification and confirmation of *Salmonella*.

#### **3.6.1 Gram staining**

Grease free slide was taken and a thin smear of purified colony in sterilized normal saline solution was prepared and heat fixed. For staining, crystal violet stain (HiMedia) was poured for 1 min followed by washing under tap water. After that, Gram's iodine (HiMedia) was poured for 1 min followed by washing with water. Further the smear was decolorized by acid alcohol for 15-20 seconds followed washing and counterstaining with safranin (HiMedia) for 30 sec and washing under tap water. The smears were air dried and visualized under microscope. The bacteria appearing pink with rod shaped morphology are Gram negative. *Salmonella* are Gram negative bacteria.

#### **3.6.2 Biochemical analysis**

The Gram negative isolates were further subjected to following biochemical reactions to identify *Salmonella* isolates:

#### **3.6.3 Catalase test**

The colony from nutrient agar was picked up with the help of a wooden toothpick and mixed with 3% hydrogen peroxide solution on a clean grease free slide. Positive test is indicated by immediate appearance of gas bubbles. *Salmonella* is a catalase positive bacterium.

#### **3.6.4 Oxidase test**

A loopful of purified bacterial colony was picked up from Nutrient agar with the help of a wooden tooth pick and rubbed on oxidase disc (HiMedia). Positive oxidase test is indicated by presence of dark blue colour within 20 seconds while no colour development or late colour development is a negative reaction. *Salmonella* is an oxidase negative bacterium.

#### **3.6.5 Indole test**

5ml of tryptone water broth (HiMedia) was inoculated with small growth of test organisms at 35°C for 24 hours followed by addition of 0.2-0.3 ml Kovacs' reagent. Appearance of deep red colour at the surface of the broth is indicative of positive result

while appearance of yellowish-brown colour indicates negative reaction. *Salmonella* is indole negative bacterium.

### **3.6.6 Methyl-Red (MR) test**

A small growth of suspected bacteria colony was inoculated in 5 ml glucose phosphate peptone broth (Hi-media) followed by incubation at 35°C for 96 hours. 5-6 drops of methyl red indicator were added. Appearance of diffuse red colour in the medium is a positive reaction. *Salmonella* is positive for methyl red test.

### **3.6.7 Voges-Proskauer (VP) test**

Presumed colony was inoculated in 5 ml glucose phosphate peptone broth (Hi-media) followed by incubation at 37°C for 48 hours. After that, 0.6 ml of alpha naphthol and 0.2ml of 40% KOH solution were added. Appearance of cherry red colour is a positive reaction. *Salmonella* have negative reaction in this test.

### **3.6.8 Citrate utilization test**

The suspected colony was inoculated on Simmon's citrate agar (Hi-media) using stab wire by streaking slant first and then stabbing the butt followed by incubation at 35°C for 96 hours. Change in colour from green to blue is positive for this test. *Salmonella* is positive for citrate utilization test.

### **3.6.9 Triple sugar iron test (TSI)**

TSI agar (HiMedia) slants were prepared and test organism was inoculated by stabbing the butt and streaking the slant followed by incubated at 35°C for 24 hours. Appearance of red slant and yellow butt with blackening of agar is positive for *Salmonella* in TSI test.

### **3.6.10 Lysine Iron Agar**

A small growth of suspected colony was inoculated in LIA slant with the help of a stab wire by stabbing the butt twice and then streaking the slant of LIA. Incubated the test culture at 35°C for 24 hours. Purple reaction in the butt is positive for LIA test for *Salmonella*.

### **3.6.11 Urease test**

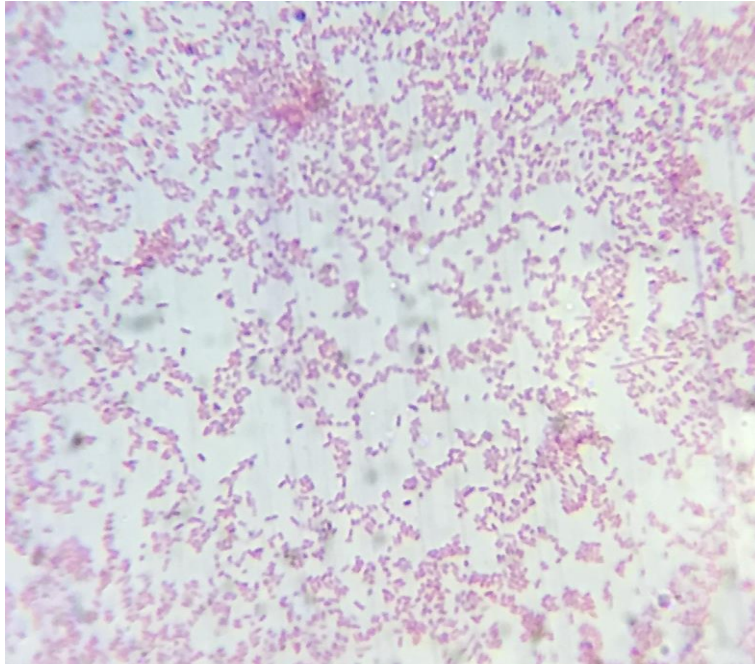
Test colony was inoculated in urease medium with incubation at 35°C for 24 hours. Appearance of purple red colour after incubation is a positive reaction. *Salmonella* is a urease negative bacteria.



**Plate 2: Typical blue green colonies of *Salmonella* with black Centre on HE agar plate**



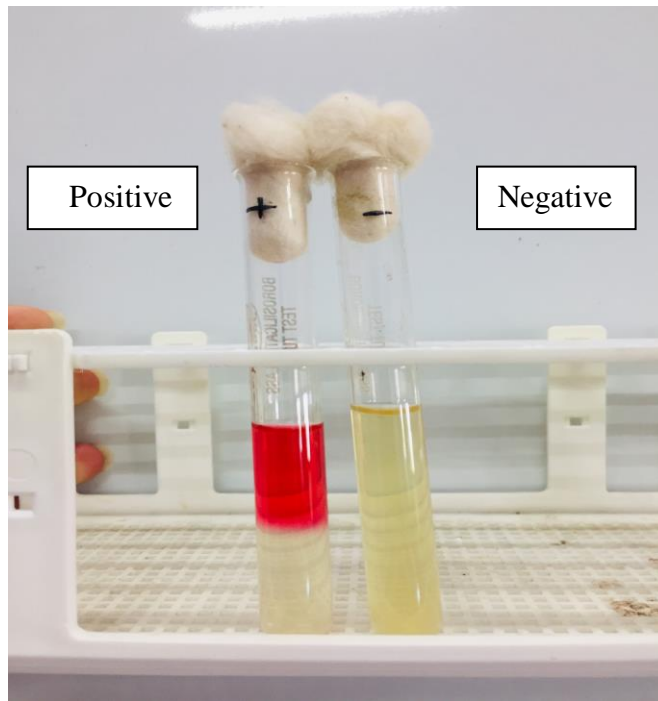
**Plate 3: Typical pink colonies of *Salmonella* with black Centre on XLD agar plate**



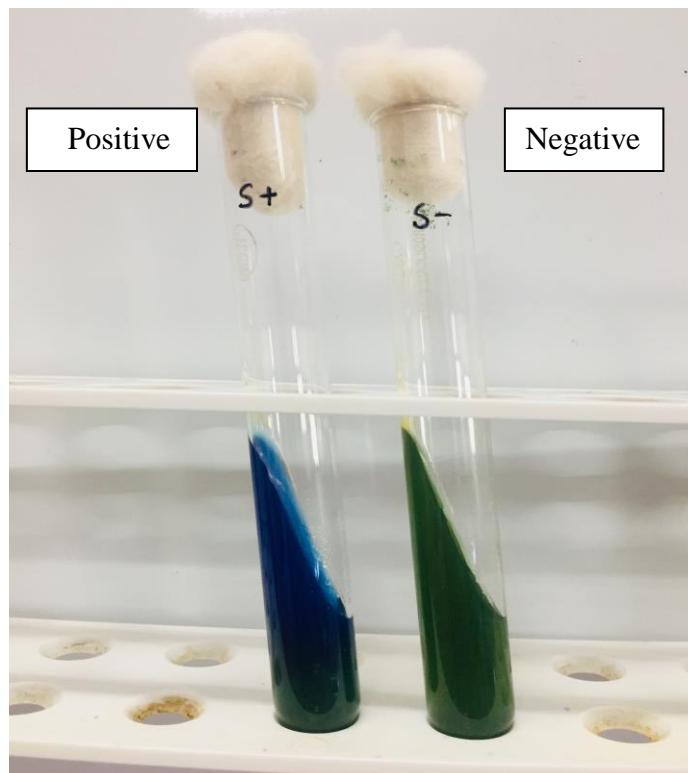
**Plate 4: Gram staining of *Salmonella* (1000X)**



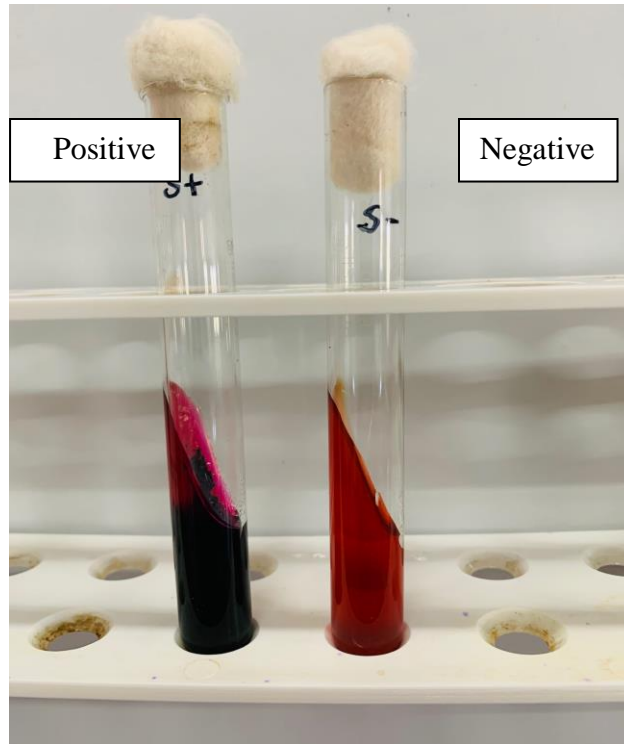
**Plate 5: Catalase test for *Salmonella***  
(*Salmonella* is catalase positive)



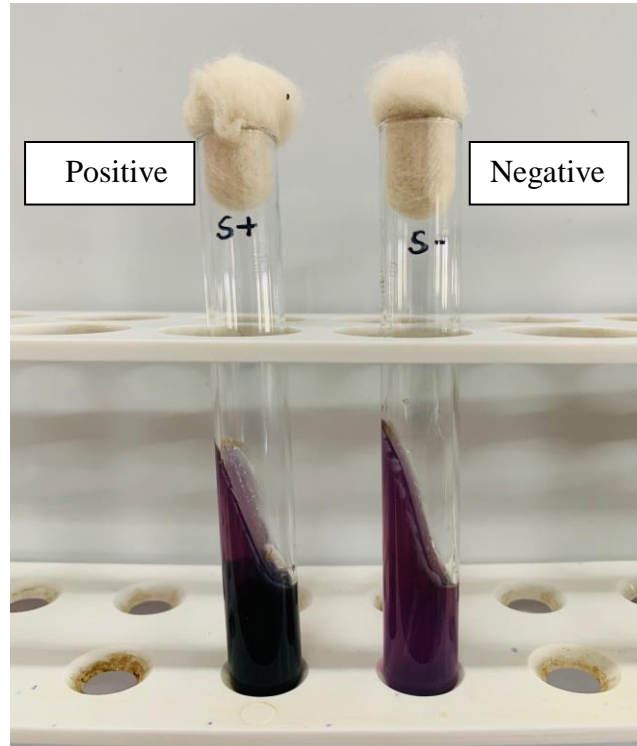
**Plate 6: Methyl red test for *Salmonella***  
(*Salmonella* is Methyl red positive)



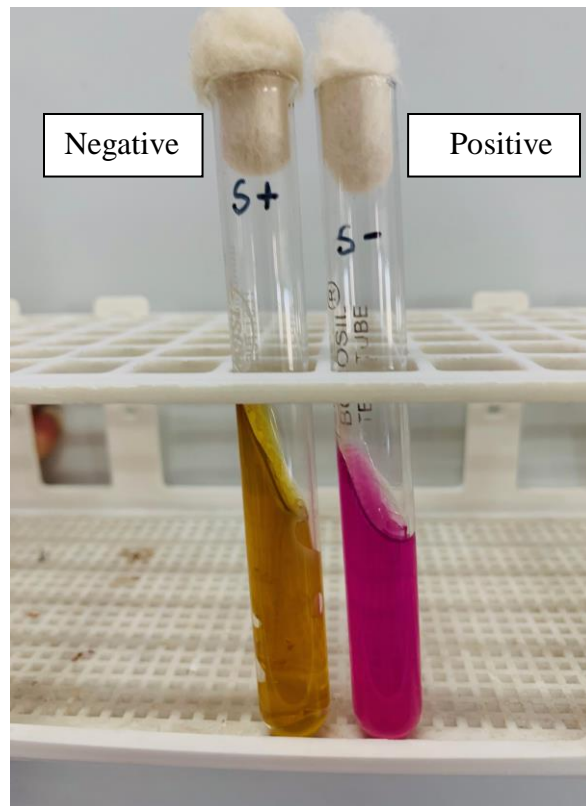
**Plate 7: Citrate Utilization test for *Salmonella***  
(*Salmonella* is Citrate utilization positive)



**Plate 8: Triple sugar Iron test for *Salmonella***  
(*Salmonella* is TSI positive)



**Plate 9: Lysine Iron Agar test for *Salmonella***  
(*Salmonella* is LIA positive)



**Plate 10: Urease test for *Salmonella***  
(*Salmonella* is Urease test Negative)



**Plate 11: Growth of *Salmonella* on Nutrient agar plate**

### **3.7 Molecular confirmation of *Salmonella* isolates**

*Salmonella* isolates confirmed by phenotypic methods were further subjected to PCR for *invA* gene to confirm *Salmonella* genotypically. The *invA* gene is *Salmonella* specific with unique sequences. It is a suitable PCR target having well established diagnostic application (Pathmanathan *et al.*, 2003). The gene is recognized internationally for detection of *Salmonella* (Jamshidi *et al.*, 2008).

#### **3.7.1 Preparation of DNA template**

DNA template was prepared by boiling method. *Salmonella* isolates were streaked on nutrient agar and incubated at 37°C for 24 h. On the following day, in 500 µl molecular grade water, peanut size inoculum from grown colony was taken. The microcentrifuge tube was kept in a hot water bath (temperature 95°C) for 10 minutes followed by centrifugation at 15,000 rpm for 5 minutes. The supernatant was transferred in a sterilized microcentrifuge tube and was preserved at -20°C to be used as template DNA in PCR assays.

#### **3.7.2 Standardization of PCR protocol for *invA* gene**

The *Salmonella* strain maintained at Division of Veterinary Public Health and Epidemiology, FVSC & AH, SKUAST-Jammu, India was used in PCR standardization. The details of standardized PCR for *invA* gene are given Table 4. PCR assay was run on Biorad T100 thermocycler.

**Table 4: PCR details for detection of *invA* gene**

|  |   |
|--|---|
| <p style="text-align: center;">Primers<br/>(Rahn <i>et al.</i>,1992)</p>           | <p style="text-align: center;">Forward primer (<i>invA</i> forward)<br/>5'-GTGAAATTATCGCCACGTTTCGGGCAA-3'</p> <p style="text-align: center;">Reverse Primer(<i>invA</i> reverse)<br/>5'TCATCGCACCGTCAAAGG AACC-3'</p>   |
| <p style="text-align: center;">PCR reaction mixture<br/>(25 <math>\mu</math>l)</p> | <p style="text-align: center;">1x PCR Buffer (Promega, USA)<br/>MgCl<sub>2</sub> –1.5 mM<br/>dNTP each – 200 <math>\mu</math>M<br/>Primer each – 0.4 <math>\mu</math>M<br/>Taq polymerase–1 unit<br/>Template -2 <math>\mu</math>l</p>  |
| <p style="text-align: center;">PCR conditions</p>                                  | <p style="text-align: center;">Initial denaturation -94<sup>o</sup>C for 3 min<br/>30 cycles of denaturation - 94<sup>o</sup>C for 1 min, annealing -<br/>51<sup>o</sup>C for 1min and extension -72<sup>o</sup>C for 1 min<br/>Final extension - 72<sup>o</sup>C for 5 min</p> |

### 3.7.3 Analysis of PCR Products

The amplified products were subjected to Agarose gel electrophoresis by using 1.5% agarose gel in 1x TAE buffer with ethidium bromide @ 0.5 $\mu$ g/ml gel. The electrophoresis was carried out at 8V/cm in Hoefer electrophoresis system with 100 bp gene ruler as marker and photographed with Eppendorfgel documentation system.



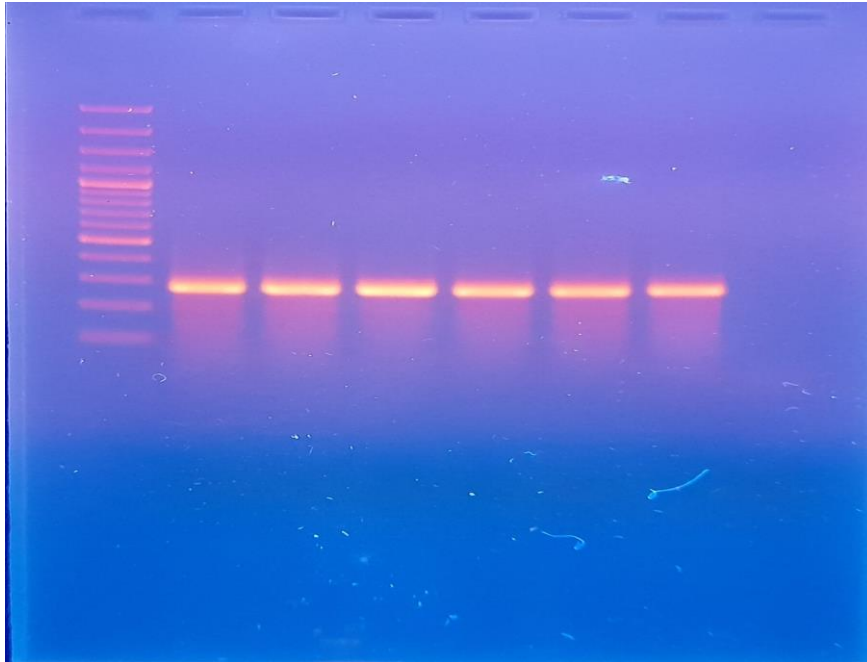
(a)



(b)

**Plate 12: Preparation of DNA template for PCR**

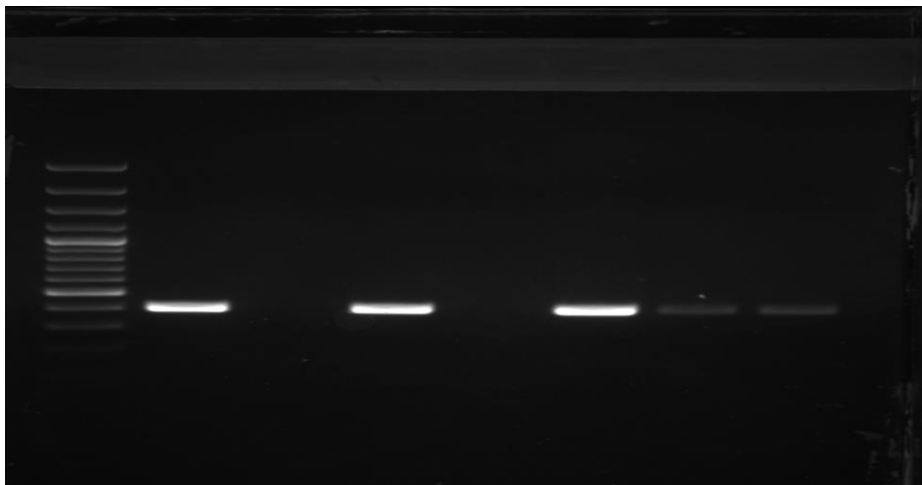
M 1 2 3 4 5 6 7



**Plate 13: PCR amplification of *invA* gene**

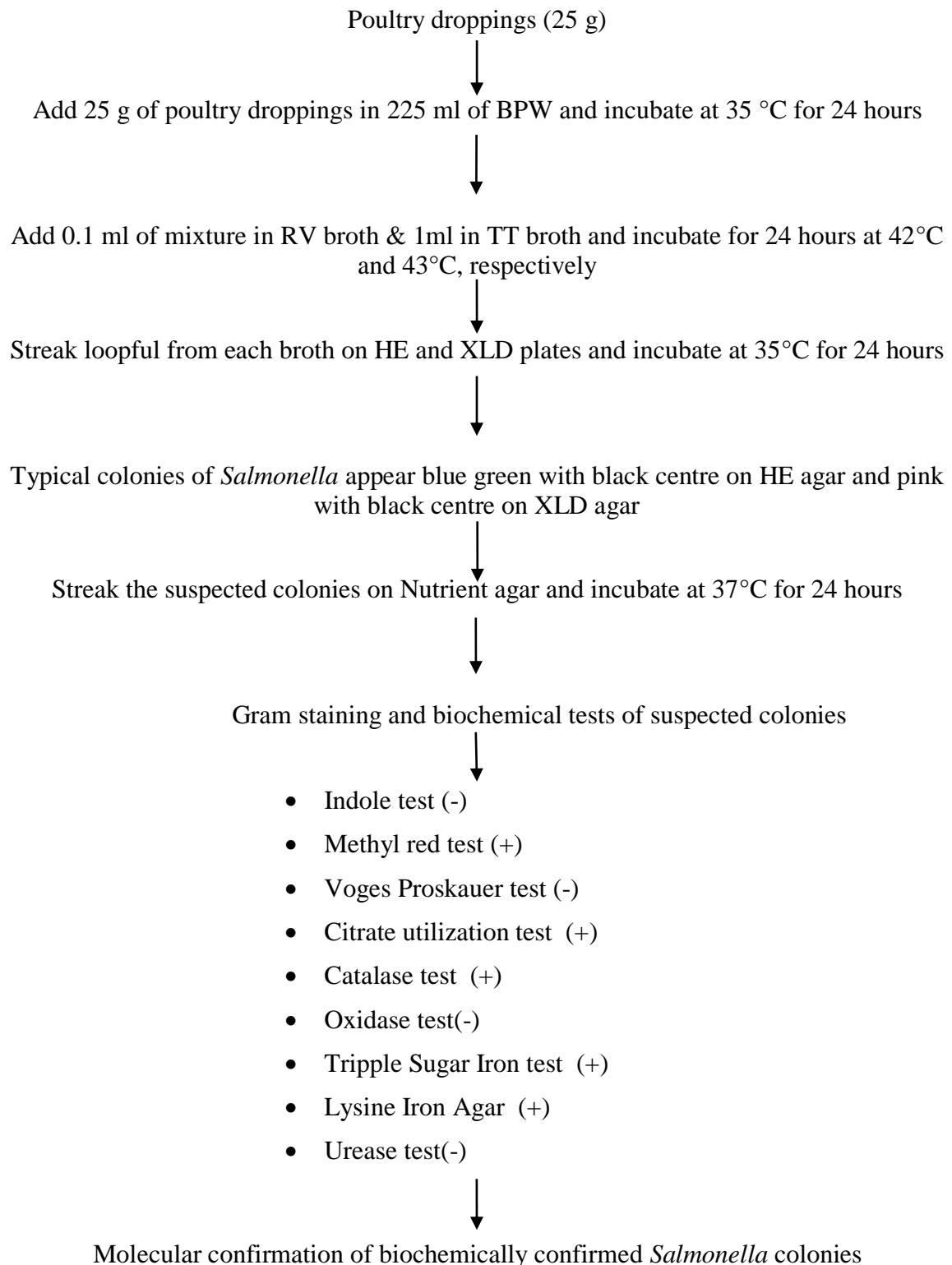
Lane M: Marker; 1: Positive Control; 2-6: Positive isolates; 7: Negative control

M 1 2 3 4 5 6 7



**Plate 14: PCR amplification of *spy* gene**

Lane M: Marker; 1: Positive Control; 3, 5, 6 & 7: Positive isolates; 2: Negative Control; 4: Negative isolate

**FLOWDIAGRAM FOR ISOLATION OF *Salmonella* FROM POULTRY DROPPINGS**

### 3.8 Serotyping of *Salmonella* isolates

*Salmonella* isolates obtained in the study were serotyped using serological tests and PCR

#### 3.8.1 Serotyping of *Salmonella* isolates by serological testing

For serotyping of confirmed *Salmonella* isolates using serological tests, *Salmonella* isolates were sent to National *Salmonella* Center (Vety.), Division of Bacteriology and Mycology, ICAR-Indian Veterinary Research Institute, Bareilly, India. National *Salmonella* Center (Vety.) is the national reference laboratory dealing with *Salmonella* serotyping.

#### 3.8.2 PCR assay for *Salmonella* serotyping

*Salmonella* isolates were also subjected to uniplex PCRs for serotyping of *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum*.

#### 3.8.3 PCR assays to identify *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum*

The primers used for *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum* are given in Table 5. For *S. Typhimurium*, *spy* gene fragment was used for identification which encodes for a specific bacterial periplasmic protein, whereas for *S. Enteritidis*, a 304 bp fragment of the *SdfI* gene, related to invasiveness and infection of poultry and eggs was used. In case of *S. Gallinarum*, 187 bp *rfbS* allele specific gene was used. PCR reaction mixture and PCR conditions for each PCR were same and are depicted in Table 6.

**Table 5: Primers used in PCR detection of *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum***

| Serotype              | Primers  | PCR Product size | Reference                       |
|-----------------------|--|------------------|---------------------------------|
| <i>S. Typhimurium</i> | Forward primer<br>5'-TTGTTCACTTTTTACCCCTGAA-3'<br>Reverse Primer<br>5'-CCCTGACAGCCGTTAGATATT-3'      | 401 bp           | De Freitas <i>et al.</i> (2010) |
| <i>S. Enteritidis</i> | Forward primer<br>5'-TGTGTTTTATCTGATGCAAGAGG-3'<br>Reverse primer<br>5'-TGAACACTACGTTTCGTTCTTCTGG 3' | 304 bp           | De Freitas <i>et al.</i> (2010) |
| <i>S. Gallinarum</i>  | Forward primer<br>5'-GTATGGTTATTAGACGTTGTT-3'<br>Reverse Primer<br>5'-TATTCACGAATTGATATACTC-3'       | 187 bp           | Shah <i>et al.</i> (2005)       |

**Table 6: PCR reaction mixture and conditions used in detection of *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum***

|  |  |
|--|--|
| <p style="text-align: center;">PCR reaction mixture<br/>(25 <math>\mu</math>l)</p> | <p style="text-align: center;">1x PCR Buffer (Promega, USA)<br/><br/>MgCl<sub>2</sub> –1.5 mM<br/><br/>dNTP each – 200 <math>\mu</math>M<br/><br/>Primer each – 0.4 <math>\mu</math>M<br/><br/>Taq polymerase–1 unit<br/><br/>Template -3 <math>\mu</math>l</p>                                  |
| <p style="text-align: center;">PCR conditions</p>                                  | <p style="text-align: center;">Initial denaturation -94<sup>o</sup>C for 3 min<br/><br/>35 cycles of denaturation - 94<sup>o</sup>C for 30sec,<br/>annealing - 57<sup>o</sup>C for 1min and extension -<br/>72<sup>o</sup>C for 1.5 min<br/><br/>Final extension - 72<sup>o</sup>C for 5 min</p> |

### 3.9 Antibiotic Sensitivity assay of *Salmonella* isolates

In this procedure, one pure colony from each confirmed isolate was transferred in 10 ml Muller Hinton broth followed by incubation for growth at 37°C overnight. Next morning, the growth was adjusted to 0.5 McFarland standard. The adjusted growth was swabbed on the Muller Hinton agar. The antibiotic discs of known concentrations (Table 7) were placed on the agar by maintaining suitable distance between the discs and incubated at 37°C for 16 to 18 hours. Results were measured by measuring the zone diameter and interpreted in reference to antimicrobial susceptibility testing for Enterobacterales as per Clinical and Laboratory Standards Institute guidelines (CLSI, 2021).

**Table 7: Antimicrobials used in the study and their interpretation criteria**

| S. No | Antimicrobial Agent            | Antimicrobial class         | Disc content | Zone Diameter Breakpoints (nearest whole mm) |       |              |
|-------|--------------------------------|-----------------------------|--------------|--|-------|--------------|
|       |                                |                             |              | S ( $\geq$ )                                 | I     | R ( $\leq$ ) |
| 1.    | Amoxyclav (AMC)                | Penicillin/ $\beta$ -lactam | 30mcg        | 18   | 14-17 | 13           |
| 2.    | Ampicillin (AMP)               | $\beta$ -lactam             | 10mcg        | 17   | 14-16 | 13           |
| 3.    | Cefoperazone (CPZ)             | Cephalosporin               | 75mcg        | 21   | 16-20 | 15           |
| 4.    | Cefotaxime (CTX)               | Cephalosporin               | 30mcg        | 26   | 23-25 | 22           |
| 5.    | Ceftriaxone (CTR)              | Cephalosporin               | 30mcg        | 23   | 20-22 | 19           |
| 6.    | Chloramphenicol (C)            | Amphenicol                  | 30mcg        | 18   | 16-17 | 12           |
| 7.    | Ciprofloxacin (CIP)            | Fluoroquinolone             | 5mcg         | 31   | 21-30 | 20           |
| 8.    | Doxycycline Hydrochloride (DO) | Tetracycline                | 30mcg        | 14   | 11-13 | 10           |
| 9.    | Gentamicin (GEN)               | Aminoglycoside              | 10mcg        | 15   | 13-14 | 12           |
| 10.   | Nalidixic Acid (NA)            | Quinolone                   | 30mcg        | 19   | 14-18 | 13           |
| 11.   | Sulfadiazine (SZ)              | Sulphonamide                | 100mcg       | 17   | 13-16 | 12           |
| 12.   | Tetracycline(TE)               | Tetracycline                | 30mcg        | 15   | 12-14 | 11           |

## Chapter-4

# *Results*

**4.1 Objective 1.** To study the distribution and serotype diversity of *Salmonella enterica* in poultry farms of Jammu.

To determine distribution and serotype diversity of *Salmonella enterica*, a total of 101 samples of poultry droppings from 30 poultry farms of Jammu region were collected and processed for isolation of *Salmonella*. Four districts viz., Jammu, Samba, Kathua and Udhampur were sampled. The samples were processed for isolation as per BAM followed by molecular characterization. The confirmed *Salmonella* isolates were serotyped at National *Salmonella* Centre (Vety.), IVRI, Bareilly and by PCR.

Out of 30 farms sampled, 4 farms were in contract with a poultry products company, one farm was governmental and 25 farms were independent in operations. District-wise, out of 30 farms, 16 farms were of Jammu district followed by Kathua (n=9), Udhampur (n=3) and Samba (n=2). All farms sampled were rearing broilers and were having Cobb 430 breed.

On processing the 101 samples of poultry droppings of 30 poultry farms, 5 poultry farms were positive for *Salmonella* with the apparent prevalence of 16.67% of salmonellosis among poultry farms. From these 5 positive farms, 19 *Salmonella* isolates were obtained. All positive farms were having independent operations and none of farms on contract with private company was positive for salmonellosis. The only government farm sampled in the study was also negative for salmonellosis. Out of these 5 positive farms, two farms were from Jammu district and one from Samba, Kathua and Udhampur district each. District-wise, the apparent prevalence of salmonellosis was 12.5%, 11.11%, 33.33% and 50% in Jammu, Kathua, Udhampur and Samba districts, respectively (Table 8).

**Table 8: Apparent prevalence of salmonellosis in poultry farms (n=30)**

| S. No | Farm Location (District) | Poultry farms analysed | No. of Farms Positive (%) | No of isolates obtained |
|-------|--------------------------|------------------------|---------------------------|-------------------------|
| 1     | Jammu                    | 16                     | 2 (12.5%)                 | 3                       |
| 2     | Kathua                   | 9                      | 1 (11.11%)                | 4                       |
| 3     | Udhampur                 | 3                      | 1 (33.33%)                | 7                       |
| 4     | Samba                    | 2                      | 1 (50%)                   | 5                       |
|       | <b>Total</b>             | <b>30</b>              | <b>5 (16.67%)</b>         | <b>19</b>               |

These isolates were serotyped at National *Salmonella* Centre (Veterinary), ICAR-IVRI, Izzatnagar. Also, the isolates were serotyped by uniplex PCRs for *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum*. On serotyping of 19 *Salmonella* isolates obtained from 5 salmonellosis positive poultry farms, four serotypes viz., *S. Kentucky*, *S. Infantis*, *S. Agona* and *S. Typhimurium* were obtained.

Among the serotypes obtained, *S. Kentucky* was obtained from 2 farms of Jammu district. *S. Infantis* was obtained from one farm of Samba district. *S. Agona* was isolated from one farm of Kathua district. *S. Typhimurium* was the isolation from one farm of Udhampur district (Table 9).

**Table 9: Details of *Salmonella* serotypes in poultry farms (n=30) of Jammu region**

| Farm No.                | District (no of farms) | Block      | Farm Location     | Facility type | Age of birds (Days) | <i>Salmonella enterica</i> serotype isolated | No. of isolates            |
|-------------------------|------------------------|------------|-------------------|---------------|---------------------|--|----------------------------|
| 1                       | Jammu (n=16)           | Satwari    | Balicharana       | Government    | 60                  | -  | -                          |
| 2                       |                        | Bhalwal    | Barnai            | Contracted    | 22                  | -  | -                          |
| 3                       |                        | Akhnoor    | Mandrian 1        | Contracted    | 20                  | -  | -                          |
| 4                       |                        |            | Mandrian 2        | Contracted    | 20                  | -  | -                          |
| 5                       |                        |            | Barui             | Contracted    | 13                  | -  | -                          |
| 6                       |                        | R. S. Pura | Kadyal            | Independent   | 15                  | Kentucky                                     | 2                          |
| 7                       |                        |            | Gazipur 1         | Independent   | 7                   | -  | -                          |
| 8                       |                        |            | Gazipur 2         | Independent   | 7                   | -  | -                          |
| 9                       |                        |            | RangpurMalani an1 | Independent   | 14                  | -  | -                          |
| 10                      |                        |            | RangpurMalani an2 | Independent   | 14                  | -  | -                          |
| 11                      |                        |            | Chohala           | Independent   | 20                  | -  | -                          |
| 12                      |                        |            | Satrayan Kalan    | Independent   | 6                   | Kentucky                                     | 1                          |
| 13                      |                        |            | Chak Roi          | Independent   | 20                  | -  | -                          |
| 14                      |                        |            | Darsopur          | Independent   | 25                  | -  | -                          |
| 15                      |                        |            | Magowali          | Independent   | 20                  | -  | -                          |
| 16                      |                        | Chak Roi   | Independent       | 21            | -                   | -  |                            |
| 17                      | Samba (n=2)            | Samba      | Sangwal 1         | Independent   | 18                  | Infantis                                     | 5                          |
| 18                      |                        |            | Sangwal 2         | Independent   | 18                  | -  | -                          |
| 19                      | Kathua (n=9)           | Kathua     | Muthi khurd       | Independent   | 33                  | -  | -                          |
| 20                      |                        |            | Virpur            | Independent   | 15                  | -  | -                          |
| 21                      |                        |            | Mahi chak         | Independent   | 25                  | -  | -                          |
| 22                      |                        |            | Jandore 1         | Independent   | 14                  | -  | -                          |
| 23                      |                        |            | Jandore 2         | Independent   | 25                  | -  | -                          |
| 24                      |                        |            | Barwal            | Independent   | 14                  | -  | -                          |
| 25                      |                        |            | Lachipur          | Independent   | 14                  | -  | -                          |
| 26                      |                        |            | Palli morh        | Independent   | 3                   | Agona  | 4                          |
| 27                      |                        |            | Nangal            | Independent   | 22                  | -  | -                          |
| 28                      | Udhampur (n=3)         | Ramnagar   | Ramnagar 1        | Independent   | 25                  | Typhimurium                                  | 7                          |
| 29                      |                        |            | Ramnagar 2        | Independent   | 25                  | -  | -                          |
| 30                      |                        |            | Thaplal           | Independent   | 19                  | -  | -                          |
| <b>Total Farms = 30</b> |                        |            |                   |               |                     | <b>No. of serotypes – 4</b>                  | <b>Total isolates = 19</b> |

## **4.2 Objective 2: To determine the antibiotic sensitivity pattern of *Salmonella enterica* isolates.**

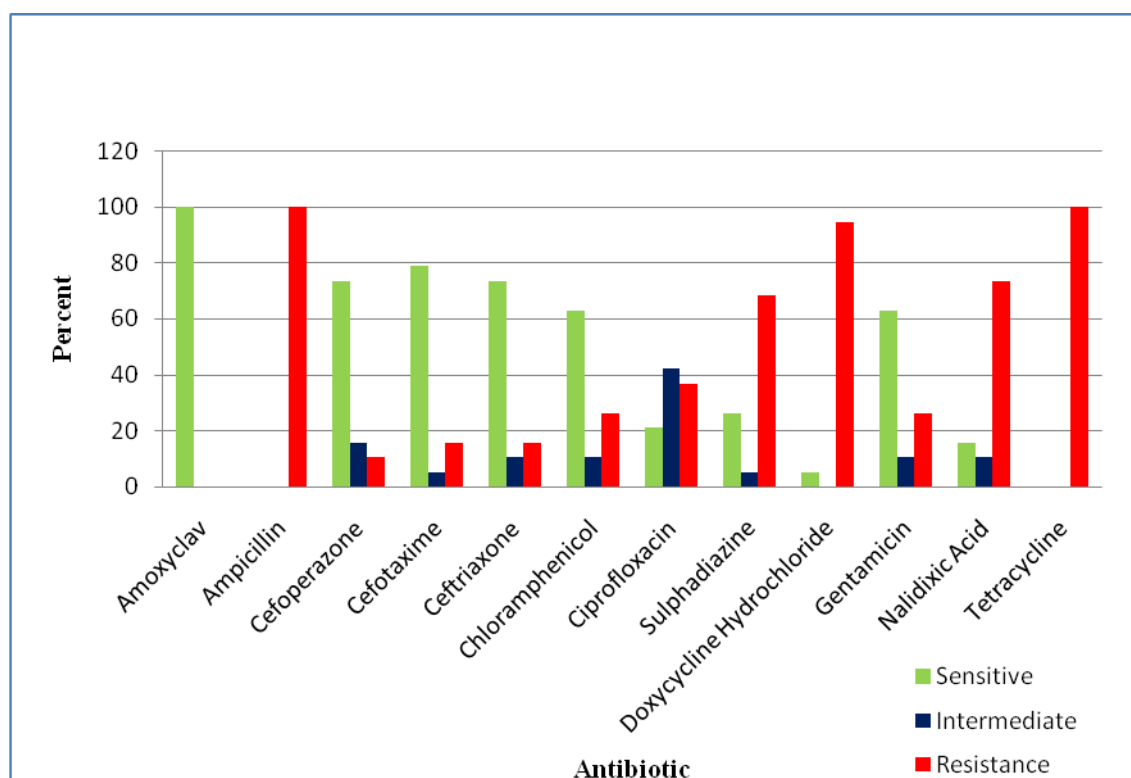
The *Salmonella* isolates (n=19) obtained in objective 1 were analyzed for their antibiotic sensitivity pattern by disc diffusion assay (Kirby-Bauer disc diffusion method). Twelve antibiotics were used. The results were interpreted as per CLSI (2021). The selection of antibiotics was primarily based upon the antibiotics used in the poultry farms sampled.

### **4.2.1 Overall Antibiotic sensitivity pattern of *Salmonella* isolates**

In antibiotic sensitivity assay, all isolates studied were found to be multi-drug resistant. Of 19 *Salmonella* isolates, only 2 (10.52%) isolates were moderately resistant (resistance to 2-4 drugs) and rest of the 17 (89.47%) isolates showed extreme drug resistance (resistance to more than 4 drugs). All isolates were resistant to ampicillin and tetracycline (100%). Further, resistance pattern in descending order was doxycycline (94.73%) followed by nalidixic acid (73.64%), sulphadiazine (68.42%), gentamicin (26.31%), chloramphenicol (26.31%), ciprofloxacin (36.84%), cefotaxime (15.78%), ceftriaxone (15.78%) and cefoperazone (10.53). However, all 19 isolates were sensitive to amoxyclav (Table 10, Fig. 1). The results for each isolate are shown in Table 11.

**Table 10: Overall Antibiotic sensitivity pattern of *Salmonella* isolates (n=19)**

| S.No. | Antibiotic (Abbreviation)      | Concentration | Sensitive   | Intermediate | Resistant   |
|-------|--------------------------------|---------------|-------------|--------------|-------------|
| 1.    | Amoxyclav (AMC)                | 30mcg         | 19 (100%)   | 0.0%         | 0.0%        |
| 2.    | Ampicillin (AMP)               | 10mcg         | 0.0%        | 0.0%         | 19 (100%)   |
| 3.    | Cefoperazone (CPZ)             | 75mcg         | 14 (73.68%) | 3 (15.79%)   | 2 (10.53%)  |
| 4.    | Cefotaxime (CTX)               | 30mcg         | 15 (78.94%) | 1 (5.26%)    | 3 (15.79%)  |
| 5.    | Ceftriaxone (CTR)              | 30mcg         | 14 (73.69%) | 2 (10.53%)   | 3 (15.79%)  |
| 6.    | Chloramphenicol (C)            | 30mcg         | 12 (63.16%) | 2 (10.53%)   | 5 (26.31%)  |
| 7.    | Ciprofloxacin (CIP)            | 5mcg          | 4 (21.05%)  | 8 (42.11%)   | 7 (36.84%)  |
| 8.    | Sulphadiazine (SZ)             | 100mcg        | 5 (26.31%)  | 1 (5.26%)    | 13 (68.42%) |
| 9.    | Doxycycline Hydrochloride (DO) | 30mcg         | 1 (5.26%)   | 0.0%         | 18 (94.74%) |
| 10.   | Gentamicin (GEN)               | 10mcg         | 12 (63.16%) | 2 (10.53%)   | 5 (26.31%)  |
| 11.   | Nalidixic Acid (NA)            | 30mcg         | 3 (15.79%)  | 2 (10.53%)   | 14 (73.68%) |
| 12.   | Tetracycline (TE)              | 30mcg         | 0.0%        | 0.0%         | 19 (100%)   |

**Fig.:** Bar diagram depicting overall antibiotic sensitivity pattern of *Salmonella* isolates (n=19)

**Table 11: Antibiotic sensitivity pattern of individual *Salmonella* isolate against 12 antibiotics**

| S.NO | Isolate ID | <i>Salmonella</i> Serotype | Farm No. | AMC | AMP | CPZ | CTX | CTR | C | CIP | SZ | DO | GEN | NA | TE |
|------|------------|----------------------------|----------|-----|-----|-----|-----|-----|---|-----|----|----|-----|----|----|
| 1    | S1         | Infantis                   | 17       | S   | R   | S   | S   | S   | S | I   | R  | R  | I   | R  | R  |
| 2    | S2         | Infantis                   | 17       | S   | R   | S   | S   | S   | S | I   | R  | R  | S   | R  | R  |
| 3    | S3         | Infantis                   | 17       | S   | R   | S   | S   | S   | S | I   | R  | R  | I   | R  | R  |
| 4    | S4         | Infantis                   | 17       | S   | R   | S   | S   | S   | S | I   | R  | R  | S   | R  | R  |
| 5    | S5         | Infantis                   | 17       | S   | R   | S   | S   | S   | S | I   | R  | R  | S   | R  | R  |
| 6    | S6         | Agona                      | 26       | S   | R   | S   | S   | I   | R | R   | R  | R  | S   | R  | R  |
| 7    | S7         | Agona                      | 26       | S   | R   | S   | S   | I   | I | R   | R  | R  | S   | R  | R  |
| 8    | S8         | Agona                      | 26       | S   | R   | I   | S   | S   | S | R   | R  | R  | S   | R  | R  |
| 9    | S9         | Agona                      | 26       | S   | R   | I   | S   | S   | S | R   | R  | R  | S   | R  | R  |
| 10   | 3A         | Kentucky                   | 6        | S   | R   | R   | R   | R   | R | R   | R  | R  | R   | R  | R  |
| 11   | 4A         | Kentucky                   | 6        | S   | R   | R   | I   | S   | R | R   | S  | R  | R   | R  | R  |
| 12   | 9A         | Kentucky                   | 12       | S   | R   | S   | R   | R   | S | S   | R  | S  | S   | S  | R  |
| 13   | T1         | Typhimurium                | 28       | S   | R   | S   | S   | S   | I | I   | S  | R  | S   | S  | R  |
| 14   | T2         | Typhimurium                | 28       | S   | R   | I   | S   | S   | S | R   | S  | R  | S   | S  | R  |
| 15   | T3         | Typhimurium                | 28       | S   | R   | S   | S   | S   | S | I   | S  | R  | S   | I  | R  |
| 16   | T4         | Typhimurium                | 28       | S   | R   | S   | S   | S   | R | S   | R  | R  | R   | R  | R  |
| 17   | T5         | Typhimurium                | 28       | S   | R   | S   | R   | R   | R | I   | R  | R  | S   | I  | R  |
| 18   | T6         | Typhimurium                | 28       | S   | R   | S   | S   | S   | S | S   | I  | R  | R   | R  | R  |
| 19   | T7         | Typhimurium                | 28       | S   | R   | S   | S   | S   | S | S   | S  | R  | R   | R  | R  |

S-sensitive, I-intermediate, R-resistant

#### 4.2.2 Antibiotic sensitivity pattern of specific *Salmonella* serotype

Three isolates of *S. Kentucky* from 2 poultry farms [farm 6 (n=2) and farm 12 (n=1)] of R.S.Pura block of Jammu district were obtained. The isolates from two farms differ in their antibiotic sensitivity pattern (Table 12). The two isolates from farm 6 were resistant to majority of antibiotics used; these isolates differ in their sensitivity against 3<sup>rd</sup> generation cephalosporins. The only isolate from farm 12 showed sensitivity towards ciprofloxacin, doxycycline, gentamicin, nalidixic acid and sulphadiazine, unlike the isolates of farm 2. Overall, *S. Kentucky* isolates were 100% resistant to ampicillin and tetracycline followed by doxycycline, nalidixic acid, sulphadiazine, gentamicin, chloramphenicol, ciprofloxacin, cefotaxime, ceftriaxone and cefoperazone with 66.66% resistance for each antibiotic (Table 13).

*S. Agona* (n=4 from farm 26) isolates showed complete resistance towards ampicillin, ciprofloxacin, sulphadiazine, doxycycline nalidixic acid and tetracycline while only one isolate showed resistance towards chloramphenicol. *S. Agona* isolates were sensitive towards gentamicin and amoxyclav. For cephalosporins, pattern varied from sensitive to intermediate.

In *S. Infantis* (n=5 from farm 17), all 5 isolates have almost consistent pattern. The isolates were resistant to ampicillin, tetracycline, sulphadiazine, doxycycline and nalidixic acid while sensitivity was observed to cephalosporins, chloramphenicol and amoxyclav. Intermediate resistance was seen to ciprofloxacin in all *S. Infantis* isolates (Table 14).

*S. Typhimurium* isolates (n=7 from farm 28) showed 100 percent resistance to ampicillin and tetracycline. Three isolates were resistant to gentamicin and nalidixic acid while two isolates were resistant to chloramphenicol and sulphadiazine. One isolate was resistant to ceftriaxone, cefotaxime and ciprofloxacin (Table 15).

**Table 12: Antibiotic sensitivity pattern of *S. Kentucky* isolates (n=3)**

| S.No. | Antibiotic (Abbreviation)      | Concentration | Sensitive  | Intermediate | Resistant  |
|-------|--------------------------------|---------------|------------|--------------|------------|
| 1.    | Amoxyclav (AMC)                | 30mcg         | 3 (100%)   | 0.0%         | 0.0%       |
| 2.    | Ampicillin (AMP)               | 10mcg         | 0.0%       | 0.0%         | 3 (100%)   |
| 3.    | Cefoperazone (CPZ)             | 75mcg         | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 4.    | Cefotaxime(CTX)                | 30mcg         | 0.0%       | 1 (33.33%)   | 2 (66.67%) |
| 5.    | Ceftriazone (CTR)              | 30mcg         | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 6.    | Chloramphenicol (C)            | 30mcg         | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 7.    | Ciprofloxacin (CIP)            | 5mcg          | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 8.    | Sulphadiazine(SZ)              | 100mcg        | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 9.    | Doxycycline Hydrochloride (DO) | 30mcg         | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 10.   | Gentamicin (GEN)               | 10mcg         | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 11.   | Nalidixic Acid (NA)            | 30mcg         | 1 (33.33%) | 0.0%         | 2 (66.67%) |
| 12.   | Tetracycline(TE)               | 30mcg         | 0.0%       | 0.0%         | 3 (100%)   |

**Table 13: Antibiotic sensitivity pattern of *S. Agona* isolates (n=4)**

| S.No | Antibiotic (Abbreviation)      | Concentration | Sensitive | Intermediate | Resistant |
|------|--------------------------------|---------------|-----------|--------------|-----------|
| 1.   | Amoxyclav (AMC)                | 30mcg         | 4 (100%)  | 0.0%         | 0.0%      |
| 2.   | Ampicillin (AMP)               | 10mcg         | 0.0%      | 0.0%         | 4 (100%)  |
| 3.   | Cefoperazone (CPZ)             | 75mcg         | 2 (50%)   | 2 (50%)      | 0.0%      |
| 4.   | Cefotaxime(CTX)                | 30mcg         | 4 (100%)  | 0.0%         | 0.0%      |
| 5.   | Ceftriazone (CTR)              | 30mcg         | 2 (50%)   | 2 (50%)      | 0.0%      |
| 6.   | Chloramphenicol (C)            | 30mcg         | 2 (50%)   | 1 (25%)      | 1 (25%)   |
| 7.   | Ciprofloxacin (CIP)            | 5mcg          | 0.0%      | 0.0%         | 4 (100%)  |
| 8.   | Sulphadiazine(SZ)              | 100mcg        | 0.0%      | 0.0%         | 4 (100%)  |
| 9.   | Doxycycline Hydrochloride (DO) | 30mcg         | 0.0%      | 0.0%         | 4 (100%)  |
| 10.  | Gentamicin (GEN)               | 10mcg         | 4 (100%)  | 0.0%         | 0.0%      |
| 11.  | Nalidixic Acid (NA)            | 30mcg         | 0.0%      | 0.0%         | 4 (100%)  |
| 12.  | Tetracycline(TE)               | 30mcg         | 0.0%      | 0.0%         | 4 (100%)  |

**Table 14: Antibiotic sensitivity pattern of *S. Infantis* isolates (n=5)**

| S. No | Antibiotic                     | Concentration | Sensitive | Intermediate | Resistant |
|-------|--------------------------------|---------------|-----------|--------------|-----------|
| 1.    | Amoxyclav (AMC)                | 30mcg         | 5 (100%)  | 0.0%         | 0.0%      |
| 2.    | Ampicillin (AMP)               | 10mcg         | 0.0%      | 0.0%         | 5 (100%)  |
| 3.    | Cefoperazone (CPZ)             | 75mcg         | 5 (100%)  | 0.0%         | 0.0%      |
| 4.    | Cefotaxime(CTX)                | 30mcg         | 5 (100%)  | 0.0%         | 0.0%      |
| 5.    | Ceftriazone (CTR)              | 30mcg         | 5 (100%)  | 0.0%         | 0.0%      |
| 6.    | Chloramphenicol (C)            | 30mcg         | 5 (100%)  | 0.0%         | 0.0%      |
| 7.    | Ciprofloxacin (CIP)            | 5mcg          | 0.0%      | 5 (100%)     | 0.0%      |
| 8.    | Sulphadiazine (SZ)             | 100mcg        | 0.0%      | 0.0%         | 5 (100%)  |
| 9.    | Doxycycline Hydrochloride (DO) | 30mcg         | 0.0%      | 0.0%         | 5 (100%)  |
| 10.   | Gentamicin (GEN)               | 10mcg         | 3 (60%)   | 2 (40%)      | 0.0%      |
| 11.   | Nalidixic Acid (NA)            | 30mcg         | 0.0%      | 0.0%         | 5 (100%)  |
| 12.   | Tetracycline(TE)               | 30mcg         | 0.0%      | 0.0%         | 5 (100%)  |

**Table 15: Antibiotic sensitivity pattern of *S. Typhimurium* isolates (n=7)**

| S. No | Antibiotic (Abbreviation)      | Concentration | Sensitive  | Intermediate | Resistant  |
|-------|--------------------------------|---------------|------------|--------------|------------|
| 1.    | Amoxyclav (AMC)                | 30mcg         | 7 (100%)   | 0.0%         | 0.0%       |
| 2.    | Ampicillin (AMP)               | 10mcg         | 0.0%       | 0.0%         | 7 (100%)   |
| 3.    | Cefoperazone (CPZ)             | 75mcg         | 6 (85.71%) | 1 (14.28%)   | 0.0%       |
| 4.    | Cefotaxime(CTX)                | 30mcg         | 6 (85.71%) | 0.0%         | 1 (14.28%) |
| 5.    | Ceftriazone (CTR)              | 30mcg         | 6 (85.71%) | 0.0%         | 1 (14.28%) |
| 6.    | Chloramphenicol (C)            | 30mcg         | 4 (57.14%) | 1 (14.28%)   | 2 (28.57%) |
| 7.    | Ciprofloxacin (CIP)            | 5mcg          | 3 (42.85%) | 3 (42.85%)   | 1 (14.28%) |
| 8.    | Sulphadiazine (SZ)             | 100mcg        | 4 (57.14%) | 1 (14.28%)   | 2 (28.57%) |
| 9.    | Doxycycline Hydrochloride (DO) | 30mcg         | 0.0%       | 0.0%         | 7 (100%)   |
| 10    | Gentamicin (GEN)               | 10mcg         | 4 (57.14%) | 0.0%         | 3 (42.85%) |
| 11    | Nalidixic Acid (NA)            | 30mcg         | 2 (28.57%) | 2 (28.57%)   | 3 (42.85%) |
| 12    | Tetracycline (TE)              | 30mcg         | 0.0%       | 0.0%         | 7 (100%)   |

### **4.2.3 Comparison of resistance profiles of *Salmonella* serotypes**

*Salmonella* serotypes were also compared exclusively for their resistance profile. All four serotypes were resistant to ampicillin while all were sensitive to amoxyclav. For cephalosporins resistance was higher in *S. Kentucky* than other three serotypes. For ciprofloxacin, resistance was higher in *S. Kentucky* and *S. Agona* than *S. Infantis* and *S. Typhimurium*. Resistance to doxycycline was found in all four serotypes. For gentamicin, the resistance was observed in *S. Kentucky* and *S. Typhimurium* but not in *S. Agona* and *S. Infantis*. For nalidixic acid and tetracycline, resistance was observed in all four serotypes.

**Table 16: Resistance profile of *Salmonella* serotypes obtained in the study**

| <b>S.No</b> | <b>Antibiotic<br/>(Abbreviation)</b> | <b>Concentration</b> | <b><i>S. Kentucky</i><br/>(n=3)</b> | <b><i>S. Agona</i><br/>(n=4)</b> | <b><i>S. Infantis</i><br/>(n=5)</b> | <b><i>S. Typhimurium</i><br/>(n=7)</b> |
|-------------|--------------------------------------|----------------------|-------------------------------------|----------------------------------|-------------------------------------|--|
| 1.          | Amoxyclav (AMC)                      | 30mcg                | 0 (0.0%)                            | 0 (0.0%)                         | 0 (0.0%)                            | 0 (0.0%)                               |
| 2.          | Ampicillin (AMP)                     | 10mcg                | 3 (100%)                            | 4 (100%)                         | 5 (100%)                            | 7 (100%)                               |
| 3.          | Cefoperazone (CPZ)                   | 75mcg                | 2 (66.66%)                          | 0 (0.0%)                         | 0 (0.0%)                            | 0 (0.0%)                               |
| 4.          | Cefotaxime (CTX)                     | 30mcg                | 2 (66.66%)                          | 0 (0.0%)                         | 0 (0.0%)                            | 1 (14.28%)                             |
| 5.          | Ceftriazone (CTR)                    | 30mcg                | 2 (66.66%)                          | 0 (0.0%)                         | 0 (0.0%)                            | 1 (14.28%)                             |
| 6.          | Chloramphenicol (C)                  | 30mcg                | 2 (66.66%)                          | 1 (25%)                          | 0 (0.0%)                            | 2 (28.57%)                             |
| 7.          | Ciprofloxacin (CIP)                  | 5mcg                 | 2 (66.66%)                          | 4 (100%)                         | 0 (0.0%)                            | 1 (14.28%)                             |
| 8.          | Sulphadiazine (SZ)                   | 100mcg               | 2 (66.66%)                          | 4 (100%)                         | 5 (100%)                            | 2 (28.57%)                             |
| 9.          | Doxycycline<br>Hydrochloride (DO)    | 30mcg                | 2 (66.66%)                          | 4 (100%)                         | 5 (100%)                            | 7 (100%)                               |
| 10.         | Gentamicin (GEN)                     | 10mcg                | 2 (66.66%)                          | 0 (0.0%)                         | 0 (0.0%)                            | 3 (42.85%)                             |
| 11.         | Nalidixic Acid (NA)                  | 30mcg                | 2 (66.66%)                          | 4 (100%)                         | 5 (100%)                            | 3 (42.85%)                             |
| 12.         | Tetracycline (TE)                    | 30mcg                | 3(100%)                             | 4 (100%)                         | 5 (100%)                            | 7 (100%)                               |

#### 4.2.4 Data collected on attributes of poultry farms (n=30) sampled in the study

During the course of our study data related to various attributes of poultry farms and antimicrobial usage was collected (Table 17). Out of 30 poultry farms samples, 25 farms were independent in operations (83.3%), 4 were in contract with private companies (13.3%) and one was of government (3.3%). Out of these three categories, only independent farms were positive for *Salmonella*.

All farms were rearing broilers with age range from 3 days to 60 days. The farms were categorized into four age categories viz., <1 week (n=3, 10%), 1 to 3 weeks (n=18, 60%), 3 to 6 weeks (n=8, 26.7%) and >6 weeks (n=1, 3.3%). Two flocks from <1 week of age and 1 to 3 weeks of age were positive for *Salmonella* while one flock was of 3 to 6 weeks age group.

Data related to antimicrobial usage revealed that 86.7% (n=26) farms were actively using antibiotics for disease treatment and prevention whereas 13.3% (n=4) were reluctant towards their use. Multiple classes of antibiotics were used in these farms including tetracyclines (n=21, 70%), fluoroquinolones (n=17, 56.7%), aminoglycosides (n=11, 36.7%), Sulphadiazine (n=6, 20%) and Tylosin (n=4, 13.3%).

Data related to biosecurity measures and hygiene practices at farm level showed that 17 (56.7%) farms were not employing any biosecurity measure at the entrance while 13 (43.3) farms were using foot rub having quick lime at the entrance. None of the farms was using foot dip or any other precautions such as gloves and mask.

Isolation of sick birds to prevent spread of disease on farm was followed by majority of the farms (n=27, 90%). The owners of these independent farms were well aware about the contagious nature of diseases in poultry. Therefore they considered isolation of sick birds as priority.

Demographics related to the level of education of farm owners revealed that 46.7% (n=14) of the owners were matriculate or less followed by 30% (n=9) who were 10+2 passed while 13.3% (n=4) were illiterate. 10% (n=3) of farm owners were graduate.

**Table 17: Data collected on various attributes of poultry farms studied (n=30)**

| S.No | Farm attribute   | Category    | No of responses (%) |
|------|--|-------------|---------------------|
| 1.   | Type of Farm   | Broiler     | 30 (100%)           |
|      |  | Layer       | 0 (0.0%)            |
| 2.   | Type of farm   | Independent | 25 (83.3%)          |
|      |  | Contracted  | 4 (13.3%)           |
|      |  | Government  | 1 (3.3%)            |
| 3.   | Age of birds at farm   | <1 week     | 3 (10%)             |
|      |  | 1-3 week    | 18 (60%)            |
|      |  | 3-6week     | 8 (26.7%)           |
|      |  | >6 week     | 1 (3.3%)            |
| 4.   | Antimicrobial Usage<br>(Disease treatment/ Disease prevention) | Yes         | 26 (86.7%)          |
|      |  | No          | 4 (13.3%)           |
| 5.   | Usage of specific antimicrobials                               |             |                     |
|      | Tetracyclines  |             | 21 (70%)            |
|      | Fluoroquinolones   |             | 17 (56.7%)          |
|      | Aminoglycosides  |             | 11 (36.7%)          |
|      | Sulphadiazine  |             | 6 (20%)             |
|      | Tylosin  |             | 4 (13.3%)           |
| 6.   | Precautions taken while entering the farms                     |             |                     |
|      | No any precaution  |             | 17 (56.7%)          |
|      | Foot rubs (quick lime)   |             | 13 (43.3%)          |
| 7.   | Isolation of sick bird   | Yes         | 27(90%)             |
|      |  | No          | 3(10%)              |
| 8.   | Education level of respondent                                  |             |                     |
|      | Illetrate  |             | 4 (13.3%)           |
|      | ≤ matric   |             | 14 (46.7%)          |
|      | 10+2   |             | 9 (30%)             |
|      | Graduation   |             | 3 (10%)             |

Chapter-5

*Discussion*

Salmonellosis is one of the most common foodborne zoonotic diseases. In USA, salmonellosis has been the second most important infection (incidence 18.3 per 100000 human population) transmitted via contaminated food to humans, just after campylobacteriosis whose incidence is 19.5 per 100000 human population in USA (Tack *et al.*, 2019). Poultry is one of the largest reservoirs of *Salmonella*. It may harbor the *Salmonella* serotypes which infect poultry exclusively or multiple hosts including humans. Therefore, it is always pertinent to identify *Salmonella* serotypes circulating in the poultry of the region and the present study was aimed in this direction for Jammu region, Jammu and Kashmir.

In the present study, 30 poultry farms of 4 districts of Jammu region of Jammu and Kashmir were sampled. Poultry droppings were the samples and were processed as per FDA- BAM method (2020). To increase the isolation rate from a sample, PCR at enrichment step was also used. DNA from enrichment broths (Tetrathionate broth and Rappaport Vassiliadis broth) was extracted using boiling method and was subjected to PCR for *invA* gene (data not shown). The study identified the strategy to be useful for successful isolation of *Salmonella*.

Out of 30 poultry farms, 5 farms were positive for salmonellosis yielding an apparent prevalence of 16.67%, which is much higher than the study of Mir *et al.* (6.88%) conducted in Kashmir region. Prevalence of 14.40% has been reported in North-Eastern region of India by Murugkar *et al.* (2005). The poultry farms positive in the present study were independent in operations and were purchasing day old chicks from hatcheries spread over the Northern part of India. In study, four farms samples were in contract with a private company and none of those farms was positive for salmonellosis.

From 5 positive farms, 19 isolates were obtained. The isolates were got serotyped at National *Salmonella* Centre (Veterinary), ICAR-IVRI, Izzatnagar and by uniplex PCRs for *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum*. The isolates belonged to four serotypes *viz.*, *S. Kentucky*, *S. Infantis*, *S. Agona* and *S. Typhimurium*. *S. Kentucky* was obtained from 2 farms of Jammu district. *S. Infantis*

was obtained from one farm of Samba district. *S. Agona* was isolated from one farm of Kathua district while *S. Typhimurium* was isolated from one farm of Udhampur district. The study didn't report *S. Enteritidis* and *S. Gallinarum* which have been frequently isolated from poultry worldwide.

Isolation of four different non-typhoidal *Salmonella* (NTS) serotypes namely, *S. Agona*, *S. Infantis*, *S. Kentucky* and *S. Typhimurium* is in contrary to the earlier reports which suggest *S. Gallinarum* as the most predominant serotype in North India (Mir *et al.*, 2010). These NTS serotypes are known worldwide to infect humans through contaminated food products while very few reports related to *S. Agona*, *S. Infantis* and *S. Kentucky* are available in India. Therefore, existence of these three serotypes in Jammu region is an alarming bell. In fact, at a major *Salmonella* repository of our country - National *Salmonella* and *Escherichia* Centre, Kasauli - *S. Enteritidis*, *S. Typhimurium* and *S. Gallinarum* have been the most common serotypes isolated from poultry from 2011 to 2016 (Kumar *et al.*, 2019). Later, Sharma *et al.* (2019) reported presence of *S. Kentucky* and *S. Virchow* in addition to *S. Typhimurium* in chicken shops from North India. Study of Sharma *et al.* (2019) and the present study is probably indicating about the shift in the serotype being harbored by poultry of the region and indicates about the possible emergence of previously unknown NTS serotypes in human foodborne outbreaks.

In antibiotic sensitivity assay, all isolates studied were found to be multi-drug resistant. Of 19 *Salmonella* isolates, only 2 (10.52%) isolates were moderately resistant (resistance to 2-4 drugs) and rest all (n=17, 89.47%) were showing extreme drug resistance (resistance to more than 4 drugs). All isolates were resistant to ampicillin and tetracycline (100%). *Salmonella* showing resistant towards tetracycline is common. This resistance to tetracycline has also been observed by Waghmare *et al.* (2018) in South India. In their study, 78.57% of isolates were resistant towards tetracycline. Zhao *et al.* (2017) has also reported high resistance to tetracycline and ampicillin.

Further resistance pattern in *Salmonella* isolates of present study was doxycycline (94.73%) followed by nalidixic acid (73.64%), sulphadiazine (68.42%), gentamicin (26.31%), chloramphenicol (26.31%), ciprofloxacin (36.84%), cefotaxime (15.78%), ceftriaxone (15.78%) and cefoperazone (10.53). However, all 19 isolates were sensitive to Amoxyclav.

In the study, three isolates of *S. Kentucky* from 2 poultry farms [farm 6 (n=2) and farm 12 (n=1)] of Jammu district were obtained. The isolates from two farms differ in their antibiotic sensitivity pattern. The two isolates from farm 6 were resistant to majority of antibiotics used while the only isolate from farm 12 showed sensitivity towards ciprofloxacin, doxycycline, gentamicin, nalidixic acid and sulphadiazine. Multi-drug resistance in *S. Kentucky* isolates has been reported by Andoh *et al.* (2016). The resistance in *Salmonella* to fluoroquinolones and third generation cephalosporins is an issue of concern, as these are the most frequent drugs used in treatment of human salmonellosis cases. There is increase in isolation of fluoroquinolone resistant *S. Kentucky* from human diarrheic hospitalized cases in India (Sharma *et al.*, 2020). Bai *et al.* (2015) has also reported resistance of *S. Kentucky* to ciprofloxacin and cephalosporins.

*S. Kentucky* is an emerging human pathogen. It is the common serotype reported in human salmonellosis cases in USA (Sandt *et al.*, 2013). Reports suggest *S. Kentucky* to be the common serotype in poultry also (CDC, 2011). In another study, *S. Kentucky* was most predominant serotype isolated from Chicken farm (Magdy *et al.*, 2019). *S. Kentucky* has also been isolated from poultry farms of Punjab region by Mahindroo *et al.* (2019). During their study, all *S. Kentucky* isolates were found to be ciprofloxacin resistant (ST198) whereas in our study, 66.66% (n=2) of *S. Kentucky* isolates obtained were resistant to ciprofloxacin. Similar findings have been reported by Sharma *et al.* (2019) in north India. In our study, two isolates of *S. Kentucky* were resistant to third generation cephalosporins while Mahindroo *et al.* (2020) didn't detect cephalosporin resistance in *S. Kentucky* isolates (Mahindroo *et al.*, 2020). As all poultry farms positive in our study purchased the day old chicks from hatcheries of North India, there is great possibility that the ciprofloxacin resistant *S. Kentucky* strain ST198 could be circulating here as well which need whole genome sequencing of the isolated *S. Kentucky* strains.

In the study, five isolates of *S. Infantis* from one farm of Samba district. In a Japanese study, *S. Infantis* was the most common serotype from chicken samples (Furukawa *et al.*, 2017). In an Indian study, Bangera *et al.* (2019) has reported *S. Infantis* to be the most common serotype in poultry in Manipal. However, reports related to *S. Infantis* in poultry in India are scanty. The significance of *S. Infantis* in causing NTS in humans in India is not well known. All the 5 isolates had almost

uniform antibiotic sensitivity pattern. The isolates were resistant to ampicillin, tetracycline, sulphadiazine, doxycycline and nalidixic acid while sensitive to cephalosporins, chloramphenicol and amoxycyclav. Intermediate resistance was seen to ciprofloxacin in all *S. Infantis* (n=5). Similar results have been reported by Ghodduzi *et al.* (2015) in their study in which all *S. Infantis* isolates from backyard chickens were resistant to tetracycline and doxycycline. In another study, Shahada *et al.* (2006) reported 95.6% of resistance in *S. Infantis* isolates to sulfamethoxazole.

In our study, *S. Agona* (4 isolates) is reported from one farm of Kathua district. As per knowledge of authors, this is the first study documenting *S. Agona* in poultry in the country. However, in other countries, the association of *S. Agona* with poultry is well established. In Australia, Manning *et al.* (2015) has isolated *S. Agona* with poultry while in Morocco, it has been done by El Allaoui *et al.* (2017). *S. Agona* isolates in the present study isolates showed complete resistance towards ampicillin, ciprofloxacin, sulphadiazine, doxycycline nalidixic acid and tetracycline. Similar reports of high resistance of *S. Agona* to ampicillin were reported by Manning *et al.* (2015). For cephalosporins, pattern varied from sensitive to intermediate resistance, which is in agreement with the findings of El Allaoui *et al.* (2017).

In the study seven isolates of *S. Typhimurium* were obtained from one farm of Udhampur district. The isolates had 100 percent resistance to ampicillin and tetracycline. Three isolates were resistant to gentamicin and nalidixic acid while two isolates were resistant to chloramphenicol and sulphadiazine. One isolate was resistant to ceftriaxone, cefotaxime and ciprofloxacin. *S. Typhimurium* is known to cause NTS in humans all over the world (Helms *et al.*, 2005). Resistance towards ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline in multidrug-resistant *S. Typhimurium* DT104 is well known (Rajashekara *et al.*, 2000). In our study, two isolates were resistant simultaneously to ampicillin, chloramphenicol, sulfonamides and tetracycline. Thus, there is great probability that a multi drug resistant strain of *S. Typhimurium* might also be circulating in poultry farms of Jammu.

The poultry farms sampled in the study were also questioned about biosecurity measures adopted on the farms. Surprisingly, none of the farms were using foot bath at the entry of farm while 13 (43.3%) farms were using Quick lime foot rub at the entrance. Majority of farms (90%) were isolating the sick birds

indicating about the knowledge of farmers about the contagious nature of the infectious diseases. Further, majority of farms were using antibiotics for prevention of diseases.

Foodborne pathogens are a challenge of public health globally. Foods of animal origin greatly contribute to the foodborne cases and thus require the interventions to prevent spread of infections by their consumption. Poultry and poultry products have been documented as one of the main sources of NTS transmission in humans. To prevent such occurrence require studying epidemiology and surveillance of salmonellosis in poultry. The present study was an attempt in this direction for Jammu region of Jammu and Kashmir. The study found the poultry being harboring salmonellosis in the region emphasizing the need to use intervention strategies at farm and from farm to fork to prevent NTS infection in humans.



## Chapter-6

# *Summary and Conclusion*

## CHAPTER-6

### SUMMARY AND CONCLUSIONS

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Salmonellosis is a major public health concern worldwide. It involves wide range of hosts including cold blooded animal, warm blooded animals and humans. Poultry is one of the largest reservoirs of *Salmonella*. It may harbor the *Salmonella* serotypes which infect poultry exclusively and multiple hosts including humans. In this regard, the present study identified the serotypes of *Salmonella* circulating in the poultry of Jammu region of Jammu and Kashmir.

A total of 101 samples of poultry droppings from 30 poultry farms of Jammu region were processed for isolation of *Salmonella*. Samples belonged to four districts viz., Jammu, Samba, Kathua and Udhampur. The samples were processed for isolation as per BAM followed by molecular characterization.

25 grams of poultry droppings were added in 225 ml sterile buffered peptone water, mixed well and incubated at 35°C for 24 hours. From the mixture, 0.1 ml inoculum was transferred to 10 ml Rappaport–Vassiliadis broth and incubated at 42°C for 24 hours while 1 ml inoculum was transferred to 10 ml Tetrathionate broth and incubated at 43°C for 24 hours. After incubation, the inoculum from each broth was streaked on HE agar and XLD agar plates followed by incubation at 35°C for 24 hours. Blue green colonies with black center and pink colonies with black center on HE and XLD, respectively, were suspected for *Salmonella*. The suspected colonies were subjected to phenotypic identification and biochemical tests to identify *Salmonella* isolates.

Phenotypically identified *Salmonella* isolates were confirmed by PCR for *invA* gene (Rahn *et al.*, 1992). *Salmonella* isolates were also subjected to uniplex PCRs for serotyping of *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum*. These isolates were also sent to National *Salmonella* Centre (Veterinary), ICAR-IVRI, Izzatnagar for serotyping. The *Salmonella* isolates were also analyzed for their antibiotic sensitivity pattern against 12 antibiotics.

On processing the 101 samples of poultry droppings of 30 poultry farms, 5 poultry farms were positive for *Salmonella* with an apparent prevalence of 16.67% of salmonellosis among poultry farms. From these 5 positive farms, 19 *Salmonella* isolates

were obtained. All positive farms were having independent operations and none of farms on contract with private company was positive for salmonellosis. Out of these 5 positive farms, two farms were from Jammu district and one from Samba, Kathua and Udhampur district each. District-wise, the apparent prevalence of salmonellosis was 12.5%, 11.11%, 33.33% and 50% in Jammu, Kathua, Udhampur and Samba districts, respectively

On serotyping of 19 *Salmonella* isolates obtained from 5 salmonellosis positive poultry farms, four serotypes viz., *S. Kentucky*, *S. Infantis*, *S. Agona* and *S. Typhimurium* were obtained. *S. Kentucky* was obtained from 2 farms of Jammu district. *S. Infantis* was obtained from one farm of Samba district. *S. Agona* was isolated from one farm of Kathua district. *S. Typhimurium* was the isolation from one farm of Udhampur district

In antibiotic sensitivity assay, all isolates were resistant to ampicillin and tetracycline (100%). For other antibiotics, resistance pattern in descending order was doxycycline (94.73%) followed by nalidixic acid (73.64%), sulphadiazine (68.42%), gentamicin (26.31%), chloramphenicol (26.31%), ciprofloxacin (36.84%), cefotaxime (15.78%), ceftriaxone (15.78%) and cefoperazone (10.53). However, all 19 isolates were sensitive to Amoxyclav. Serotypes differ in their antibiotic sensitivity pattern. *S. Kentucky* and *S. Agona* were having higher resistance for cephalosporins and fluoroquinolones than *S. Infantis* and *S. Typhimurium*.

None of the poultry farms sampled was using foot bath at the entry of farm while 13 (43.3%) farms were using Quick lime foot rub at the entrance. Majority of farms (90%) were isolating the sick birds. Further, majority of farms were using antibiotics for prevention of diseases.

The present study led to the following conclusions:

- The study isolated *S. Kentucky*, *S. Infantis*, *S. Agona* and *S. Typhimurium* from poultry which have been known to cause non-typhoidal salmonellosis in humans.
- As per authors, this is the first report on isolation of *S. Agona* from poultry in the country
- The study didn't report *S. Enteritidis* and *S. Gallinarum* which have been frequently isolated from poultry worldwide, probably indicating shift in the

serotype pattern in poultry of the region.

- Antibiotic resistance was high in the *Salmonella* isolates.
- The study indicates the risk of foodborne salmonellosis on consumption of contaminated poultry and poultry products in the region.



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## CERTIFICATE-IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled "**Study on distribution of *Salmonella enterica* serotypes in poultry farms of Jammu**", submitted by **Mr. Arvindepal Singh**, Registration No. **J-19-MV-601**.



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