Serotyping, Antimicrobial Resistance Profile and Virulence Genes of Salmonella Serovars Isolated from Human, Animals and Birds

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The study was undertaken to investigate the prevalence, antimicrobial susceptibility, antimicrobial resistance and virulence genes of Salmonella isolates recovered from human and different species of animals and birds. Out of 88 (7.15%), 21 (23.86%) belonged to Salmonella enterica subsp. entericarWeltevreden, 22 (25%) to serovar Enteritidis, 16 (18.2%) to serovar Typhi and 14 (15.9%) to serovar Newport, while 7 (7.95%) isolates were found to be unypatable. Among the 88 isolates, 45.45% showed resistance to ampicillin, 61.36% to tetracycline, 61.18% to cefotaxime, 65.90% to gentamicin, 48.86% to trimethoprim, 11.36% to ceftioxacin, 10.22% to chloramphenicol, and 7.95% each to ciprofloxacin and cefepime. Most of the isolates were susceptible to a low MIC (≤0.25 µg/ml) of Cefepime, Cefotaxime, Ciprofloxacin, Ceftiraxone and Co-trimoxazole and a moderate MIC (0.5µg/ml – 4µg/ml) of Ampicillin, Tetracycline, Gentamicin and Chloramphenicol. The resistance genes, blatem, tetA and dfrA12 were most prevalent, irrespective of the host of origin of the isolates. While invA was used for molecular detection of Salmonella, other virulence genes, viz. sipA, sipB, sipC, stn and T2544 were also detected in all (100%) the Salmonella isolates. Total 69.32% of tested samples were found to be contaminated with multi-drug resistant (MDR) Salmonella and various virulence genes were present among the isolated serovars. Another virulence-associated gene, T2544 (pagN) could also be found in all the isolates, irrespective of serovar or host of origin suggesting the possibility of using this gene as a marker for identification of pathogenic Salmonella isolates. This study highlights the importance of continuous monitoring and surveillance for pathogenic salmonellae and their potential risks to both human and animal.

Introduction

Bacteria under the genus Salmonella are found in the intestinal tract of many animals, including cattle, pigs, horses, other mammals, reptiles, amphibians, and poultry (e.g., chickens, ducks, geese, and turkeys) are capable of causing infections in these species (Hale et al. 2012). Salmonella infection in human typically manifests as acute gastroenteritis that develops 12–72 hours after exposure. Young children, persons >65 years of age, and immuno-compromised persons are at greater risk for serious complications, including septicemia, joint or brain infections, and death (Giannella et al. 1996). The genus Salmonella consists of two species, namely Salmonella bongori and Salmonella enterica (Issenhuth-Jeanjean et al. 2014). Salmonella bongori is classically called as the Salmonella of lizards and it is mostly found in reptiles causing diarrhea. Salmonella enterica is predominantly found in human and animals causing severe diarrhoea and fever. The species S. enterica has six subspecies namely enterica, salamae, arizonae, houtonae, indica and diarizonae. All of these subspecies have different serotypes. So far, a total of 2659 serotypes of S. enterica have been reported. Out of that, S. enterica subsp. enterica has the highest 1586 serotypes (Issenhuth-Jeanjean et al. 2014). Although all serotypes must be considered as potential human pathogens, only a limited number of serotypes are attributed to be the cause of infection in humans and animals. Most of the enteric diseases in human and animals with severe diarrhoea and fever are caused by different serotypes of S. enterica subsp. enterica. This subsp. is one of the leading causes of zoonotic food-borne disease worldwide (Voetsch et al. 2004). It is estimated that in 2019, there were 2,12,500 and 4,100 cases of infections due to drug resistant non-typhoidal Salmonella and drug resistant Salmonella Typhi, respectively only in USA (CDC report; 2019). Antimicrobial Resistance Surveillance and Research Network (AMRSN) of India has categorized Salmonella (typhoidal and non-typhoidal) into enteric fever pathogens and diarrhoeagenic bacterial organisms groups out of a total of six groups chosen by ICMR (Walia et al. 2019) for developing a comprehensive plan on Anti-microbial resistance (AMR) surveillance in India. Salmonella species, specifically those which are fluoroquinolone resistant are categorized as “high priority” by WHO (WHO Report, 2017). MDR Salmonella carrying several classes of virulence genes have been detected in duck meat in China (Chen et al. 2020). Outbreaks of S. Typhi recently occurred in Bangladesh (Tanmoy et al. 2018) and Pakistan (Kleem et al. 2018) with the presence of XDR strains resistant to Ceftiraxone and several other antibiotics. Salmonella Typhimurium showed a high frequency of occurrence in poultry (41.40%) and humans (43%), and S. Weltevreden was found to be of zoonotic significance in India and has been recorded as one of the five most frequently isolated serovars (Kumar et al. 2009). In Assam, isolation of S. Weltevreden, S Choleraesuis, S Paratyphi B and S. Typhimurium from pigs (Rajkhowa et al. 2018), and S Enteritidis, S Gallinarum, S. Typhimurium, S. Newport and S. Indiana from poultry (Rahman et al. 1997; Rajkhowa et al. 2018) have been reported.

Emergence and spread of antimicrobial resistance among zoonotic Salmonella has become a public health threat. Importantly, Salmonella strains having “clinically important resistance” to some agents like extended spectrum cephalosporins and fluoroquinolones have been isolated from livestock (Li et al. 2013). In most developing countries, misuse and overuse of antibiotics has contributed to the increasing trend of multi-resistance in Salmonella (Eddra et al. 2017). Salmonella with antibiotic resistance in contaminated products could infect humans directly or transmit their resistance genes to human pathogens through the food chain, leading to failure of antibiotic treatment and may pose a serious threat to human health. The aim of the current study was to investigate the prevalence, antimicrobial resistance and virulence gene profiles of Salmonella serovars isolated from human and different species of animals and birds.

Materials And Methods

Collection of samples

A total of 1231 different samples consisting of faecal swabs from diarrhoeic (83) and apparently healthy (60) human, apparently healthy (101) and diseased (165) cattle, diarrhoeic (60) and apparently healthy (30) pigs, diarrhoeic (53) and apparently healthy (50) goats, diarrhoeic (302) and apparently healthy (103) poultry, apparently healthy (208) wild birds, apparently healthy (8) Gecko gecko, apparently healthy (6) tigers and mice (2) were collected from different parts of Assam. Type of samples was either faecal matter or part of intestine, in case of dead animals. Fresh samples were collected in sterile sample containers or in Cary-Blair medium, in case of anticipated delay from collection to processing by not more than 48 hours.

Isolation

Immediately after receiving the samples in the laboratory, they were put in pre-enrichment non-selective medium (sterile buffered peptone water broth) and incubated at 37°C for overnight. It was followed by inoculation of 1 ml overnight broth into 9 ml of selective enrichment (Selenite broth or Rappaport
Vassiliadis soy peptone broth and incubated at 37°C for overnight. Both broth cultures were kept at constant shaking at 250 rpm in a shaking incubator. Overnight turbid broth was inoculated to Brilliant Green Agar (BGA) and suspected positive colonies were picked and streaked on MacConkey’s Lactose Agar (MLA). Both plates were incubated overnight at 37°C. For preliminary identification of Salmonella, the suspected cultures were subjected to biochemical tests, viz. indole, urease and H₂S production, as well as production of lysine and ornithine decarboxylase. Additionally, growth characteristics on Triple Sulphate Iron (TSI) agar slants were also studied for determination of K/A reaction.

**Molecular detection of Salmonella**

Reference strains of Salmonella used in this study (Table 1) were obtained from Microbial Type Culture Centre (MTCC), Chandigarh, American Type Culture Centre, USA and National Institute of Cholera and Enteric Diseases, ICMR, (NICED), Kolkata. The isolates were grown in Luria Bertani (LB) broth for overnight at 37°C and 2 ml of the overnight cultures were centrifuged at 10,000g for 10 minutes. The supernatants were discarded and the pellets were suspended in a total volume of 100 µl of 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and boiled at 100°C for 10 minutes. After boiling, the cell suspensions were immediately cooled on ice for 10 minutes and centrifuged at 15,000g for 10 minutes at 4°C. The supernatant was then collected without disturbing the sediment. Isolated DNA was quantified in Nanodrop 1000 and stored in -20°C for future use as template DNA for PCR (Choudhury et al. 2016).

The isolates were confirmed as Salmonella by detecting the specific gene invA by simplex PCR. The specific primer pairs used (Galan et al. 1992) for this purpose was 5'\-ACCAGCTTTCTGCTGG-3' and 5'\-GAACGTAGTACGTAGGCTC-3'. A reaction mixture of 25 µl was prepared with 12.5 µl of Dream Taq PCR master mix (Fermentus), 0.5 µl each primer (10 pmol/µl), 1.0 µl of template DNA and 10.5 µl of nuclease-free water. PCR condition was standardized at 94°C for 5 minutes followed by 30 cycles at 94°C for 30 seconds, at 56°C for 1 minute and at 72°C for 1 minute. Final extension was given at 72°C for 5 minutes followed by infinite halt at 4°C. The amplified products were run on 1.5% agarose gel, and visualized and documented in Gel Doc XR+ (Bio-Rad).

**Serotyping**

The Salmonella isolates were sent for serotyping at National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India, and National Salmonella and Escherichia Centre (NSEC), Central Research Institute, Kasauli, Himachal Pradesh, India.

**Antibiotic Susceptibility Testing and MIC**

Antibiotic sensitivity and MIC values were determined by disc dilution method and E-test using 6 mm antibiotic disc (HiMedia) and Ezy MIC™ (HiMedia) strips, respectively, according to Clinical and Laboratory Standards Institute guideline (CLSI Guideline, 2017) [19]. Pure colonies from agar plate were inoculated in Luria Bertani broth for 6-8 hours to get log phase growth. Following incubation, the turbidity was adjusted to match that of a 0.5 McFarland standard. The container of discs and strips were removed from the -20°C to equilibrate to room temperature for 30 minutes. Sterile 20% Muller Hinton Agar (SRL) plates were inoculated by swabbing with sterile cotton swab (HiMedia) evenly. Antibacterial discs and Ezy MIC™ strips were placed on the surface aseptically as per manufacturer's instructions and kept for incubation at 37°C for overnight. Zone of inhibition and breakpoint values were recorded after the overnight incubation. A total of nine antimicrobial agents were used for both the tests. Ampicillin (AMP), Tetracycline (TET), Cefotaxime (CTX), Ciprofloxacin (CIP), Gentamicin (GEN), Chloramphenicol (CHL), Cefepime (CPM) and Ceftriaxone (CTR) were common for both, whereas Trimethoprim (TR) used in disc diffusion method was replaced by Co-trimoxazole (COT) in E-test (Table 2).

**Resistance and virulence gene screening**

*Salmonella* isolates exhibiting resistance to different antimicrobial agents were then subjected to PCR for detecting resistance genes of concerned antibiotics. Resistance genes blaOXA, blaTEM, blaqPSEκ, dfrA1, dfrA12, tet(A), tet(B) and tet(G) were detected using reported primers and PCR conditions (Table 3). PCR reaction mixture constituents were same as mentioned earlier for invA gene detection. All the Salmonella isolates were screened for the presence of a total of 11 virulence genes including invA universally present in all isolates. The primer sets used for PCR amplification of sipA, sipB, sipC, stn, sopB, sopE, pefA, sefC, fepA and pagN along with PCR conditions are listed in Table 4. PCR reaction mixture constituents were same as mentioned earlier. The amplified products were run on 1.5% agarose gel and visualized under Gel Doc XR+ (Bio-Rad, USA).

**Results**

**Isolation and identification**

Out of 1231 samples examined, *Salmonella* was recovered from 88 (7.15%). Among the 663 clinical samples collected from man and animals with a history of diarrhoea, 68 (10.26%) were positive for *Salmonella*, while out of 568 samples collected from apparently healthy animals and birds, 20 (3.52%) were positive for *Salmonella* (Table 5). All the 88 *Salmonella* isolates recovered from different sources fermented glucose, mannitol and dulcitol, but did not ferment lactose and sucrose. All the isolates were positive for methyl red test, citrate utilization and hydrogen sulphide production but were negative for indole production, Voges-Proskauer (VP) test and urease production. The isolates showed yellow butt, black middle and pink top on inoculation into Triple Sugar Iron (TSI) Agar slant.
Table 1
List of Reference strains of *Salmonella* serotypes

| Sl no | Serotype name            | Strain no |
|-------|--------------------------|-----------|
| 1     | Salmonella Typhimurium   | MTCC 98   |
| 2     | Salmonella Typhi         | MTCC 8767|
| 3     | Salmonella Gallinarum    | MTCC 2992|
| 4     | Salmonella Newport       | MTCC 3229|
| 5     | Salmonella Virchow       | MTCC 1166|
| 6     | Salmonella Enteritidis   | ATCC 13076|
| 7     | Salmonella Poona         | NCTC 4840|
| 8     | Salmonella Vellore       | ATCC 15611|
| 9     | Salmonella Enteritidis   | ATCC 13076|
| 10    | Salmonella Abony         | NCTC 6017|
| 11    | Salmonella Idikan        | NICED 503984|
| 12    | Salmonella Infantis      | NICED 505330|
| 13    | Salmonella Paratyphi A   | NICED C6915|
|       | Salmonella Paratyphi B   | NICED NK3727|

Table 2
Selected antimicrobial agents used for Antibiotic Susceptibility Testing and MIC

| Sl. no. | Disc diffusion | E-test |
|---------|----------------|--------|
|         | Antimicrobial agent | Con. (µg) | Antimicrobial agent | Con. gradient(µg/ml) |
| 1       | Ampicillin (AMP) | 10      | Ampicillin (AMP) | 0.016–256 |
| 2       | Tetracycline (TE) | 30      | Tetracycline (TET) | 0.016–256 |
| 3       | Cefotaxime (CTX) | 30      | Cefotaxime (CTX) | 0.016–256 |
| 4       | Ciprofloxacin (CIP) | 5      | Ciprofloxacin (CIP) | 0.002–32 |
| 5       | Gentamicin (GEN) | 30      | Gentamicin (GEN) | 0.016–256 |
| 6       | Chloramphenicol (C) | 30      | Chloramphenicol (CHL) | 0.016–256 |
| 7       | Cefepime (CPM) | 30      | Cefepime (CPM) | 0.016–256 |
| 8       | Ceftriaxone (CTR) | 30      | Ceftriaxone (CTR) | 0.002–32 |
| 9       | Trimethoprim (TR) | 5      | Co-trimoxazole (COT) | 0.002–32 |
### Table 3

**List of primers used in the study for detection of resistance genes in Salmonella**

| R-genes     | Primer sequence      | Amplicon size (bp) | Annealing temp (°C) | Reference               |
|-------------|----------------------|--------------------|---------------------|-------------------------|
| *blaOXA* (AMP) | F-AGCAAGCCAGCTGATCA  | 708                | 60                  | Guerra et al. (2001)    |
|             | R-ATTGACCCCAAGTTGCC  |                    |                     |                         |
| *blaTEM* (AMP) | F-TTGGGTGCAAGTTGGGT | 504                | 57                  | Arlet and Phillippon (1991) |
|             | R-TAATTGTGCCGGGAAGC  |                    |                     |                         |
| *blaPSE-1* (AMP) | F-CGCTTGCCGTTAACAAAGTAC | 420             | 58                  | Sandvang et al. (1997)  |
|             | R-CTGTTCTATTCAGATAGGC |                    |                     |                         |
| *dfrA1* (TR)   | F-GTGAACACTATCATAATGG | 470                | 50                  | Guerra et al. (2001)    |
|             | R-CCCTTTGCCAGATTTTG  |                    |                     |                         |
| *dfrA12* (TR)  | F-ACTCGGAATCAGTACGCA | 463                | 50                  | Guerra et al. (2001)    |
|             | R-GTGTACGGAATCAGCT   |                    |                     |                         |
| *tet (A)*    | F-GCTACATCTGTCTGCCTT | 210                | 57                  | Ng et al. (1999)        |
|             | R-CATAAGTCCGCGTGGAAGA |                    |                     |                         |
| *tet (B)*    | F-TTGGTTAGGGGCAAAGTTTG | 659              | 57                  | Ng et al. (1999)        |
|             | R-GTAATGGGCGGAAATAACCCG |                  |                     |                         |
| *tet (G)*    | F-GCTCGGGTGTATCTCGC  | 468                | 57                  | Ng et al. (1999)        |
|             | R-AGCAACAGAATCGGGAAC  |                    |                     |                         |

### Table 4

**List of primers used in the study for detection of virulence genes in Salmonella**

| Target Gene | Primer sequence (5’ – 3’)                     | Primer concentration (pMol/µl) | Product size (bp) |
|-------------|------------------------------------------------|-------------------------------|-------------------|
| sipA        | Forward –CGCAATCATAAAAAAGATGTCGCTTCTTT       | 20                            | 646               |
|             | Reverse-CGCACTTTATCAATCGTCTT                  |                               |                   |
| sipB        | Forward –CTAACGTCTGAACAGTTTCG                | 10                            | 160               |
|             | Reverse-AACGCCACTTTATTTAGGGTG                |                               |                   |
| sipC        | Forward –TCTTAATGATGCGAGCGCTTA               | 10                            | 365               |
|             | Reverse-TGTCGATTTACGCTGAACCTC               |                               |                   |
| strn        | Forward -ATTTGAGGGGTTTTATCTCCTT             | 10                            | 543               |
|             | Reverse-GCTTTGAACTCTGACCTGT                  |                               |                   |
| sopB        | Forward -GCATCTCTAAACGCTACTG                | 10                            | 470               |
|             | Reverse-GCTTCTATACGCTCGTCA                  |                               |                   |
| sopE        | Forward -GTAGGGCATATGAAACCAG                | 10                            | 254               |
|             | Reverse-TTATATGGCTCCTACTAGGCCC              |                               |                   |
| pefA        | Forward -CCAAAGTACTGGTGGGAAAG               | 20                            | 185               |
|             | Reverse-TATTTGAAACGCTCGCAA                  |                               |                   |
| sefC        | Forward -GCAGGTCCAAAACCTATAACA              | 10                            | 609               |
|             | Reverse-CGATAACGAAACACCCATT                  |                               |                   |
| fepA        | Forward-CGACGTGCAGAAATCATCC                 | 10                            | 382               |
|             | Reverse-GCCTCTGTATCTTTATATGTTCC             |                               |                   |
| pagN        | Forward-GAATCAACTCAACCTTCAGC                | 10                            | 105               |
|             | Reverse-AGCTACAACCTGAGTACACC                |                               |                   |
Table 5

Isolation of *Salmonella* from human and different species of animals and birds

| Host      | No. of samples examined | No. of isolates recovered |   |
|-----------|------------------------|---------------------------|---|
|           | Total                  | Diarrhoeic                | Apparently Healthy | Diarrhoeic | Apparently Healthy |
| Poultry   | 405                    | 302                       | 103 | 17 (5.63%) | 7 (6.8%) |
| Cattle    | 266                    | 165                       | 101 | 18 (10.91%) | - |
| Wild Birds| 208                    | -                         | 208 | - | 10 (4.81%) |
| Human     | 143                    | 83                        | 60  | 28 (33.73%) | - |
| Goat      | 103                    | 53                        | 50  | - | - |
| Pig       | 90                     | 60                        | 30  | 5 (8.3%) | - |
| Gecko     | 8                      | -                         | 8   | - | 1 (12.5%) |
| Tiger     | 6                      | -                         | 6   | - | 1 (16.67%) |
| Mice      | 2                      | -                         | 2   | - | 1 (50%) |
| Total     | 1231                   | 663                       | 568 | 68 (10.26%) | 20 (3.52%) |

Serotyping of *Salmonella*

Out of the 88 isolates, 21 (23.86%) belonged to *S. enterica* subsp. *enterica* serovar Weltevreden, 22 (25%) to serovar Enteritidis, 16 (18.20%) to serovar Typhi and 14 (15.90%) to serovar Newport, while 7 (7.95%) isolates were found to be untypable. Detailed results of serotyping of the *Salmonella* strains are presented in (Table 6). In case of human samples, *S. Typhi* (62.50%) was the most frequently isolated serovar, followed by serovars Weltevreden and Enteritidis (Figure. 1). On the other hand, *S. Newport* (52.63%) was found to be the most abundant serovar in cattle followed by serovars Weltevreden and Enteritidis (Figure. 2).

Table 6

Distribution of various serovars among *Salmonella* isolates recovered from different sources

| Sl. No. | Serotype               | Antigenic Structure | No. of isolates | Source/ Host |
|---------|------------------------|---------------------|-----------------|--------------|
| 1       | *Salmonella* Enteritidis | 9,12: g,m           | 22              | Cattle (2), Pig (1), Human (2), Poultry (13), Wild Birds (4) |
| 2       | *Salmonella* Weltevreden | 3(10), rz6       | 21              | Poultry (5), Human (4), Cattle (7), Pig (1), Wild Birds (3), *Gecko gecko* (1) |
| 3       | *Salmonella* Typhi     | 9,12: V,d          | 16              | Human |
| 4       | *Salmonella* Newport   | 6,8,e, h, 1, 2     | 14              | Cattle (9), Human (2), Poultry (3) |
| 5       | *Salmonella* Typhimurium | 1,4,5,12,i,1,2    | 2               | Human |
| 6       | *Salmonella* Litchfield | 8,6,8,l,v,1,2     | 2               | Poultry |
| 7       | *Salmonella* Kentucky  | 8,8,20,l,z6        | 1               | Tiger |
| 8       | *Salmonella* Idikan    | 13,1,3,23,i,1,5   | 1               | Human |
| 9       | *Salmonella* Paratyphi B | 1,4,5,12,b,1,2    | 1               | Human |
| 10      | *Salmonella* Virchow   | 7,5,7,14,c,1,2    | 1               | Mouse |
| 11      | Untypable              |                     | 7               | Poultry (1), Pig (3), Wild Birds (3) |

Antimicrobial Resistance Detected By Disc Diffusion Test

All the 88 *Salmonella* isolates recovered from different sources in the present study were subjected to antimicrobial susceptibility test by disc diffusion method. Of the 88 isolates, 40 (45.45%) showed resistance to ampicillin, 54 (61.36%) to tetracycline, 60 (61.18%) to cefotaxime, 58 (65.90%) to gentamicin, 43 (48.86%) to trimethoprim, 10 (11.36%) to ceftriaxone, 9 (10.22%) to chloramphenicol, and 7 (7.95%) each to ciprofloxacin and cefepime. Of the 11 serovars, *S. Weltevreden* and *S. Typhi* and the untypable isolates showed resistance to all the antibiotics under test. Cefotaxime showed the highest resistance (61.18%), whereas ciprofloxacin and cefepime showed the least resistance (7.95%). *Salmonella Virchow* showed resistance to three antibiotics (Tetracycline, Gentamicin and Chloramphenicol), *S. Kentucky* to four (Ampicillin, Tetracycline, Cefotaxime and Gentamicin) and *S. Paratyphi B* to four (Tetracycline, Cefotaxime, Gentamicin and Trimethoprim) antimicrobial agents (Table 7). Ciprofloxacin resistance was shown by the isolates from poultry, wild bird, pig and human. Most of the *S. Typhi* isolates from human showed resistance to all the nine antibiotics tested. All the isolates from different hosts showed resistance to tetracycline except one *S. Weltevreden* isolate from *Gecko gecko*. None of the isolates of serovars *S. Litchfield*, *S. Newport*, *S. Kentucky*, *S. Idikan*, *S. Paratyphi B* and *S. Virchow* showed resistance to ciprofloxacin (Table 8).
## Table 7

Resistance patterns of *Salmonella* isolates of different serovars from different sources against different antimicrobial agents

| Serovars     | No. of isolates | Ampicillin (10mcg) | Tetracycline (30mcg) | Cefotaxime (30mcg) | Ciprofloxacin (5mcg) | Gentamicin (30mcg) | Chloramphenicol (30mcg) | Cefepime (30mcg) | Ceftriaxone (30mcg) | Trim (5mcg) |
|--------------|-----------------|--------------------|----------------------|---------------------|-----------------------|--------------------|--------------------------|----------------|----------------------|------------|
| *S. Litchfield* | 2               | 1 (50)             | 1 (50)               | 2 (100)             | 0                     | 2 (100)            | 0                        | 0              | 0                    | 1 (50)     |
| *S. Weltevreden* | 21              | 7 (33.34)          | 12 (57.14)           | 14 (66.67)          | 1 (4.76)              | 10 (47.61)         | 2 (9.52)                 | 2 (9.52)       | 2 (9.52)             | 10 (4)     |
| *S. Newport*   | 14              | 9 (64.28)          | 7 (50)               | 7 (50)              | 0                     | 11 (78.57)         | 3 (21.42)               | 2 (14.28)      | 3 (21.42)            | 5 (35)     |
| *S. Typhi*     | 16              | 5 (31.25)          | 13 (81.25)           | 11 (68.75)          | 3 (18.75)             | 10 (62.5)          | 1 (6.25)                | 1 (6.25)       | 3 (18.75)            | 8 (50)     |
| *S. Enteritidis* | 22              | 10 (45.45)         | 12 (54.55)           | 15 (68.18)          | 1 (4.54)              | 14 (63.64)         | 0                        | 1 (4.54)       | 1 (4.54)             | 13 (5)     |
| *S. Kentucky*  | 1               | 1 (100)            | 1 (100)              | 1 (100)             | 0                     | 1 (100)            | 0                        | 0              | 0                    | 0          |
| *S. Typhimurium* | 2               | 1 (50)             | 2 (100)              | 1 (50)              | 1 (50)                | 1 (50)             | 0                        | 0              | 0                    | 1 (50)     |
| *S. Ilikan*    | 1               | 1 (100)            | 1 (100)              | 1 (100)             | 0                     | 1 (100)            | 1 (100)                 | 0              | 0                    | 1 (10)     |
| *S. Paratyphi B* | 1             | 0                  | 1 (100)              | 1 (100)             | 0                     | 1 (100)            | 0                        | 0              | 0                    | 1 (10)     |
| *S. Virchow*   | 1               | 0                  | 1 (100)              | 0                   | 0                     | 1 (100)            | 1 (100)                 | 0              | 0                    | 0          |
| Untypable      | 7               | 5 (71.43)          | 3 (42.85)            | 7 (100)             | 1 (14.28)             | 6 (85.71)          | 1 (14.28)               | 1 (14.28)      | 1 (14.28)            | 3 (42)     |
| **Total**      | **88**          | **40 (45.45)**     | **54 (61.36)**       | **60 (61.18)**      | **7 (7.95)**          | **58 (65.9)**      | **9 (10.22)**           | **7 (7.95)**   | **10 (11.36)**        | **43 (4)** |
Resistance patterns of *Salmonella* isolates from different host species against different antimicrobial agents

| Serovars       | Host       | No. of isolates | Ampicillin (10mcg) | Tetracycline (10mcg) | Cefotaxime (30mcg) | Ciprofloxacin (5mcg) | Gentamicin (30mcg) | Chloramphenicol (25mcg) | Cefepime (30mcg) | Ceftriaxone (30mcg) |
|----------------|------------|-----------------|--------------------|----------------------|-------------------|----------------------|---------------------|--------------------------|-------------------|---------------------|
| *S. Litchfield* | Poultry    | 2               | 1 (50)             | 1 (50)               | 2 (100)           | 0                    | 2 (100)            | 0                        | 0                 | 0                   |
|                | Wild bird  | 3               | 1 (33.33)          | 0                    | 2 (66.67)         | 0                    | 1 (33.33)          | 0                        | 0                 | 0                   |
| *S. Weltevreden* | Poultry    | 5               | 2 (40)             | 3 (60)               | 5 (100)           | 1 (20)               | 4 (80)             | 1 (20)                   | 0                 | 1 (20)              |
|                | Human      | 4               | 1 (28.57)          | 4 (100)              | 4 (100)           | 0                    | 2 (50)             | 0                        | 0                 | 0                   |
|                | Cattle     | 7               | 2 (28.57)          | 4 (57.14)            | 2 (28.57)         | 0                    | 3 (42.85)          | 0                        | 1 (14.28)         | 1 (14.28)           |
|                | Pig        | 1               | 0                  | 1 (100)              | 0                 | 0                    | 0                  | 1 (100)                 | 1 (100)           | 0                   |
|                | Gecko      | 1               | 1 (100)            | 0                    | 1 (100)           | 0                    | 0                  | 0                        | 0                 | 0                   |
| *S. Newport*   | Poultry    | 3               | 1 (33.33)          | 1 (33.33)            | 1 (33.33)         | 0                    | 2 (66.67)          | 1 (33.33)                | 1 (33.33)         | 1 (33.33)           |
|                | Cattle     | 9               | 7 (77.78)          | 5 (55.55)            | 4 (44.44)         | 0                    | 7 (77.78)          | 1 (11.11)                | 0                 | 0                   |
|                | Human      | 2               | 1 (50)             | 1 (50)               | 2 (100)           | 0                    | 2 (100)            | 1 (50)                   | 1 (50)            | 2 (100)             |
| *S. Typhi*     | Human      | 16              | 5 (31.25)          | 13 (81.25)           | 11 (68.75)        | 3 (18.75)            | 10 (62.5)          | 1 (6.25)                 | 1 (6.25)          | 3 (18.75)           |
| *S. Enteritidis* | Wild bird  | 4               | 2 (50)             | 1 (25)               | 2 (50)            | 2 (50)              | 0                  | 0                        | 1 (25)            | 1 (25)              |
|                | Poultry    | 14              | 8 (57.14)          | 7 (50)               | 9 (64.28)         | 1 (7.14)             | 8 (57.14)          | 0                        | 0                 | 0                   |
|                | Cattle     | 2               | 0                  | 2 (100)              | 2 (100)           | 0                    | 2 (100)            | 0                        | 0                 | 0                   |
|                | Pig        | 1               | 0                  | 1 (100)              | 1 (100)           | 0                    | 1 (100)            | 0                        | 0                 | 0                   |
|                | Human      | 1               | 0                  | 1 (100)              | 1 (100)           | 1 (100)             | 0                  | 0                        | 0                 | 0                   |
| *S. Kentucky* | Tiger      | 1               | 1 (100)            | 1 (100)              | 1 (100)           | 0                    | 1 (100)            | 0                        | 0                 | 0                   |
| *S. Typhimurium* | Human    | 2               | 1 (50)             | 2 (100)              | 1 (50)            | 1 (50)              | 1 (50)             | 0                        | 0                 | 0                   |
| *S. Idikan*    | Human      | 1               | 1 (100)            | 1 (100)              | 1 (100)           | 0                    | 1 (100)            | 1 (100)                  | 0                 | 0                   |
| *S. Paratyphi* | Human      | 1               | 0                  | 1 (100)              | 1 (100)           | 0                    | 1 (100)            | 0                        | 0                 | 0                   |
| *S. Virchow*   | Mouse      | 1               | 0                  | 1 (100)              | 0                 | 0                    | 1 (100)            | 1 (100)                 | 0                 | 0                   |
| Untypable      | Poultry    | 1               | 1 (100)            | 1 (100)              | 1 (100)           | 0                    | 1 (100)            | 0                        | 0                 | 0                   |
|                | Pig        | 3               | 1 (33.33)          | 1 (33.33)            | 3 (100)           | 3 (100)             | 2 (66.67)          | 1 (33.33)                | 0                 | 1 (33.33)           |
|                | Wild bird  | 3               | 3 (100)            | 1 (33.33)            | 3 (100)           | 3 (100)             | 1 (33.33)          | 3 (100)                  | 0                 | 1 (33.33)           |

A total of 61 (69.32 %) isolates were found to be multi-drug resistant (MDR) and one among these isolates showed resistance to four antimicrobial agents (Cefotaxim, Tetracycline, Gentamicin and Ampicillin). *Salmonella* Weltevreden isolates showed resistance to Cefotaxime (92.31%) and Tetracycline (69.23%). Among the human isolates, 100% resistance was observed against Cefotaxime, Trimethoprim and Tetracycline. However, no resistance was shown against Ceftriaxone and Cefepime. Among the poultry isolates, 100% resistance was observed against Cefotaxime, while no resistance was shown against Cefepime. *Salmonella* Newport isolates showed 100% resistance against Gentamicin and 77.78% resistance against both Ampicillin and Cefotaxime. Among the cattle isolates, Gentamicin and Ampicillin resistance was found to be 100%, Cefotaxime resistance was 80% with no resistance to Ceftriaxone. Among *S. Enteritidis* isolates, Cefotaxime, Trimethoprim and Ampicillin resistance was found to be 100%, 92.86% and 35.71%, respectively. Among the poultry isolates, Ampicillin, Cefotaxime and Tetracycline resistance was found to be 50%, 100% and 60%, respectively. In *S. Typhi*, which is a human host-specific serovar; 83.33 % resistance was found against Cefotaxime, Gentamicin and Tetracycline each, while resistance to Chloramphenicol and Ceftriaxone were found to be 8.33% and 25%, respectively.

**Mic Determined By E- Test**

Most of the isolates had a MIC value of $≤ 0.125 \mu g/ml$ for Cefepime, Cefotaxim, Ciprofloxacin, Ceftriaxone and Co-trimoxazole followed by MIC value of 0.25 $\mu g/ml$ for Ampicillin and Tetracycline. The MIC values of *S. Newport* for all antimicrobial agents were found between 1$\mu g/ml$ and 16$\mu g/ml$, for *S. Typhi* the values were between 0.5 $\mu g/ml$ and 16 $\mu g/ml$, for *S. Weltevreden* between 0.25 $\mu g/ml$ and 16 $\mu g/ml$ with only three isolates showing MIC values greater than 256 $\mu g/ml$. For *S. Enteritidis*, Ampicillin and Tetracycline MIC values were mostly found between 0.5 $\mu g/ml$ and 8 $\mu g/ml$ with three isolates showing MIC values greater than 256 $\mu g/ml$. Chloramphenicol and Gentamicin MIC values for *S. Newport, S. Weltevreden, and S. Enteritidis* were between 0.5 $\mu g/ml$ and 4
µg/ml, whereas the corresponding values were between 0.5 µg/ml and 8 µg/ml for S. Typhi isolates (Figures 3). For S. Typhimurium, the MIC values were found to be between ≤ 0.125µg/ml and 4 µl/ml for Cefepime, Cefotaxim, Ciprofloxacin, Ceftriaxone and Co-trimoxazole, while for the rest of the drugs, it varied from 4 µl/ml to 32 µl/ml (Table 9).

| Antibiotics | Minimum Inhibitory Concentration (MIC) Distribution (µg/ml) of 88 Salmonella isolates |
|-------------|---------------------------------------------------------------------------------|
| AMP         | ≤ 0.125 0.25 0.5 1 2 4 8 16 32 64 128 ≥ 256                                    |
| TET         | 0 0 0 23 33 12 4 7 4 2 1 0 2                                               |
| CPM         | 64 15 0 3 1 0 1 0 0 0 0 4                                                   |
| CTX         | 65 19 0 0 0 0 0 0 2 1 1                                                   |
| CIP         | 65 12 1 0 0 2 2 1 5 0 0 0                                                   |
| CHL         | 0 0 1 1 49 36 1 0 0 0 0 0                                                   |
| GEN         | 1 2 22 51 6 1 5 0 0 0 0 0                                                   |
| CTR         | 83 0 1 0 0 0 0 4 0 0 0 0                                                   |
| COT         | 85 3 0 0 0 0 0 0 0 0 0 0                                                   |

**Detection Of Resistance Genes**

*Salmonella* isolates showing resistance to Ampicillin, Tetracycline and Trimethoprim were further subjected to PCR for detection of resistance genes (*blaTEM*, *blaPSE* and *blaOXA*, *tetA*, *tetB* and *tetG; drfA1, drfA12*) corresponding to their phenotypic resistance patterns. Out of 41 isolates showing resistance to ampicillin, 2 (4.88%) showed presence of *blaOXA* gene, 1 (2.44%) showed *blaPSE* and 28 (63.64%) showed *BlaTEM* gene. Only two isolates of serovar S. Enteritidis from poultry showed presence of *blaOXA* and one isolate of S. Typhi from human showed presence of *blaPSE*. The two S. Newport isolates from human and poultry did not possess *blaTEM* gene. Similarly, three S. Weltevreden isolates from poultry and one isolate each from wild birds and Gecko gecko belonging to the same serovar did not show presence of *blaTEM* gene. All the isolates (100%) of S. Litchfield, S. Typhi, S. Typhimurium, S. Kentucky and S. Idikan as well as the untypable isolates showed presence of *blaTEM* gene.

Out of 54 isolates showing resistance to tetracycline, 9 (16.67%) showed presence of *tetA* gene and 1 (1.85%) showed presence of *tetG* gene, whereas none showed presence of *tetB* gene. Only one untypable isolate from poultry showed presence of *tetG* gene. However, none of the isolates of S. Newport, S. Kentucky, S. Idikan, S. Paratyphi B and S. Virchow showed presence of *tetA* gene. Out of the 44 isolates showing resistance to trimethoprim, 5 (11.36%) showed presence of resistance gene *drfA1* but none showed presence of *drfA12*. The *drfA1* gene was detected in two isolates each of S. Typhi and S. Enteritidis, and one isolate of S. Typhimurium (Table 10).
Table 10
Screening of *Salmonella* isolates from different sources for resistance genes against different antimicrobial agents

| Serovars               | No. of isolates showing resistance | No. of isolates showing positive for ampicillin resistant genes | Serovars               | No. of isolates showing resistance | No. of isolates showing positive for tetracycline resistance genes | Serovars               | No. of isolates showing resistance | No. of trr resistance gen |
|------------------------|-----------------------------------|---------------------------------------------------------------|------------------------|-----------------------------------|---------------------------------------------------------------|------------------------|-----------------------------------|---------------------------|
| S. Litchfield          | 1                                 | 1 (100)                                                       | S. Litchfield          | 1                                 | 1 (100)                                                       | S. Newport            | 7                                  | 0                         |
| S. Newport             | 9                                 | 7 (77.78)                                                     | S. Newport             | 7                                 | 0                                                             | S. Newport            | 5                                  | 0                         |
| S. Enteritidis         | 11                                | 2 (18.18)                                                     | S. Enteritidis         | 12                                | 2 (16.67)                                                     | S. Enteritidis        | 13                                 | 2                         |
| S. Typhi               | 4                                 | 1 (25)                                                        | S. Typhi               | 13                                | 1 (7.69)                                                      | S. Typhi              | 8                                  | 2                         |
| S. Weltevreden         | 8                                 | 2 (25)                                                        | S. Weltevreden         | 12                                | 2 (16.67)                                                     | S. Typhimurium        | 1                                  | 1                         |
| Untypable              | 5                                 | 5 (100)                                                       | Untypable              | 3                                 | 2 (16.67)                                                     | S. Paratyphi B        | 1                                  | 0                         |
| S. Kentucky            | 1                                 | 1 (100)                                                       | S. Kentucky            | 1                                 | 0                                                             | S. Idikan             | 1                                  | 0                         |
| S. Typhimurium         | 1                                 | 1 (100)                                                       | S. Typhimurium         | 2                                 | 1 (50)                                                        | S. Litchfield         | 1                                  | 0                         |
| S. Idikan              | 1                                 | 1 (100)                                                       | S. Idikan              | 1                                 | 0                                                             | Untypable             | 3                                  | 0                         |
| S. Paratyphi B         | 1                                 | 1 (100)                                                       | S. Paratyphi B         | 1                                 | 0                                                             |                       |                                    |                            |
| S. Virchow             | 1                                 | 1 (100)                                                       |                       |                                    |                                                               |                       |                                    |                            |
| **Total**              | **41**                            | **28 (63.64)**                                                | **54**                 | **9**                             | **1 (1.85)**                                                  | **44**                | **5**                             |                           |

**Virulence Gene Detection**

All the 88 *Salmonella* isolates were subjected to simplex PCR for detection of 11 important virulence genes (Table 11). The different serovars of *Salmonella* showed variability in their virulence gene profiles. While invA was used as the internal control for molecular detection of *Salmonella*, virulence genes sipA, sipB, sipC, strep and T2544 were also detected in all (100%) the *Salmonella* isolates, while fepA gene was present in 57 (64.77%) isolates belonging to serovars Enteritidis (12), Weltevreden (14), Typhi (14), Newport (8), Litchfield and Idikan (one isolate each), and Typhimurium (2) and the untypable (5) isolates. The rest four virulence genes sopB (86.36%), sopE (62.5%), pefA (79.54%) and sefC (51.14%) were found to be present in varying percentage among the *Salmonella* serovars. Maximum numbers (5) of the 17 isolates carrying all the eleven genes under study were recovered from wild birds and human. Out of all the 88 isolates screened, a total of 11 (12.5%) isolates belonging to serovars Weltevreden (7) and Typhi (4) were found to be positive for all eleven genes, while three other untypable isolates also carried all eleven genes.
Salmonella infections are one of the major global public health problems. During the last decade, antimicrobial resistance and multi-drug resistance of Salmonella spp. have increased to a great extent, especially in the developing countries commensurating with increased and indiscriminate use of antimicrobial agents in the treatment of humans and animal diseases. In the present study, an attempt was made to isolate Salmonella from faecal and intestinal samples of human, animals and birds, to study serotype distribution, their antimicrobial resistance patterns, and to detect important antibiotic resistance genes and Salmonella-specific virulence genes. Out of 83 diarrhoeic stool samples collected from human, 27 (32.53%) were positive for Salmonella. While working in the same geographical region, Borah (2017) could recover Salmonella from 45.23 per cent of diarrhoeic stool samples from man, while Purkayastha (2013) recovered the organism from 46.67 per cent of diarrhoeic human stool samples. Out of the 165 faecal samples collected from diarrhoeic cattle, 18 (10.90%) yielded Salmonella, while Borah (2017) reported isolation of Salmonella from 6.34 per cent of bovine faecal samples. The percentage of recovery of the organism from faecal samples of diarrhoeic cattle observed in the present study was slightly higher, which might be due to the larger sample size included in the present study compared to the earlier studies reported from this part of the country. Out of 60 diarrhoeic faecal samples collected from pig, 5 (8.30%) were positive for Salmonella. A total of 10.63% Salmonella were isolated from the rectal swabs of 94 diarrhoeic piglets (Rahman et al. 2006).

Out of the 302 faecal/intestinal samples collected from diarrhoeic poultry, 17 (5.63%) yielded Salmonella. There have been reports of recovery of Salmonella from as low as 2.90 per cent (Esteban et al. 2008) and as high as 42% (Chen et al. 2020) from different parts of the world. The moderate to high prevalence of Salmonella in poultry recorded in this region might be attributed to the favourable climatic conditions like high humidity, high rainfall and to the fact that poultry is the major reservoir of Salmonella (Rahman et al. 2006). Out of the 88 isolates, 21 (23.86%) belonged to the serovar S. enterica subsp. enterica serovar Weltevreden with the antigenic structure 3(10), r; 22 (25%) isolates belonged to serovar Enteritidis with the antigenic structure 9,12:g,m:-; 16 (18.20%) belonged to serovar Typhi with antigenic structure 9,12,V3:5-; and 14 (15.90%) isolates belonged to serovar Newport with the antigenic structure 6,8,e,h,1,2, while 7 (7.95%) isolates were found to be untypable. Salmonella Enteritidis is reported as the most common serotype worldwide (65% of the isolates), followed by S. Typhimurium (12%) (Galantis et al. 2006). This was in close agreement with the present findings. In animals, Typhimurium is the commonest serovar recovered in India followed by Weltevreden (Kumar et al. 2009). This was also in partial agreement with the present findings.

Table 11

| Strains      | No of isolates | Genes |
|--------------|----------------|-------|
|              |                | invA | stn | t2544 | fepA | pefA | sopE | sopB | sefC | sipA | sipB | sipC |
| S. Litchfield | 2              | 2    | 2   | 2     | 1    | 1    | 0    | 1    | 0    | 2    | 2    | 2     |
|              | S. Weltevreden  | 21   | 21  | 21    | 14   | 18   | 12   | 17   | 12   | 21   | 21   | 21    |
|              | S. Newport      | 14   | 14  | 14    | 8    | 13   | 11   | 14   | 14   | 12   | 14   | 14    |
|              | S. Typhi        | 16   | 16  | 16    | 14   | 14   | 14   | 14   | 14   | 14   | 16   | 16    |
|              | S. Enteritidis  | 22   | 22  | 22    | 12   | 14   | 11   | 14   | 20   | 15   | 14   | 22    |
|              | S. Kentucky     | 1    | 1   | 1     | 1    | 1    | 1    | 0    | 1    | 0    | 1    | 0     |
|              | S. Typhimurium  | 2    | 2   | 2     | 2    | 2    | 1    | 1    | 2    | 2    | 2    | 2     |
|              | S. Idikan       | 1    | 1   | 1     | 1    | 1    | 1    | 0    | 1    | 0    | 1    | 1     |
|              | S. Paratyphi B  | 1    | 1   | 1     | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0     |
|              | S. Virchow      | 1    | 1   | 1     | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0     |
| Untypable    | 7               | 7    | 7   | 7     | 5    | 6    | 5    | 6    | 5    | 7    | 7    | 7     |
| Total        | 88              | 88   | 88  | 88    | 57   | 76   | 70   | 55   | 76   | 45   | 88   | 88    | 88    | 88    | (100%) | (100%) | (100%) | (100%) | (100%) | (100%) | (100%) | (100%) | (100%) | (100%) |

The distribution of virulence genes according to the source of recovery of the isolates revealed that invA, sipA, sipB, sipC, stn and T2544 genes were present in all the isolates irrespective of their source of origin. The sefC gene is present in 45 (51.14%) isolates from human, cattle, wild bird, pig, tiger and poultry. The genes sopB and sopE were present in 76 (86.36%) and 55 (62.50%) isolates, respectively, while pefA, sefC and pefA genes were present in 70 (79.54%), 45 (51.14%) and 57 (64.77%) isolates, respectively recovered from human, cattle, wild bird, pig, tiger, Gecko gecko and poultry. Currently, T2544 and sopE are re-annotated as pagN and sopE2, respectively.

Discussion

Salmonella infections are one of the major global public health problems. During the last decade, antimicrobial resistance and multi-drug resistance of Salmonella spp. have increased to a great extent, especially in the developing countries commensurating with increased and indiscriminate use of antimicrobial agents in the treatment of humans and animal diseases. In the present study, an attempt was made to isolate Salmonella from faecal and intestinal samples of human, animals and birds, to study serotype distribution, their antimicrobial resistance patterns, and to detect important antibiotic resistance genes and Salmonella-specific virulence genes. Out of 83 diarrhoeic stool samples collected from human, 27 (32.53%) were positive for Salmonella. While working in the same geographical region, Borah (2017) could recover Salmonella from 45.23 per cent of diarrhoeic stool samples from man, while Purkayastha (2013) recovered the organism from 46.67 per cent of diarrhoeic human stool samples. Out of the 165 faecal samples collected from diarrhoeic cattle, 18 (10.90%) yielded Salmonella, while Borah (2017) reported isolation of Salmonella from 6.34 per cent of bovine faecal samples. The percentage of recovery of the organism from faecal samples of diarrhoeic cattle observed in the present study was slightly higher, which might be due to the larger sample size included in the present study compared to the earlier studies reported from this part of the country. Out of 60 diarrhoeic faecal samples collected from pig, 5 (8.30%) were positive for Salmonella. A total of 10.63% Salmonella were isolated from the rectal swabs of 94 diarrhoeic piglets (Rahman et al. 2006).

Out of the 302 faecal/intestinal samples collected from diarrhoeic poultry, 17 (5.63%) yielded Salmonella. There have been reports of recovery of Salmonella from as low as 2.90 per cent (Esteban et al. 2008) and as high as 42% (Chen et al. 2020) from different parts of the world. The moderate to high prevalence of Salmonella in poultry recorded in this region might be attributed to the favourable climatic conditions like high humidity, high rainfall and to the fact that poultry is the major reservoir of Salmonella (Rahman et al. 2006). Out of the 88 isolates, 21 (23.86%) belonged to the serovar S. enterica subsp. enterica serovar Weltevreden with the antigenic structure 3(10), r; 22 (25%) isolates belonged to serovar Enteritidis with the antigenic structure 9,12:g,m:-; 16 (18.20%) belonged to serovar Typhi with antigenic structure 9,12,V3:5-; and 14 (15.90%) isolates belonged to serovar Newport with the antigenic structure 6,8,e,h,1,2, while 7 (7.95%) isolates were found to be untypable. Salmonella Enteritidis is reported as the most common serotype worldwide (65% of the isolates), followed by S. Typhimurium (12%) (Galantis et al. 2006). This was in close agreement with the present findings. In animals, Typhimurium is the commonest serovar recovered in India followed by Weltevreden (Kumar et al. 2009). This was also in partial agreement with the present findings.
Higher level of resistance shown by the *Salmonella* isolates to ampicillin, gentamicin, cefotaxime and tetracycline might be attributed to frequent and long-term use of these drugs as therapeutic agents both in man and in animals. Although chloramphenicol has long been used as one of the drugs of choice for treatment of salmonellosis in human, it was interesting to observe that lesser number of the isolates tested in the present study exhibited resistance to this drug. This might be attributed to the current trend of prescribing fluoroquinolones like ciprofloxacin more frequently in human medicine to treat cases of enteric fever. Comparatively higher percentage of isolates from cattle and poultry were found to exhibit resistance to most of the antimicrobial agents tested. It might be due to more frequent use of antimicrobial therapy in these two species compared to other species to control and prevent infectious diseases. Recently, Chloramphenicol, Ampicillin and Trimethoprim resistant *S. Typhi* that were also Ceftriaxone resistant created havoc in Pakistan and Bangladesh (Klemm et al. 2018). Here in our study also, we have recovered a few isolates showing the same resistance pattern, which is alarming. Higher level of resistance to Ampicillin and Tetracycline reported by many workers (Li et al. 2019) corroborated with the present findings. *Salmonella* Enteritidis causes gastroenterological disorders in human and their antimicrobial resistance is a huge concern. In the present study, Ampicillin (45.45%), Tetracycline (54.55%), Cefotaxime (68.18%), Gentamicin (63.34%) and Trimethoprim (59.09%) resistance was recorded among *S. Enteritidis* isolates. Findings by AMRSN showing a nationwide downwards trend in India for resistance to Ampicillin, Chloramphenicol and trimethoprim-sulfamethaxone among *S. Typhi* (Walia et al. 2019) was in complete agreement with the present findings. *Salmonella* Weltevreden is found equally both in human and animals, and has importance in both as a pathogen. Wang et al. (2019) stated that antibiotic resistance behaves differently in different hosts for the same serovar. In the present study, *S. Weltevreden* showed less resistance towards Ampicillin, Ciprofloxacin, Chloramphenicol and Cephalosporins. This is indeed a good sign for the region as treatment options are still available; this was in agreement with the findings of Li et al. (2018). *Salmonella* Weltevreden, *S. Typhi*, *S. Newport* and *S. Enteritis* are the most frequently isolated serovars in this study. It was observed that Cefotaxime, a 3rd generation cephapolorspin, was less effective against all these isolates. However Ceftriaxone, another 3rd generation cephalosporin, along with Cefepime, a 4th generation cephalosporin, was effective against most of the isolates, irrespective of host and serovars. Interestingly, a moderate level of resistance was shown by the isolates to Ampicillin. This may be due to the current practice of very limited use of a β lactam antibiotics without a β lactamase inhibitor (like Clavulanic acid) in the treatment regime for human and livestock diseases. Most of the isolates from human and cattle were resistant to Gentamicin but moderate resistance was observed in case of isolates from other host species. This may be due to the abundant use of the drug in these two species for treatment purpose. Ciprofloxacin, a fluoroquinolone, has still remained effective, as less resistance was shown by the isolates of all serovars against this drug. Isolates from Cattle, Poultry and Pig showed moderate level of resistance to Chloramphenicol, a lifesaving ICU category drug and very low resistance was exhibited by the isolates from human, particularly of serovars *S. Weltevreden* and *S. Newport*.

The increase in antimicrobial resistance is a threat to global health. Trimethoprim is commonly used in the treatment of urinary tract infections (UTI) in all parts of the world. However, soon after the introduction of the drug, trimethoprim resistance was reported in several species (Skold, 2001). In the present study, 41, 54 and 44 phenotypically positive *Salmonella* isolates which showed resistance to Ampicillin, Tetracycline and Trimethoprim, respectively were subjected to PCR for detection of resistance genes conferring resistance to these drugs. Out of 41 isolates showing resistance to ampicillin, 28 (63.64%) showed presence of **blaTEM** gene, while only 2 (4.88%) showed presence of **blaOXA** gene and 1 (2.44%) showed presence of **blaPSE** gene. The presence of **blaTEM** in all the isolates resistant to Ampicillin as observed in the present study suggested that Ampicillin resistance in majority of *Salmonella* is mediated by this gene. Out of 54 isolates showing resistance to tetracycline, 9 (16.67%) showed presence of **tetA** gene and 1 (1.85%) showed presence of **tetG** gene, whereas, none of the isolates showed presence of **tetB** gene. This suggested involvement of some other resistance determinants in the other isolates that conferred resistance to tetracycline. Out of the 44 isolates showing resistance to trimethoprim, 5 (11.36%) showed presence of resistance gene **drfA12** but none showed presence of **drfA1**. Trimethoprim resistance in *Salmonella* is said to be mostly mediated by **drfA1** gene (Brolund et al. 2010).

It has been observed that disruption of genes in *Salmonella* Pathogenicity Island (SPI) I of *S. Typhimurium* and *S. Dublin* blocks the secretion of *Salmonella* invasive proteins. The virulence genes of *Salmonella* spp. encoding five different Sips (*Salmonella* invasion protein) namely sipA, *B*, *C*, *D* and *E* are capable of inducing apoptosis in macrophage (Kaur et al. 2012), and hence may play a vital role in *Salmonella* pathogenesis. Since **stn** has been found to localize and transcribe in juxtaposition to the gene, which encodes the dehydrogenase regulatory protein, a common and related protein among enteric micro-organisms, it is anticipated that **stn** determinant might be prevalent among all salmonellae (Prager et al. 1995). This gene in *Salmonella* is one of the chromosomally encoded genes that codes for production of enterotoxins. Observations from the present study indicated that the **stn** gene is universally present among all the *Salmonella* isolates, irrespective of the serovars, which was in agreement with Prager et al. (1995), and Murugkar et al. (2003). In the present study, **sopB** gene was detected in 86.36% of the *Salmonella* isolates which was in agreement with the findings of Rahman et al. (2006) who reported that all 50 isolates of *S. enterica* belonging to 11 serovars carried **sopB** gene, irrespective of their serovars and source of isolation. The **sopC** gene was present in 51.14% of the isolates. The **sop**ABC genes make up part of a complex **sop** operon responsible for the expression and assembly of **SEF14** fimbriae (Clouthier et al. 1993).

Conclusions

In our study, we examined the prevalence of *Salmonella* and antimicrobial resistant isolates in the fecal samples of human, animals and birds. Our findings showed a high prevalence and serotype diversity in the area under study. Serovars *S. Enteritidis*, *S. Weltevreden* and *S. Typhi* were the most common serovars. Comparatively higher percentage of isolates from cattle and poultry exhibited resistance to most of the antimicrobial agents tested. It might be attributed to more frequent and rather indiscriminate use of antimicrobial agents in these two species to control and prevent infectious diseases. Moreover, the recovered *Salmonella* isolates exhibiting multi-drug resistance and multiple virulence genes suggested a possible risk to human and animals. Therefore, it is important to rationalize the use of antimicrobial agents to prevent vertical and horizontal transfer of MDR strains across host species. Presence of multiple virulence genes in different combinations in all the field isolates of *Salmonella* as revealed by the present study indicated their possible role as a pathogen in the host species. However, a molecular level phylogenetic analysis is needed to establish the inter-serovar and inter-host relationships among the *Salmonella* isolates.

Declarations
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Consent to participate: Verbal consent from the authority of each farm and individual was taken before collecting the samples.

Consent for publication: We give our consent for the publication of the submitted manuscript

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Figures

![Salmonella Serotypes](image)

**Figure 1**

Frequency of isolation of different serovars of Salmonella from human.
Figure 2

Frequency of isolation of different serovars of Salmonella from cattle

Figure 3

MIC data of (A) Salmonella Enteritidis, (B) Salmonella Newport, (C) Salmonella Weltevreden (D) Salmonella Typhi