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

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Abstract

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. *enterica* serovar Weltevreden, 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 untypable. 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 ceftriaxone, 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, Ceftriaxone and Co-trimoxazole and a moderate MIC (0.5µg/ml – 4µg/ml) of Ampicillin, Tetracycline, Gentamicin and Chloramphenicol. The resistance genes, *bla*TEM, *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 diarrhoea. *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 (Klemm et al. 2018) with the presence of XDR strains resistant to Ceftriaxone 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 Carry-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'-ACCACGCTCTTTCGTCTGG-3' and 5'GAACTGACTACGTAGACGCTC-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 MICTM (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 MICTM 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*, *bla*^{TEM}, *blaPSE*⁻¹, *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 Abony	NCTC 6017
10	Salmonella Idikan	NICED 503984
11	Salmonella Infantis	NICED 505330
12	Salmonella Paratyphi A	NICED C6915
13	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
<i>blaOXA</i> (AMP)	F-AGCAGCGCCAGTGCATCA R- ATTCGACCCCAAGTTTCC	708	60	Guerra <i>et al.</i> (2001)
<i>blaTEM</i> (AMP)	F- TTGGGTGCACGAGTGGGT R- TAATTGTTGCCGGAAGC	504	57	Arlet and Phillippon (1991)
<i>blaPSE-1</i> (AMP)	F-CGCTTCCCGTTAACAAGTAC R-CTGGTTCATTTAGATAGCG	420	58	Sandvang <i>et al.</i> (1997)
<i>dfrA1</i> (TR)	F- GTGAACTATCACTAATGG R- CCCTTTTGCCAGATTTGG	470	50	Guerra <i>et al.</i> (2001)
<i>dfrA12</i> (TR)	F- ACTCGGAATCAGTACGCA R- GTGTACGGAATTACAGCT	463	50	Guerra <i>et al.</i> (2001)
<i>tet (A)</i>	F- GCTACATCCTGCTTGCTT R- CATAGATCGCCGTGAAGA	210	57	Ng <i>et al.</i> (1999)
<i>tet (B)</i>	F-TTGGTTAGGGGCAAGTTTTG R-GTAATGGGCAATAACACCG	659	57	Ng <i>et al.</i> (1999)
<i>tet (G)</i>	F- GCTCGGTGGTATCTCTGC R- AGCAACAGAATCGGAAC	468	57	Ng <i>et al.</i> (1999)

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)
<i>sipA</i>	Forward – CGCAAATCATAAAAGATGGC Reverse-CGGCTATTATCAATCGTCTT	20	646
<i>sipB</i>	Forward – CTAACGTGCTGAAACAGTTG Reverse-AACGCCACTTTATTTAGGGT	10	160
<i>sipC</i>	Forward – TCTTAATGATGCGACGCTTA Reverse-TGGTCGATTTACGTGAACTT	10	365
<i>stn</i>	Forward -ATTGAGCGCTTTAATCTCCT Reverse- GCTGTTGAATCTGTACCTGA	10	543
<i>sopB</i>	Forward - GCATCTCTAAACGCTACTG Reverse- GCTTCTATCACTCAGCTTCA	10	470
<i>sopE</i>	Forward - GTAGGGCAGTATTAACCAG Reverse- TTTATCTCCCTAGGTAGCCC	10	254
<i>pefA</i>	Forward - CCAAAGTACTGGTTGAAAG Reverse- TATTTGTAAGCCACTGCGAA	20	185
<i>sefC</i>	Forward - GCAGGTCCAAAACATACA Reverse- CGATAACGAAACACCATTT	10	609
<i>fepA</i>	Forward -CGACGTGTCAGAAATCATCC Reverse- GCCTTCGTCTTTATGTTCCG	10	382
<i>pagN</i>	Forward -GAATCAACTCAACCTTCAGC Reverse-AGTCATAACCGATAGCAACC	10	105

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 Newport (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	<i>Salmonella</i> Enteritidis	9,12:g,m	22	Cattle (2), Pig (1), Human (2), Poultry (13), Wild Birds (4)
2	<i>Salmonella</i> Weltevreden	3{10},r,z ₆	21	Poultry (5), Human (4), Cattle (7), Pig (1), Wild Birds (3), <i>Gecko gecko</i> (1)
3	<i>Salmonella</i> Typhi	9,12,Vi,d	16	Human
4	<i>Salmonella</i> Newport	6, 8,e, h, 1, 2	14	Cattle (9), Human (2), Poultry (3)
5	<i>Salmonella</i> Typhimurium	1,4,5,12,i,1,2	2	Human
6	<i>Salmonella</i> Litchfield	8,6,8,l,v,1,2	2	Poultry
7	<i>Salmonella</i> Kentucky	8,8,20,l,z ₆	1	Tiger
8	<i>Salmonella</i> Idikan	13,1,3,23,i,1,5	1	Human
9	<i>Salmonella</i> Paratyphi B	1,4,5,12,b,1,2	1	Human
10	<i>Salmonella</i> Virchow	7,6,7,14,r,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)	Trimethoprim (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 (47.61)
S. Newport	14	9 (64.28)	7 (50)	7 (50)	0	11 (78.57)	3 (21.42)	2 (14.28)	3 (21.42)	5 (35.71)
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 (59.09)
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. Idikan	1	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)
S. Paratyphi B	1	0	1 (100)	1 (100)	0	1 (100)	0	0	0	1 (100)
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 (42.85)
	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 (48.86)

Table 8
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)	0	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 B	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)	1 (33.33)	3 (100)	0	1 (33.33)	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 $\mu\text{g/ml}$ for Cefepime, Cefotaxim, Ciprofloxacin, Ceftriaxone and Co-trimoxazole followed by MIC value of 0.25 $\mu\text{g/ml}$ for Ampicillin and Tetracycline. The MIC values of *S. Newport* for all antimicrobial agents were found between 1 $\mu\text{g/ml}$ and 16 $\mu\text{g/ml}$, for *S. Typhi* the values were between 0.5 $\mu\text{g/ml}$ and 16 $\mu\text{g/ml}$, for *S. Weltevreden* between 0.25 $\mu\text{g/ml}$ and 16 $\mu\text{g/ml}$ with only three isolates showing MIC values greater than 256 $\mu\text{g/ml}$. For *S. Enteritidis*, Ampicillin and Tetracycline MIC values were mostly found between 0.5 $\mu\text{g/ml}$ and 8 $\mu\text{g/ml}$ with three isolates showing MIC values greater than 256 $\mu\text{g/ml}$. Chloramphenicol and Gentamicin MIC values for *S. Newport*, *S. Weltevreden*, and *S. Enteritidis* were between 0.5 $\mu\text{g/ml}$ 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).

Table 9
Antimicrobial resistance phenotypes of Salmonella isolates (n = 88).

Antibiotics	Minimum Inhibitory Concentration (MIC) Distribution (µg/ml) of 88 <i>Salmonella</i> isolates											
	≤ 0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
AMP	0	1	5	23	27	7	6	5	2	1	0	11
TET	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	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	0	4	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 resistance genes
		blaOXA	blaPSE	blaTEM			tetA	tetB	tetG			
S. Litchfield	1	0	0	1 (100)	S. Litchfield	1	1 (100)	0	0	S. Weltevreden	11	0
S. Newport	9	0	0	7 (77.78)	S. Newport	7	0	0	0	S. Newport	5	0
S. Enteritidis	11	2 (18.18)	0	6 (54.54)	S. Enteritidis	12	2 (16.67)	0	0	S. Enteritidis	13	2
S. Typhi	4	0	1 (25)	4 (100)	S. Typhi	13	1 (7.69)	0	0	S. Typhi	8	2
S. Weltevreden	8	0	0	2 (25)	S. Weltevreden	12	2 (16.67)	0	0	S. Typhimurium	1	1
Untypable	5	0	0	5 (100)	Untypable	3	2 (16.67)	0	1 (33.33)	S. Paratyphi B	1	0
S. Kentucky	1	0	0	1 (100)	S. Kentucky	1	0	0	0	S. Idikan	1	0
S. Typhimurium	1	0	0	1 (100)	S. Typhimurium	2	1 (50)	0	0	S. Litchfield	1	0
S. Idikan	1	0	0	1 (100)	S. Idikan	1	0	0	0	Untypable	3	0
					S. Paratyphi B	1	0	0	0			
					S. Virchow	1	0	0	0			
	41	2 (4.88)	1 (2.44)	28 (63.64)		54	9 (16.67)	0	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*, *stn* 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.

Table 11
Distribution of virulence genes in *Salmonella* isolates of different serovars isolated from various sources

Strains	No of isolates	Genes										
		invA	stn	t2544	fepA	pefA	sopE	sopB	sefC	sipA	sipB	sipC
S. Litchfield	2	2 (100%)	2 (100%)	2 (100%)	1 (50%)	1 (50%)	0	1 (50%)	0	2 (100%)	2 (100%)	2 (100%)
S. Weltevreden	21	21 (100%)	21 (100%)	21 (100%)	14 (66.67%)	18 (85.7%)	12 (57.1%)	17 (80.95%)	12 (57.1%)	21 (100%)	21 (100%)	21 (100%)
S. Newport	14	14 (100%)	14 (100%)	14 (100%)	8 (57.14%)	13 (92.86%)	11 (78.57%)	14 (100%)	5 (35.71%)	14 (100%)	14 (100%)	14 (100%)
S. Typhi	16	16 (100%)	16 (100%)	16 (100%)	14 (87.5%)	13 (81.25%)	13 (81.25%)	14 (87.5%)	6 (37.5%)	16 (100%)	16 (100%)	16 (100%)
S. Enteritidis	22	22 (100%)	22 (100%)	22 (100%)	12 (54.55%)	14 (63.64%)	11 (50%)	20(90.9%)	15 (68.18%)	22 (100%)	22 (100%)	22 (100%)
S. Kentucky	1	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)
S. Typhimurium	2	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)
S. Idikan	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
S. Paratyphi B	1	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)	0	0	1 (100%)	1 (100%)	1 (100%)
S. Virchow	1	1 (100%)	1 (100%)	1 (100%)	0	0	0	0	0	1 (100%)	1 (100%)	1 (100%)
Untypable	7	7 (100%)	7 (100%)	7 (100%)	5 (71.43%)	6 (85.71%)	5 (71.43%)	6 (85.71%)	5 (71.43%)	7 (100%)	7 (100%)	7 (100%)
Total	88	88 (100%)	88 (100%)	88 (100%)	57 (64.77%)	70 (79.54%)	55 (62.5%)	76 (86.36%)	45 (51.14%)	88 (100%)	88 (100%)	88 (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 *fepA* 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, z6; 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,Vi,d;- 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%) (Galanis 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 cephalosporin, 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 *drfA1* but none showed presence of *drfA12*. 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 *sefC* gene was present in 51.14% of the isolates. The *sefABC* genes make up part of a complex *sef* operon responsible for the expression and assembly of SEF14 fimbriae (Clouthier et al. 1993).

Conclusion

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

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Figures

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