SUPPLEMENTARY MATERIAL AVAILABLE ONLINE

Indian Journal of Animal Sciences **94** (10): 831–836, October 2024/Article
https://doi.org/10.56093/ijans.v94i10.141354

Detection of virulent and ESBL-resistant *Salmonella* species from cattle and associated farm environment

SNEHAL SUDRIK[1], RAHUL KOLHE[1✉], CHANDRAKANT BHONG[1], TEJAS SHENDE[1], SAMEER JADHAV[1], PRASHANT MHASE[1], VIKAS WASKAR[1], GANESH HINGE[1] and SNEHAL GADHAVE[1]

KNP College of Veterinary Science, Shirwal, Maharashtra Animal and Fishery Sciences University, Satara, Maharashtra 412 801 India

Received: 23 August 2023; Accepted: 2 August 2024

ABSTRACT

The aim of this study was to explore the virulence characteristics and antibiotic resistance in *Salmonella* species isolated from cattle farms. During the study, 10 cattle farms were selected and from each farm, 35 samples consisting of faeces, milk, udder swabs, hand swabs, floor swabs, animal drinking water, pit water, composite fodder sample, and dairy utensil swabs were collected. Accordingly, 350 samples were collected from 10 farms and processed for the detection of *Salmonella* spp. Presumptive *Salmonella* isolates were confirmed by PCR and characterized for virulence and antibiotic resistance genes. From 350 samples, 19 (5.40%) *Salmonellae* were recovered, and out of 19 *Salmonellae,* 12 were ESBL producers. Similarly, out of 19 isolates, *spvC* was the predominant gene detected (42.10%), followed by *stn* (31.5%), and *spvR* (26.31%), whereas, *invA,* and *iroB* genes were detected in all 19 *Salmonella* isolates. Most of the *Salmonella* isolates were multi-drug resistant strains exhibiting resistance to nalidixic acid (94.73%), tetracycline (36.84%), and ceftazidime (42.10%). Out of 12 ESBL-positive *Salmonellae*, 9 (75%) isolates harboured ESBL genes, viz. bla_{SHV} and bla_{TEM} (25% each) and $bla_{CTX\text{-}M}$ and bla_{OXA} (16.66% each). Six *Salmonella* isolates showed the presence of *tetA* gene. The current study indicated healthy cattle and the associated farm environments could be a source of virulent and drug-resistant *Salmonellae.* The current study also advocates phenotypic and genotypic antibiotic resistance monitoring at farm animal premises and agricultural ecosystems.

Keywords: Antibiotic resistance, Cattle, ESBL, *Salmonella,* Virulence

Numerous animal species are the reservoirs of non-typhoidal *Salmonella* (NTS), including cattle, and *Salmonella* species have the ability to establish lifelong infection in cattle, which is characterized by an asymptomatic carrier status with intermittent periods of bacteremia and shedding. Of the known subspecies, *S. enterica* subspecies enterica is the most relevant in dairy cattle. Salmonellosis may cause severe disease in cattle and also pose a significant zoonotic risk. Sub-clinical shedding of *Salmonella* by cattle generates occupational risk to farm workers (Srednik *et al.* 2021). *Salmonella* may enter a farm from different sources like contaminated water, litter, personnel, equipment, vehicles, rodents, insects, and pets. Cattle in dairy farms could be a potential source for the contamination of the farm environment and farm products by antibiotic-resistant bacteria like *Salmonella* spp.

Antimicrobial resistance (AMR) has been a global challenge that is being addressed extensively under the One Health paradigm. The development of AMR is a complex process that involves the aggregation of several factors. *Salmonella* spp. are among the predominant bacterial communities of *Enterobacteriaceae* family, that are resistant to critically important antimicrobials for public health. Dairy cattle and farm premises could be the reservoirs of virulent as well as drug-resistant *Salmonella* spp. and the emergence of resistant strains warrants a better understanding of the epidemiology of this pathogen. Under the umbrella of One Health, the containment of AMR required collaborative efforts to address the actual AMR burden in humans, animals, food, and also from environmental reservoirs. The current study was undertaken to detect and characterize *Salmonella* spp. isolated from cattle farms for virulence and antibiotic resistance. The goal was to learn about dairy cattle's risk of excreting pathogenic and antibiotic-resistant *Salmonella* through faeces. Earlier reports from India principally focused on phenotypic AMR studies (Taneja and Sharma 2019) however, studies on cattle-origin *Salmonella* were found to be scanty (Iyer *et al*. 2019).

MATERIALS AND METHODS

Sampling: A sampling plan comprising of a collection of samples from 10 designated cattle farms located at different

Present address: [1]KNP College of Veterinary Science, MAFSU, Shirwal, Maharashtra. ✉Corresponding author email: dr_kolherahul@rediffmail.com

places in the vicinity of the KNP College of Veterinary Science, Shirwal was designed. From a farm having at least 10 dairy animals, a minimum of five animals in milking conditions were selected. During each sampling, 35 different samples were collected which included faeces, milk, udder swabs, hand swabs, floor swabs, animal drinking water, pit water, composite fodder samples, dairy utensil swabs, and environmentally exposed agar plates (air sample). Thus, a total of 350 samples were collected from 10 different farms.

Isolation and identification of Salmonella spp: Samples were first pre-enriched in Buffered Peptone Water (BPW), followed by enrichment in Rappaport Vassiliadis (RV) medium and selective plating on Xylose Lysine Deoxycholate (XLD) agar and Hektoen enteric (HE) agar. The plates were incubated at 37°C for 24 h, three to five representative *Salmonella* colonies were picked up, purified, and confirmed by biochemical tests namely catalase, oxidase, indole, methyl red, Voges Proskauer, H_2S production on TSI agar, and citrate utilization.

Molecular detection: Bacterial DNA was extracted from all the isolates using boiling and snap-chilling method. DNA concentrations and qualities were checked using NanoDrop (Thermo Scientific, USA). All the presumptive *Salmonella* isolates were screened by PCR for the presence of *invA* and *iroB* genes as per the protocol developed by Bhaskara *et al*. (2012) and Bäumler *et al*. (1997), respectively. Similarly, other *Salmonella*-specific virulent genes namely, *spvC*, *stn*, and *spvR* were detected as per the method described by Nikiema *et al*. (2021). PCR conditions and primer sequences used for the detection of virulence and AMR genes have been shown in Table 1. The uniplex reactions were conducted in a total of 25 µL of mixture volume containing 12.5 µL 2× PCR Master Mix (Takara) supplied with *Taq*DNA polymerase, buffer, $MgCl_2$ and dNTPs. For PCR, 1 µL (10 pmol/ µL) each of forward and reverse primer and 5 µL DNA was used. Amplifications were performed as per different protocols for each targeted gene. The amplicons were analyzed using 1.5% agarose gel electrophoresis and visualized by the gel documentation system (IBright CL750, Thermo Scientific).

Antimicrobial susceptibility testing: Antibiotic susceptibility patterns of *Salmonella* spp. were studied using the Kirby-Bauer disc diffusion method. The test was performed using 12 different antibiotics, viz. ciprofloxacin (CIP: 5 µg), ceftazidime (CAZ: 30 µg), gentamicin (GEN: 10 µg), azithromycin (AZM: 15 µg), tetracycline (TE: 30 µg), trimethoprim (TR:10 µg), chloramphenicol (C: 30 µg), ceftriaxone (CTR: 30 µg), amoxicillin-clavulanic acid (AMC: 30 µg), amikacin (AK: 30 µg), enrofloxacin (EX: 10 µg), and nalidixic acid (NA: 30 µg) manufactured by HiMedia Laboratories Mumbai. *Salmonella* spp. were also studied for ESBL production using a double disk synergy test (DDST). Isolates showing the difference in the zone of inhibition ≥5 mm of cephalosporin discs and cephalosporin plus clavulanic acid-containing discs were considered potential ESBL producers.

Detection of AMR genes: Phenotypically confirmed ESBL-producing *Salmonella* were subjected to multiplex PCR targeting the beta-lactamase genes, viz. bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{OXA} using the method described by Fang *et al*. (2008). For the bla_{CMY2} protocol described by Mthembu *et al.* (2019), and for the detection of *tetA*, the protocol described by Ng *et al.* (2001) was used (Table 1).

Table 1. Oligonucleotide sequences used for the detection of virulence and AMR genes

Genes targeted	Oligonucleotide sequences (3’-5’)	Product size (bp)	PCR conditions
invA	F-TCA TCG CAC CGT CAA AGG AAC C R-GTG AAA TTA TCG CCA CGT TCG GGC AA	284	ID-94°C/1 min; D-94°C /1 min; A-58.3°C/30 s; E-72/1 min; FE-72/7 min
iroB	F- CGC CGCATA CAC TAT TCT CAG GA R-ACGCTC ACCGGCTCCAGATTTAT	606	ID-94°C/3 min; D-94°C/40 sec; A- 55°C/40 s; E-72°C/40 s; FE-72°C/5 min
spvC	F-ACTCCTTGCACAACCAAATGCGGA R-TGT CTT CTG CATTTCGCCACCATCA	571	ID-94°C//5 min; D-94°C/30 s; A-63°C/30 s; E-72°C//30 s; FE-72°C/7 min
stn	F-CTTTGGTCGTAAAATAAGGCG R-TGCCCAAAGCAGAGAGATTC	260	ID-94°C/5 min; D-94°C//30 s; A- 94°/30 s; E-72°C/30 s; FE- 72°C/7 min
spvR	F-CAGGTTCCTTCAGTATCGCA R-TTTGGCCGGAAATGGTCAGT	310	
tetA	F-GCTACATCCTGCTTGCCT R-CATAGATCGCCGTGAAGA	210	ID-94°C/3 min; D-95°C/30 s; A-60°C/30 s; E-72°C/1 min; FE-72°C/8 min
bla_{TEM}	F-CGC CGCATA CAC TAT TCT CAGAATGA R-ACGCTC ACCGGCTCCAGATTTAT	440	
$bla_{CTX\text{-}M}$	F-ATGTGCAGYACCAGTAARGTKATGGC R-GGGTRAARTARGTSACCAGAAYCAGCG	593	ID-94°C/5 min; D-94°C/45 s; A-63°C/1 min; E-72°C/1 min; FE-- 72°C/7 min
bla_{SHV}	F-CTT TAT CGG CCC TCA CTC AA R-AGGTGCTCATCATGGGAAAG	237	
bla_{OXA}	F-ACA CAA TAC ATA TCA ACT TCG C R-AGTGTGTTT AGA ATG GTGATC	813	
bla_{CMY2}	F-ATAACCACCCAGTCACGC R-CAGTAGCGAGACTGCGCA	631	ID-94°C/5 min; D-94°C//30 s; A- 60°/30 s; E-72°C/1 min; FE- 72°C/5 min

RESULTS AND DISCUSSION

The present study focussed on the isolation and molecular characterization of *Salmonella* species from cattle and their associated farm environment. Diverse types of samples collected during the study were faeces, milk, udder swabs, hand swabs, floor swabs, dairy utensil swabs, fodder, animal pit water, drinking water, and air samples. Out of 350 samples, 19 (5.42%) were positive for *Salmonella* spp. Variation in the distribution of *Salmonella* at different farms was observed, and the majority of the isolates were from the fodder. *Salmonella* could be isolated from almost all types of samples except milk and air samples. Out of 10 farms, hand swabs of two dairy farmers were also positive for *Salmonella,* which was an indication of poor personal hygiene. A study from Southern Ethiopia documented a moderate prevalence of salmonellosis in dairy cows with poor body condition, farm's husbandry hygiene, and management system (Asefa *et al.* 2023). Another study from Ethiopia recorded a high prevalence (11.2%) of *Salmonella* in various samples (udder swabs, faeces, milk, bucket swabs, etc.) collected from dairy farms (Abunna *et al.* 2018). *Salmonella* spp. are food and water-borne zoonotic pathogens infecting a wide range of vertebrates including humans and animals. Most of the animal and human infections of *Salmonella* are caused by serotypes of *S. enterica* subspecies *enterica.* In cattle, *Salmonella* can be isolated from apparently healthy animals. In our study, *Salmonella* was detected in the animal fodder samples, indicating the possible risk of *Salmonella* transmission from feed to animals. An exhaustive review of *Salmonella* in animal feeds has been published, which highlighted the importance of animal feed and fodder in the transmission of *Salmonella* (Sargeant *et al.* 2021).

All the *Salmonella* isolates confirmed based on cultural, morphological, and biochemical characteristics were further studied by uniplex PCR to detect species-specific *invA* gene (Fig. 1). Out of 19 *Salmonella* isolates, all isolates possessed both genes. In *Salmonella*, *invA* acts as a biomarker for detection purposes. It is involved in the invasion of host epithelial cells, plays a vital role in systemic infections, and plays a significant role in the virulence of the organism in the intestine (El-Sebay *et al.* 2017). Salmonellosis is rarely recorded in bovines however it may act as a career for *Salmonella* spp. Lanzas *et al.* (2010) investigated an outbreak of Salmonellosis in cattle to describe the transmission dynamics of MDR Salmonella Typhimurium after the onset of a clinical outbreak in a dairy herd. The outbreak was limited to five clinical cases, and only 18 animals out of 500 cows shed *Salmonella* in their faeces. The longest shedder was culture-positive for Salmonella for at least 68 days. The findings of the current study also affirmed that virulent *Salmonella* is prevalent in the dairy farm environment at a lesser frequency.

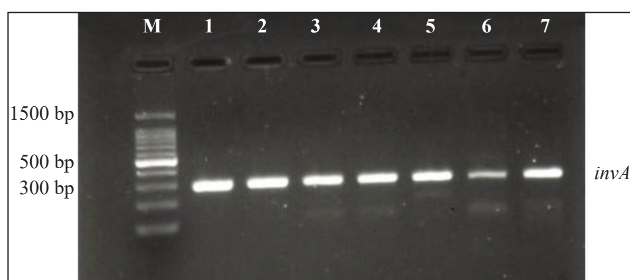

Fig. 1. Detection of *invA* gene in *Salmonella* spp [M: 100 bp DNA ladder; Lane 1-7: *invA* gene (284 bp)].

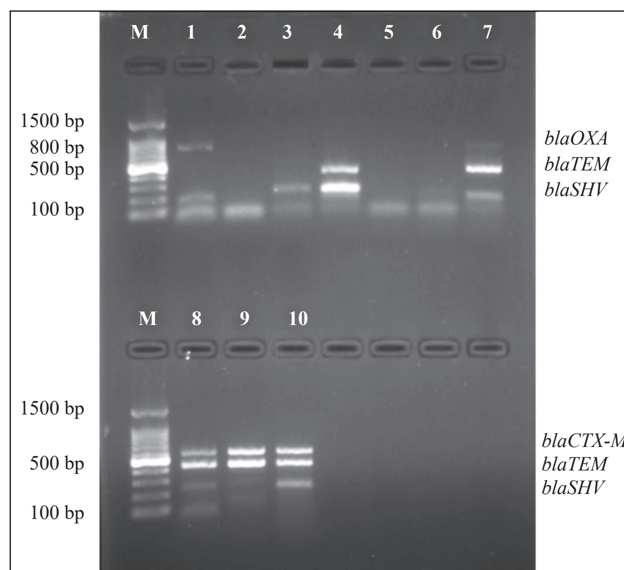

Fig. 2. Detection of ESBL genes in *Salmonella* spp. [M- 100 bp DNA ladder; Lane 1: bla_{OXA} (813 bp) gene; Lane 3: bla_{SHV} (237 bp) gene; Lane 4: bla_{TEM} (440 bp) and bla_{SHV} (237 bp) genes; Lane 7: bla_{OXA} (813 bp), bla_{TEM} (440 bp) and bla_{SHV} (237 bp) genes; Lane 8: bla_{CTX-M} (593 bp), bla_{TEM} (440 bp), and bla_{SHV} (237 bp) genes; Lane 9: bla_{CTX-M} (593 bp), bla_{TEM} (440 bp) genes; Lane 10: bla_{CTX-M} (593 bp), bla_{TEM} (440 bp), bla_{SHV} (237 bp) genes].

Another gene used for the detection of *Salmonella enterica* in the present study was *iroB*, a fur-regulated gene that is a member of the *iroA* (*iroBCDEN*) gene cluster (Supplementary Fig. 1). All the 19 isolates showed its presence. The specific role of *iroB* is to encode enterobactin glucosylation that contributes to the virulence of the bacteria by preventing the host antimicrobial protein (lipocalin-2) from sequestering the siderophore (Hantke *et al.* 2003, Deguenon *et al.* 2019, Mthembu *et al.* 2019). In short, *invA* and *iroB* genes are conserved in all *Salmonella* spp. and found in SPI-1 (*Salmonella* pathogenicity islands). The present study has raised a serious concern due to the presence of invasive strains of *Salmonella* in dairy farms, and the surrounding environment, which is of public health significance and hence needs to be monitored scrupulously.

Another objective of the study was to detect the genes concerned with virulence in *Salmonella* namely *spvC*, *spvR* and, *stn* genes (Supplementary Figs. 2, 3, 4). Out of the 19 isolates, *spvC* was the predominant gene detected (8/19; 42.10%), followed by *stn* (6/19; 31.5%), and *spvR* (5/19; 26.31%). Similar studies on bovine salmonellosis were scanty. Ownagh *et al.* (2023) evaluated the presence of *fimA*, *stn*, and *invA* genes in *Salmonella* present in faecal

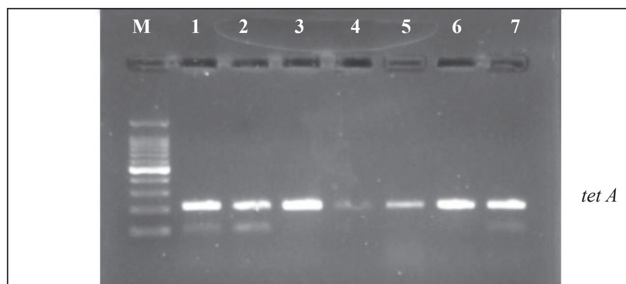

Fig. 3. Detection of *tetA* gene in *Salmonella* spp. [M: 100 bp DNA ladder; Lanes 1-7: *tetA* gene (210 bp)].

samples of buffaloes from Iran, and PCR results of the study documented that 50 (11.90%) faecal samples were positive for the genes. The spv locus is strongly associated with strains that cause non-typhoidal bacteremia. The spv region contains three genes that are required for virulence phenotype. These are positive transcriptional regulator *spvR* and two structural genes, *spvB* and *spvC*. These *Salmonella*-plasmid virulence (spv) factors are characteristics of *Salmonella* serotypes implicated in systemic diseases and thus important for spreading of the systemic infections (Nikiema *et al.* 2021). Thus, the detection of *spvC* and, *spvR*-positive *Salmonella* strains are extremely important in relation to public health. The *Salmonella stn* gene encodes for an enterotoxin and is associated with infection with serotypes of *Salmonella* Typhi, Typhimurium, and Enteritidis. The *stn* gene is a clinically important biomarker that is used to differentiate *Salmonella enterica* strains (stn+), from *Salmonella bongori* and other *Enterobacteriaceae* family (Nikiema *et al.* 2021). Few documented studies targeting these genes were available. Nikiema *et al.* (2021) studied a total of 106 *Salmonella* isolates, out of them, 70 (66%) isolates had *invA*, and stn genes, and 39 (36.8%) isolates had *spvR* gene and 51 (48.1%) had *spvC* gene.

In conjunction with this study, Deguenon *et al.* (2019) detected the virulence genes of *Salmonella* spp. in 406 animal faecal samples. The findings of the study documented that 2.46% of samples were *Salmonella* positive and out of them, *spvC* gene was present in 10% of isolates, whereas, *spvR* was found in 20% and all the isolates were positive for *invA* and *stn* genes. In contrast to our findings, a study from India by Chaudhary *et al.* (2015) demonstrated that out of 37 chicken-origin *Salmonella,* 30 isolates possessed *spvR* gene, but none of the isolates was found positive for *spvC* gene. Ranjbar *et al.* (2020) isolated a large number of *Salmonella enteritidis* from fecal samples of cattle from Iran. The observations of the current study highlighted the fact that food animals are a potential source of virulent *Salmonella* species and transmission risk from animal or environmental sources to humans cannot be ruled out. Also, the study emphasized the need for monitoring of *Salmonella* serotypes associated with apparently healthy animals and their predominance in the associated environment.

All the 19 *Salmonella* isolates were screened for the detection of antibiotic susceptibility patterns (Table 2). High resistance was noted for Nalidixic acid (94.73%), followed by Tetracycline (36.84%) and Ceftazidime (42.10%). Most of the isolates were sensitive to Gentamicin (89.47%), Enrofloxacin (89.47%), and Amikacin (89.47%). Abunna *et al.* (2018) demonstrated the antibiotic susceptibility pattern of 34 *Salmonella* isolates from dairy cattle (Ethiopia) and in the study, resistance was reported to Ampicillin (97.06%), amoxicillin, (91.18%), Nalidixic acid (67.65%), and Chloramphenicol (50%). *Salmonella* isolates of the present study were also resistant to Nalidixic acid and Ceftazidime. Geletu *et al.* (2022) revealed the susceptibility profile of 13 *Salmonella* isolates from fecal and environmental samples of dairy cattle. The study documented that most of the isolates were sensitive to Gentamicin and Nitrofurantoin, while resistance was noted towards Tetracycline and Nalidixic acid in accordance with the current study. Ampicillin, Nalidixic acid, and Nitrofurantoin resistant *Salmonella* isolates from cattle faeces and milk were also documented in other studies elsewhere (Addis *et al.* 2011). Results of the study revealed that the *Salmonella* spp. have developed resistance to 3rd generation Cephalosporins, and Nalidixic acid.

Table 2. Antibiogram profile of *Salmonella* species

Antimicrobial agent	Abbreviation	Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Ciprofloxacin	CIP	0	0	10	52.63	9	47.36
Ceftazidime	CAZ	4	21.05	7	36.84	8	42.10
Gentamicin	GEN	17	89.47	0	0	2	10.52
Azithromycin	AZM	8	42.10	8	42.10	3	15.78
Tetracycline	TE	9	47.36	3	15.78	7	36.84
Trimethoprim	TR	5	26.31	13	68.42	1	5.26
Chloramphenicol	C	10	52.63	4	21.05	4	21.05
Ceftriaxone	CTR	4	21.05	7	36.84	8	42.10
Amoxyclauv	AMC	0	0	10	52.63	9	47.36
Amikacin	AK	17	89.47	0	0	2	10.52
Enrofloxacin	EX	17	89.47	0	0	2	10.52
Nalidixic acid	NA	0	0	1	5.26	18	94.73

All the confirmed *Salmonella* isolates were also studied for the presence of ESBL-types, and out of the 19 isolates, 12 (63.15%) were ESBL-producers. Out of the four isolates, from fodder, three were ESBL positive. Very few studies have documented the prevalence of ESBL-positive *Salmonella* associated with bovines. A study from Nigeria surprisingly detected a very high percentage of ESBL-positive *Salmonella* (55.00%) in cattle faeces (Ogefere *et al.* 2017). We could not find any report from India on the isolation and characterization of *Salmonella* spp. in terms of ESBL pattern from dairy cattle.

All the 12 phenotypically ESBL-positive *Salmonella* isolates were further investigated for the presence of ESBL genes, viz. bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{OXA}, and $bla_{CMY\text{-}2}$ as described in the previous section. Out of the 12 isolates, nine (75%) harbored ESBL genes (Fig. 2). Out of them, three (33.33%) isolates were positive for bla_{SHV} and bla_{TEM} genes, while $bla_{CTX\text{-}M}$ and bla_{OXA} genes were also present in two isolates each. Sample-wise distribution showed that three fodder (33.33%), and two pit water samples were found positive for the presence of beta-lactam genes. A combination of bla_{SHV}+ bla_{TEM} was also detected in *Salmonella* isolated from one pit water sample. The findings of Wang *et al.* (2020) were in accordance with the current study. They found the predominance of $bla_{CTX\text{-}M}$ in all the ESBL-positive *Salmonella* species recovered from dairy cattle. In the present study, the prevalence of $bla_{CTX\text{-}M}$ was reported low in comparison with bla_{SHV} and bla_{TEM}. There were no reports from India on the characterization of cattle/bovine-origin *Salmonella* species for ESBL genes. Out of the 19 *Salmonella* isolates, *tetA* gene was present in six (31.57%) isolates (Fig. 3). Bag *et al.* (2021**)** demonstrated the prevalence of *tetA* gene from dairy cattle farms. In their study, the prevalence of *tetA* was found to be very high (100%). Resistance to tetracycline is very common in Gram-negative microbes and is globally prevalent. Tetracycline is a broad-spectrum antibiotic that is extensively used in animals and humans, as well as in aquaculture production. There are at least 40 different variants of *tet* genes, which governs resistance to tetracycline. The present study attempted to detect the presence of $bla_{CMY\text{-}2}$ gene in *Salmonella* spp., but all the isolates were found to be negative.

The current study indicated that cattle and their associated farm environment could be the source of virulent and drug-resistant strains of *Salmonella* spp. Cross-contamination of Salmonella from one animal to another and occupational risk of acquiring infection to farm workers cannot be ruled out. The study also advocates that phenotypic and genotypic AMR monitoring at farm animal premises and agricultural ecosystems is the need of the hour.

REFERENCES

Abunna F, Ngusie G, Beyene T, Ayana D, Wakjira B, Waktole H and Duguma R. 2018. Occurrence and antimicrobial susceptibility profile of *Salmonella* from dairy farms in and around Meki Town, Oromia, Ethiopia. *Biomedical Journal of Scientific and Technical Research* **6**(4): 5389–395.

Addis Z, Kebede N, Worku Z, Gezahegn H, Yirsaw A and Kassa T. 2011. Prevalence and antimicrobial resistance of Salmonella isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: A cross sectional study. *BMC Infectious Diseases* **11**: 222–28.

Asefa I, Legabo E, Wolde T and Fesseha H. 2023. Study on *Salmonella* isolates from fresh milk of dairy cows in selected districts of wolaita zone, Southern Ethiopia. *International Journal of Microbiology* **2023**: 6837797.

Bag M A S, Khan M S R, Sami M D H, Begum F, Islam M S, Rahman M M, Rahman M T and Hassan J. 2021. Virulence determinants and antimicrobial resistance of *E. coli* isolated from bovine clinical mastitis in some selected dairy farms of Bangladesh. *Saudi Journal of Biological Sciences* **28**(11): 6317–323.

Bäumler A J, Tsolis R M, Valentine P J, Ficht T A and Heffron F. 1997. Synergistic effect of mutations in *invA* and *lpfC* on the ability of *Salmonella typhimurium* to cause murine typhoid. *Infection and Immunity* **65**(6): 2254–259.

Chaudhary J H, Nayak J B, Brahmbhatt M N and Makwana P P. 2015. Virulence genes detection of *Salmonella* serovars isolated from pork and slaughter house environment in Ahmedabad, Gujarat. *Veterinary World* **8**(1): 121–24.

Deguenon E, Dougnon V, Lozes E, Maman N, Agbankpe J, Abdel-Massih R M, Djegui F, Baba-Moussa L and Dougnon J. 2019. Resistance and virulence determinants of faecal *Salmonella s*pp. isolated from slaughter animals in Benin. *BMC Research Notes* **12**(1): 317–23.

El-Sebay N A, Abu Shady H M, El-Rashed El-Zeedy S A and Samy A A. 2017. *InvA* gene sequencing of Salmonella Typhimurium isolated from Egyptian poultry. *Asian Journal of Scientific Research* **10**: 194–02.

Fang H, Ataker F, Hedin G and Dornbusch K. 2008. Molecular epidemiology of extended-spectrum beta-lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *Journal of Clinical Microbiology* **46**(2): 707–12.

Geletu U S, Usmael M A and Ibrahim AM. 2022. Isolation, identification, and susceptibility profile of *E. coli*, *Salmonella*, and *S. aureus* in dairy farm and their public health implication in Central Ethiopia. *Veterinary Medicine International* **2022**: 1887977.

Hantke K. 2003. Is the bacterial ferrous iron transporter FeoB a living fossil?. *Trends in Microbiology* **11**(5): 192–95.

Iyer V, Ravalia A, Bhavsar K, Cottagiri S A, Sharma A, Vegad M, Shah P, Shah B, Solanki B, Soni S and Mavalankar D. 2019. Antimicrobial resistance surveillance in typhoidal *Salmonella* in Ahmedabad in an era of global antimicrobial resistance surveillance systems. *Journal of Global Infectious Diseases* **11**(4): 153–59.

Lanzas C, Warnick L D, James K L, Wright E M, Wiedmann M and Gröhn Y T. 2010. Transmission dynamics of a multidrug-resistant *Salmonella typhimurium* outbreak in a dairy farm. *Foodborne Pathogens and Disease* **7**(4): 467–74.

Mthembu T P, Zishiri O T and El Zowalaty M E. 2019. Molecular detection of multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. *Infection and Drug Resistance* **12**: 3537–548.

Ng L K, Martin I, Alfa M and Mulvey M. 2001. Multiplex PCR for the detection of tetracycline resistant genes. *Molecular and Cellular Probes* **15**(4): 209–15.

Nikiema M E M, Kakou-Ngazoa S, Ky/Ba A, Sylla A,

Bako E, Addablah A Y A, Ouoba J B, Sampo E, Gnada K, Zongo O, Traoré K A, Sanou A, Bonkoungou I J O, Ouédraogo R,Barro N and Sangaré L. 2021. Characterization of virulence factors of *Salmonella* isolated from human stools and street food in urban areas of Burkina Faso. *BMC Microbiology* **21**(1): 338–50.

Ogefere H O and Ibadin E E. 2017. Detection of extended spectrum beta-lactamases among gram negative bacilli recovered from cattle faeces in Benin city, Nigeria. *Notulae Scientia Biologicae* **9**(2): 177–81.

Ownagh A, Etemadi N, Khademi P and Tajik H. 2023. Identification of *Salmonella* carriers by amplification of *FimA*, *Stn* and *InvA* genes and bacterial culture methods in fecal samples of buffalo. *Veterinary Research Forum* **14**(1): 21–28.

Ranjbar R, Ardashiri M, Samadi S and Afshar D. 2018. Distribution of extended-spectrum β-lactamases (ESBLs) among *Salmonella* serogroups isolated from pediatric patients. *Iranian Journal of Microbiology* **10**(5): 294–99.

Sargeant J M, Totton S C, Plishka M and Vriezen E R. 2021. *Salmonella* in animal feeds: a scoping review. *Frontiers in Veterinary Science* **8**: 727495.

Srednik M E, Lantz K, Hicks J A, Morningstar-Shaw B R, Mackie T A and Schlater L K. 2021. Antimicrobial resistance and genomic characterization of *Salmonella* Dublin isolates in cattle from the United States. *PloS One* **16**(9): e0249617.

Wang J, Xue K, Yi P, Zhu X, Peng Q, Wang Z, Peng Y, Chen Y, Robertson I D, Li X, Guo A and Aleri J W. 2020. An abattoir-based study on the prevalence of *Salmonella* fecal carriage and ESBL related antimicrobial resistance from culled adult dairy cows in Wuhan, China. *Pathogens (Basel, Switzerland)* **9**(10): 853–63.