Antimicrobial Resistance Profile of *Salmonellaenterica* Isolated from Improved Poultry Breed Farming Chain, Maharashtra, India

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ABSTRACT

Aims: With rising demand for poultry meat, globally backyard farms have adopted semi-intensive systems using improved birds. Food safety is a prime issue in antimicrobial-resistant *Salmonella* spp. found in meat, which has often been traced back to farms.
Study design: We carried out a cross-sectional prospective study.
Place and Duration of Study: The study was conducted in selected districts of Maharashtra India during October 2023 to March 2024.

Methodology:A total of 364 samples were collected from improved indigenous poultry breed hatcheries (n=5), improved indigenous breed commercial farms (n=5), and improved breed rearing backyard poultry setups (n=30) in Marathwada, Maharashtra state, India. The isolation of *Salmonella* spp. was carried out as per IS-5887 (Part 3): 1999 protocol. The bacterial isolates were further identified by cultural, microscopic morphology and biochemical characteristics (BAM 2007). Confirmation of *Salmonella* spp. was done by polymerase chain reaction (PCR) assays with primers designed for the *invA* gene. All *Salmonella* isolates were subjected for antibiotic susceptibility testing by disk diffusion methodandwere characterized for the presence of *blaTEM, blaSHV,blaOXA, blaCTXM, tetA,* and *Sul1*genes.
Results:A total of 15 isolates recovered with prevalence rates of 4.83, 6.36, and 1.53 percent in hatcheries, farms, and backyard households, respectively. Five isolates that were randomly analyzed showed a homologous sequence as *Salmonellaenterica*. Antimicrobial susceptibility testing of all isolates revealed higher resistance against Erythromycin (100%), followed by Ceftazidime (40%), while sensitivity (93.33%), was recorded against Ampicillin/Sulbactam, Amoxicillin/Sulbactam, and Enrofloxacin. The average multiple antibiotic resistance (MAR) index, of *Salmonella* isolates was 0.117. Genotypic resistance pattern revealed that all isolates (100%) were carrying the *blaTEM* gene, while none were harbouring broad spectrum extended-spectrum beta-lactamases (*blaSHV, blaOXA,* and*blaCTXM)* genes. All isolates were positive for the *tetA* gene (100%) but none of them was positive for *Sul1* genes.
Conclusion: The study highlights the low antimicrobial resistance in *Salmonella* isolates isolated from Improved Poultry Breed Farming Chain, which might be due to low usage of antimicrobials. However, monitoring of multidrug-resistant *Salmonellaenterica* in improved poultry breed farming chains is essential to protect human health.

Keywords: improved poultry breed, farming chain,Salmonella, antimicrobial resistance, genotypic resistance

1. INTRODUCTION

Poultry meat consumption is on the rise in world among all animal-derived food items (Waghamare et al. 2021). Meat, due to its high nutritive value containing essential amino acids and various macro- and micronutrients, plays a crucial role in a balanced diet (Van Boeckel et al. 2019). In recent years with the surge in chicken demand, backyard poultry farms have transformed themselves into semi-intensive farming systems with the help of improved native or coloured variety birds and good quality feed (Chaiban et al. 2020). The intervention of backyard poultry farming with improved native or coloured poultry varieties suitable for backyard production can be a source of a supportable food production structure (Singh et al., 2022). Breeder stock and hatcheries can be an important source of *Salmonella* spp. and can be improved through the execution of effective intervention methods (Sivaramalingam et al. 2013).

Salmonellosis stands as a major global foodborne illness, contributing to 93.8 million gastroenteritis cases and 155,000 deaths per annum globally (Heredia and Gracia, 2018). *Salmonella* in food chains is emerging worldwide and poultry is recognized as a foodborne pathogen reservoir, with several reports highlighting the occurrence of *Salmonella* linked to the backyard and commercial live poultry, production settings (Samanta et al. 2014,Elmonir et al. 2023).

In the past few years, there has been a significant surge in phenotypic and genotypic resistance among non-typhoidal *Salmonella* isolates to β-lactams, tetracycline, and sulphonamides(Eguale et al. 2017). The extensive use of tetracycline in poultry has caused the advance of tetracycline resistance in *Salmonella* spp. due to selective pressure (Waghamare et al. 2018). CDC, 2013, and WHO, 2017 reported that Gram-negative microorganisms that harvest beta-lactamases as one of the world's most insistent threats. Recent food safety studies have shown that *Salmonella* strains isolated from poultry and other foods possess extended-spectrum β-lactamases (ESBLs), which become a potential threat to human health as they may impact treatment regimens for ESBL-producing pathogens (Orabi et al. 2022; Dinh et al. 2023).

Broiler meat harvested from organic and pastured poultry farming production facilities has high demand (Van Loo et al. 2011; Rothrock et al. 2016). The appearance of antimicrobial resistance (AMR) in *Salmonella* from backyard poultry farming systems may pose a significant growing threat to anthropoid and animal health (Van Boeckel et al. 2019;

Hedman et al. 2020). Similarly, studies conducted by other researchers reported significantly lower *Salmonella* contamination of fecal matter and bird feed (Siemon et al. 2007; Alali et al. 2010). An additional hypothesis is that improved breed poultry production systems work on antibiotic-free rearing, which might potentially affect the antimicrobial resistance profile and presence of antibiotic resistance genes in the *Salmonella* populations along the hatchery-to-backyard farm continuum.

Therefore, to better understand the prevalence of antimicrobial resistant (AMR) *Salmonella* in these improved native breed poultry farms and backyard poultry management systems, some more work is essential; especially since market demand and production for backyard poultry has been increased. Thus, the present study was carried out to evaluate the antimicrobial resistance profile of *Salmonella* spp. isolated from the backyard poultry production system. This information will provide vital data on the prevalence, and AMR profile of *Salmonella* spp. in improved indigenous poultry breed farming chains which help to improve backyard poultry food safety systems.

2. MATERIAL AND METHODS

2.1 Sample collection

In the current study, a total of 364 samples were collected from improved indigenous poultry breed hatcheries (n=5), improved indigenous breed commercial farms (n=5), and improved breed-rearing backyard poultry setups (n=30) in Marathwada, Maharashtra state, India. The samples from hatcheries (n=124) were comprised of deceased chicks (25), unhatched eggs (25), shell waste (12), and tray swabs (12). A total of 110 samples (10 each of feed, water, litter, feces, walls swab, utensils swab, worker's hands swab, and cages; and 30 cloacal swabs) were selected from five different farms in Pathardi, Maharashtra, India. Similarly, 30 household backyard farms from the Parbhani, Vasmat, and Selu areas of Marathwada, Maharashtra, India were screened by collecting 130 samples (30 each of cloacal swabs, water and feces, 15 each of cage dust swabs and utensils swab and 10 feed samples). All the samples were collected aseptically, using sterilized polypropylene bags, and transported to the laboratory under a cold chain as per standard methods.

2.2Isolation and characterization of *Salmonella* spp.

Isolation of *Salmonella* spp. was performed as per IS-5887 (Part 3): 1999 protocol with slight modifications based on the samples collected. The sample was processed (pre-enrichment) by inoculating a 5gm sample of dead chick and unhatched egg in 45 ml of buffered peptone water (BPW), swabs and feces (1gm) were pre-enriched with 9 ml buffered peptone water, in sterile test tubes, whereas 25 g of each feed and water was inoculated in 225 ml of buffered peptone water andall samples were incubated at 37°C ± 1°C for 24 hours.Further, enrichment was done by transferring 0.1 ml of inoculum from BPW into 10 ml Rappaport-Vassiliadis (RV) medium and incubated at 42°C for 24 hrs. A loop-full of inoculum from RV broth was streaked on Xylose Lysine Deoxycholate (XLD) Agar and Brilliant Green Sulpha (BGSA) Agar, plates by four quadrant streaking method. The inoculated plates were incubated at 37°C ± 10°C for 24 hrs. for the development of colonies of *Salmonella* spp. The bacterial isolates were further identified by cultural, microscopic morphology, and biochemical characteristics (BAM 2007).

Black center smooth and round or large with black center colonies of *Salmonella* spp. were characterized by polymerase chain reaction (PCR) assays with primers designed for the *invA* gene (Nair et al. 2015). DNA of *Salmonella* isolates was extracted using heat lysis DNA Isolation protocol (Dashti et al. 2023). The details of PCR primers and cycling conditions used in the present study are mentioned in Table 1 and Table 2.

2.3 Sequencing of the *invA* gene amplified product

Amplified *invA* gene products of selected isolates from hatchery, farm, and backyard households were sequenced from an outsourced agency (Submitted to GeneOmbio Technologies Pvt Ltd, India) for sequencing. The resulting sequences were analyzed, and sequence homology searches were conducted using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch).

2.4Antimicrobial susceptibility test

As per the Clinical and Laboratory Standards Institute (CLSI) protocol, the Kirby–Bauer drug-sensitive disk technique was used to test the sensitivity of PCR-positive *Salmonella* isolates to 14 commonly used antimicrobials. Antimicrobial tested were Ampicillin/Sulbactam (A/S) 10/10 µg, Amoxycillin/Sulbactam (AMS) 30/15 µg, Amoxiclav (AMC), Tetracycline (TE) 30 µg, Enrofloxacin (EX) 10 µg, Ceftazidime (CAZ) 30 µg, Levofloxacin (LE) 5 µg, Chloramphenicol (C) 30 µg, Erythromycin (E) 15 µg, Gentamicin (GEN) 10 µg, Amikacin (AK) 10 µg, Ciprofloxacin (CIP) 5 µg, Co-Trimoxazole (COT) 25 µg and Nalidixic Acid (NA) 30 µg. Briefly, bacterial suspensions were achieved from overnight-grown cultures, which adjusted to the 0.5 McFarland turbidity standard. The broth culture was spread on the surface of a Muller Hinton agar plate using a sterile cotton swab. After about 20 min, the disks were applied to the plates and incubated at 37°C for 18 hr. Finally, using a scale (Himedia) the diameter of the inhibition zone was measured to categorize the resistance pattern. The results of phenotypic antimicrobial resistance patterns were analyzed as per the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines (2021).

2.5 Multiple Antibiotic Resistance (MAR) Index

The Multiple Antibiotic Resistance (MAR) index of individual isolates was calculated according to the method described by Krumperman (1983) by dividing the number of antibiotics to which the isolates were found resistant to the total number of antibiotics to which the isolate was exposed.

2.6 Molecular characterization of AMR genes in *Salmonella* spp.

All the 15 isolates were screened for the presence of 06 antimicrobial resistance genes. The genes that encode resistance to β-lactamases (*blaTEM*, *blaSHV*, *blaCTX-M,* and *blaOXA)*, tetracyclines (*tetA),* and sulfonamides (*Sul1)* were evaluated.

Genomic DNA of *Salmonella* spp. isolates were extracted by boiling method described by Anejo-Okopi*et al*. (2016). All *Salmonella* isolates were screened for the presence of antimicrobial resistance genes through singleplex and multiplex PCR protocol described by Ng *et al*. (2001) for *tetA*, Ma *et al.* (2017) for *Sul1* and Fang *et al.* (2008) for a group of β-lactamase genes *(blaSHV, blaCTXM, blaOXA and blaTEM)*.Primers used are listed in Table 1. The cycling conditions for PCR are mentioned in Table 2.

The singleplex PCR for *invA*, *sul1* and *tetA* was performed in 25 µl reaction volume containing 12.5 µl of 2x PCR master mix (HiMedia Laboratories Pvt. Ltd., Mumbai), 1.5 µl of each primer (10pmol/µl) (Eurofins Genomics India Pvt. Ltd., Bangalore), 1 µl of genomic DNA and 8.5µl molecular biology grade water (HiMedia Laboratories Pvt. Ltd., Mumbai) used to make desired volume. Whereas, multiplex PCR reaction was performed as described by Fang *et al*. (2008) in a 25 µl volume containing 12.5 µl 2x PCR Master Mix (Takara Bio Inc., Shiga, Japan) supplied with Taq DNA polymerase, buffer, $MgCl_2$, and dNTPs. In this PCR 1 µl (10 pmol/µl) each of forward and reverse primer was used. Similarly, the reaction mixture contained 3 µl DNA template and 7.5 µl nuclease-free water to make a final volume of 25 µl.

Table 1. Primers used for genotype characterization of *Salmonella* spp.

Sr. no	Primer	Target	Primer sequence (5’- 3’)	Product Size (bp)	Reference
1	*invA*	Invasion-associated protein	**F:**TCGTGACTCGCGTAAATGGCGATA **R:**GCAGGCGCACGCCATAATCAATAA	423	(Nair et al. 2015)
2	*tetA*	Tetracycline	**F:** GCT ACA TCC TGC TTG CCT TC **R:** CAT AGA TCG CCG TGA AGA GG	210	(Ng et al. 2001)
3	*Sul1*	Sulphonamide	**F:** TTTCCTGACCCTGCGCTCTAT **R:**GTGCGGACGTAGTCAGCGCCA	425	(Ma et al. 2007)
4	*blaTEM*	Broad Spectrum β-lactamase	**F:**CGCCGCATACACTATTCTCAGAATGA **R:** ACGCTCACCGGCTCCAGATTTAT	445	(Fang et al. 2008)
5	*blaSHV*		**F:** CTTTATCGGCCCTCACTCAA	237	

		R: AGG TGC TCA TCA TGG GAA AG	
6	*blaCTX-M*	**F:** ATGTGCAGYACCAGTAARGTKATG GC **R:**TGGGTRAARTARGTSACCAGAAYCAGCGG	593
7	*blaOXA*	**F:** ACA CAA TAC ATA TCA ACT TCG C **R:** AGT GTG TTT AGA ATG GTG ATC	813

Table 2. PCR cycling conditions used under study for different primers

PCR steps	**PCR conditions**	**Target genes**			**Broad Spectrum □-lactamase**
		invA	***tetA***	***sul1***	***blaSHV, blaCTXM,*** **blaOXA*blaTEM*** **(Multiplex PCR)**
Initial Denaturation	Temperature (°C)	94	95	94	94
	Time (min.)	5	3	3	5
Denaturation	Temperature (°C)	94	95	94	94
	Time (sec.)	30	30	30	45
Annealing	Temperature (°C)	56	60	60	63
	Time (sec.)	60	30	30	60
Extension	Temperature (°C)	72	72	72	72
	Time (sec)	90	60	45	60
Final Extension	Temperature (°C)	72	72	72	72
	Time (min.)	10	8	8	7
Number of cycles		35	35	30	30

3. RESULTS AND DISCUSSION

3.1 Prevalence of *Salmonella* isolates:

As is shown in Table 3, a total of 15 PCR *invA* gene positive *Salmonella* isolates were recovered from 364 samples, in which *Salmonella* was detected from Dead chick, Unhatched egg, Eggshell waste, Cloacal swabs, Faeces and Litter. All *Salmonella* isolates yielded desired amplified products of 423 bp for the *invA* gene (Fig. 1). The PCR-based method utilizing the *invA*genus-specific primer is a reliable approach due to its rapidity, high specificity, and sensitivity in confirming the isolates (Salehi et al. 2005).

Among the isolates, *Salmonella* strains were observed in 4.83% (6/124) of those from Hatcheries, 6.76% (7/110) of those from commercial farms, and 1.53% (2/130) of those from backyard household farms.

Salmonella can be spread horizontally and through fertilized eggs and it is zoonotic in nature (Zhao et al. 2021).Considering this, in obligation to better measure the noticeable infection by *Salmonella* spp., we obtained several samples from hatcheries, farms and backyard farms from various locations by maintaining the supply chain in improved indigenous poultry breed farming.

Results of prevalence from hatcheries are in agreement with earlier studies of Muhammad *et al*. (2010) and Sohail*et al.* (2021) who reported occurrence of 4.4% and 10.34% in hatchery samples, whereas, the present similar reported by Mulika *et al.* (2011), Xu *et al.* (2020) and Withenshaw*et al.* (2021) wherein, the prevalence of *Salmonella* spp.in hatchery samples ranging from 16.6 to 33.3% was recorded. Decontamination, sanitation, waste disposal, biosecurity measures, and proper monitoring are necessary to prevent *Salmonella* transmission from hatchery to another setting. The improvement of hatchery hygiene and the application of efficient pathogen detection and disease control strategies would improve *Salmonella* control (Fahmy et al. 2023).

The prevalence of *Salmonellaenterica* in Indigenous Poultry farms agrees with previous studies by Samanta*et al*. (2014), who reported a prevalence of 6.1 percent respectively. Abunna*et al.* (2016) stated that the distribution of *Salmonella* spp. varied depending on the sample type, poultry growth stage, and breeds. Soil and fecal matter present on the feathers and feet of birds serve as significant contributors to the microbial contamination observed in poultry carcasses (Orji et al. 2005). The results of the Prevalence of *Salmonella enterica* in Household Backyard Poultry Framing are contrast with previous reports by Jajere*et al*. (2019), Koro *et al*. (2022) and Elmonir*et al*. (2023). In household backyard poultry farming *Salmonella* spp. was observed only in cloacal swab samples (6.67%). The findings are not per the findings of Bhowmick*et al*. (2023) and Eid *et al*. (2023) who reported 30.82 and 31.00% prevalence, respectively in backyard poultry farm cloacal swabs. The presence of *Salmonella* in the backyard poultry might have been the sequel of a long-term persistence of *Salmonella* in these backyards or it could be the summary of laying hens purchased directly from contaminated hatcheries or other households (Trampel et al. 2014). The variances in sample types, topographical locations, sampling protocols, or isolation methods resulted in a multifarious and diverse isolation assessment.

Table 3. Prevalence of *Salmonella* in Backyard Poultry Farming System (n = 364)

Category	Type of Samples analyzed	Total Number	Positive Sample type	Total Number	Overall prevalence

		of Samples			(%)
Hatchery	Dead chick (internal organ) Unhatched Egg (Yolk and Embryo) Tray swab Eggshell waste	124	Dead chick (2) Unhatched Egg (3) Shell waste (1)	6	**4.83%**
Indigenous poultry breed farms	Cloaca swab Utensils swab Wall dust swab Workers hand swab Feed Water Faeces Litter	110	Cloaca swab (3) Faeces (2) Litter (2)	7	**6.36%**
Household backyard poultry	Cloaca swab Cage dust swab Utensils swab Feed Water Faeces	130	Cloacal swab	2	**1.53%**

3.2 Sequencing of selected *invA* gene PCR Products for serotype confirmation

A total of five selected isolates from hatcheries (A5/S/2 and A5/U/3), farms (B1/Cl/2) and household backyard poultry (C8/Cl/1 and C11/Cl/1) were sequenced using amplified *invA* gene (423bp) product. The sequencing homologous was observed on NCBI as *Salmonellaenterica* and showed homology of 99.49%, 99.69% and 99.49% with sequences accession no. AP020332.1, CP074202.1, CP051329.1, respectively which were available on NCBI.

Pavon and Rivera (2021), reported the possible use of the *invA* virulence gene for molecular typing of *Salmonella* through sequencing and phylogenetic. Amongst various types, Serovars Enteritidis and Typhimurium were the most informed serovars. *S. enterica* is responsible for infections in humans and animals (Andino&Hanning., 2015) which makes it a serious concern.

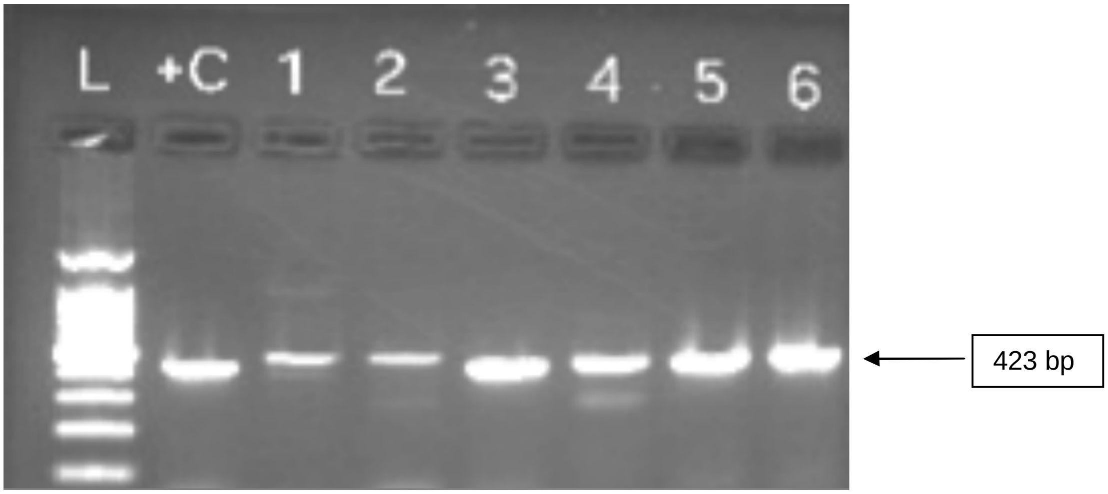

Fig. 1 Agarose gel showing PCR amplified product of *invA* gene of *Salmonella* spp.

3.3 Antimicrobial resistance pattern of *Salmonella* isolates

In the present study antimicrobial susceptibility testing discovered upper resistance against Erythromycin (100%), followed by Ceftazidime (40%), while sensitivity was recorded against Ampicillin/Sulbactam (93.33%), Amoxicillin/Sulbactam (93.33%), Enrofloxacin (93.33%), Levofloxacin (93.33%), and Nalidixic Acid (93.33%), whereas, intermediate sensitivity recorded against Ceftazidime (53.34%), followed by Tetracycline (46.67%) and Amoxiclav (26.67%). In the present study, a higher resistance pattern against Erythromycin (100%) is in agreement with the findings of Bhuvaneswa et al. (2015) and Al Mamum et al. (2017). The resistance pattern of Ceftazidime (40%) was noticed by Herrera-Sánchez et al. (2020), wherein the resistance reported was 75.5 percent. The results about Ampicillin/Sulbactam (0%), Gentamicin (0%), Amikacin (0%), Co-Trimoxazole (0%), Nalidixic acid (0%), Amoxicillin/Sulbactam (6.67%), Amoxyclav (6.67%), Tetracycline (6.67%), Enrofloxacin (6.67%), Levofloxacin (6.67%), Chloramphenicol (6.67%), Ciprofloxacin (6.67%) resistance patterns differs from results of with Akond et al. (2012), Thakur et al. (2013), Samanta et al. (2014) and Waghamare et al. (2018). Bhuvaneswa et al. (2015) reported that β-lactam and macrolide antibiotics are used routinely for curbing bacterial infection among chickens in commercial farms compared to backyard farms. The resistance of *Salmonella* Enteritidis to ampicillin decreased in our study this might be due to the reduction in antibiotic selective pressure (Varijakshapanicker et al. 2019). The exercise of using antimicrobial agents for growth, and treatment in livestock decreases their efficacy and is found to be a significant factor in the emergence of multidrug-resistant *Salmonella* (Thakur et al. 2013).

3.4 Multiple Antibiotic Index (MAR) for *Salmonella* isolates

In the present study, only 3 *Salmonella* isolates were resistant to 3 or more than 3 antibiotics. The MAR index of A5/S/2, B1/CL/2, and B3/Fe/2 were 0.21, 0.50, and 0.21, respectively (Table 4). The MAR index of the remaining 12 isolates varies from 0.07 to 0.14 which indicates lower selective pressure for antimicrobial resistance. The results of the present study do not agree with the studies carried out by Yoke-Kqueen et al. (2008) and Talukder et al. (2021) who reported that 91.01 and 100 percent *Salmonella* isolates have a MAR index of more than 0.2. The lower multidrug resistance pattern displayed by *Salmonella* isolates demonstrates that anthropoids consuming meat and meat products from concerned geographical areas are at low risk of multidrug-resistant *Salmonella* infection.

Table 4. Multiple antibiotic resistance [MAR] index and antibiogram of *Salmonella* isolates

Sr. no.	Source of samples	Sample code	Resistance to the number of Antibiotics	Antibiogram	MAR index
1.	Hatchery	A5/S/2	3	CAZ, C, E	0.21
2.		A5/U/3	1	E	0.07
3.		A5/U/4	2	CAZ, E	0.14
4.		A5/U/5	1	E	0.07
5.		A5/C/2	1	E	0.07
6.		A5/C/4	1	E	0.07
1.	Improved breed poultry farm	B2/L/1	1	E	0.07
2.		B4/L/2	1	E	0.07
3.		B1/Cl/2	7	AMS, AMC, EX, CAZ, LE, E, CIP	0.5
4.		B2/Cl/1	2	CAZ, E	0.14
5.		B2/Cl/3	1	E	0.07
6.		B1/Fe/1	2	CAZ, E	0.14
7.		B3/Fe/2	3	CAZ, E, TE	0.21
1.	Household backyard poultry	C8/Cl/1	1	E	0.07
2.		C11/Cl/1	1	E	0.07
				Total average	0.117

3.5 Characterization of Antimicrobial Resistance Genes: Extended Spectrum Beta Lactamase genes, tetracycline gene and sulphonamides gene

The carriage status of *Salmonella* strains for 04 antimicrobial Beta Lactamase resistance genes, 01 tetracycline (*tetA*) and 01 sulphonamides (*Sul1)* were screened by PCR.

All the *Salmonella* isolates were screened for ESBL genes *blaTEM*, *blaSHV*, *blaOXA* and *blaCTX-M*, with an amplicon size of 445bp, 237bp, 813bp and 593bp respectively. Among the 15 isolates, all (100 %) isolates harboured the *blaTEM* gene (Fig. 2), whereas none of them were found to be carrying *blaSHV*, *blaOXA,* and *blaCTX-M* genes. However, the *tetA* gene encoding resistance to tetracycline was also detected in all the *Salmonella* isolates (Fig 3).With regard to sulphonamides none of them were found to carry *Sul1* gene encoding resistance to sulphonamides

The results of the *blaTEM* gene are in agreement with reports of Bae et al. (2013) who reported 90.91% of isolates harbouring the *blaTEM* gene. Similarly, Eguale et al. (2017) reported the *blaTEM* gene in 79.1%, Several workers reported a lower prevalence of the *blaTEM* gene in *Salmonella* isolates isolated from poultry (Thakur et al. 2013; Elumalai et al. 2014; Herrera-Sánchez et al. 2020). In our study, none of the *Salmonella* isolates were found to carry *blaSHV*, *blaOXA,* and *blaCTX-M* genes. The study subsequently did not identify MDR *Salmonella* spp. with sequences of the ESBL broad-spectrum β-lactamases (*blaSHV*, *blaOXA,* and *blaCTX-M*) in these isolates. The *blaTEM* gene in the *Salmonella* strains could result in the widespread prevalence of *blaTEM*positive. *Salmonella* by transfer of gene to *blaTEM* negative strains through plasmid conjugation, transformation, and transduction(Lai et al. 2023).

Our results for carriage of the *tetA*gene are similar with earlier reports of Adesiji*et al.* (2018), Waghamare et al.(2018), and Soufi et al. (2012) who reported *Salmonella* isolates carrying the *tetA*gene in 100.00, 100.00 and 71.00 percent, respectively. Amplified expression of intrinsic resistance mechanisms for tetracycline resistance in microbes is due to the acquisition of mobile genetic elements, ribosomal binding place mutations, and chromosomal mutations (Pavelquesi et al. 2021).

The *sul1* gene is a sulphonamide-resistant dihydropteroate synthase of Gram-negative microorganisms(Alcock et al. 2023).The *Sul1* gene is found related to other resistance genes in class 1 integrons(Sköld 2021). A high percentage of *Sul1* genepercent in *Salmonella* isolates was reported by Adesiji*et al.*(2014) who reported 100 percent presence in *Salmonella* spp. isolated from humans and poultry. Machado *et al*. (2013) and Ma *et al*. (2017)reported that no one of the isolates was carrying sulfonamide-resistant *Sul* genes. The absence of *Sul1* in all isolates, indicates bacteria are not subjected to selective pressure by sulfonamides, a valuable means for the conservation and further addition of resistance to other antimicrobial elements.

Several factors contribute to the progress of antimicrobial resistance in bacteria, including alterations in bacterial cell permeability, enzymatic modification of drugs, and the exclusion of antimicrobials by membrane-bound efflux pumps (Chen et al. 2004). Antibiotic resistance often stems from genetic changes encoded by chromosomal and plasmid genes, with these genes primarily located on integrons, plasmids, and transposons, which are mobile genetic elements (Thong and Modarressi 2011).

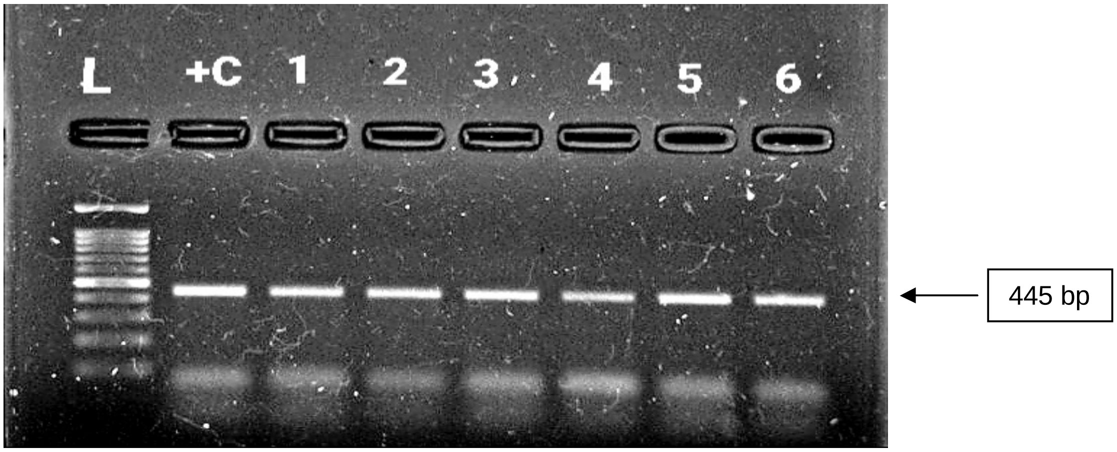

Fig. 2 Agarose gel showing PCR product of *blaTEM* gene of *Salmonella* spp.

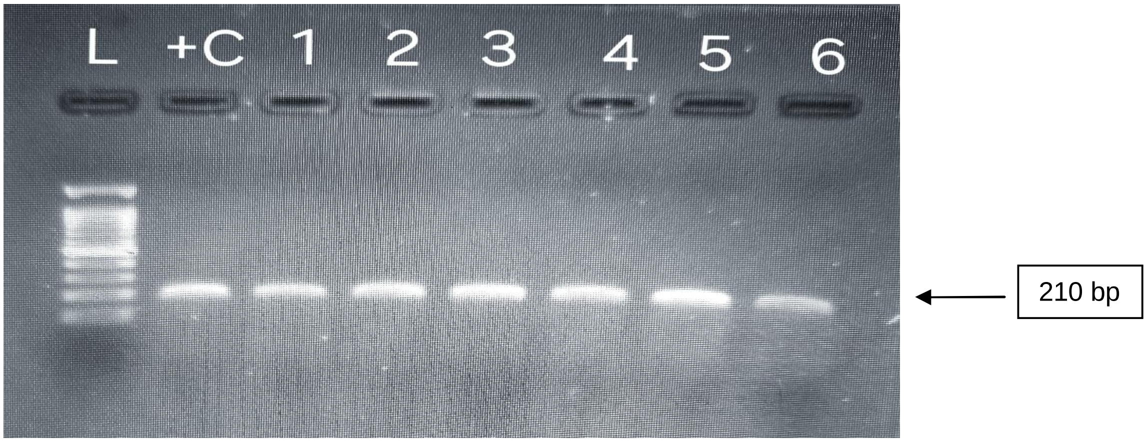

Fig. 3 Agarose gel showing PCR product of *tetA* gene of *Salmonella* spp.

4. CONCLUSION

The findings of the existing study showed that an improved native poultry breed supply chain is a potential source of virulent *Salmonella* Enterica uncovering humans to potential zoonotic infections via meat, egg, or direct exposure. But lesser MARs along with the absence of genotypic resistance for ESBL broad-spectrum β-lactamases in *Salmonella* isolates was found under study. Minor use of β-lactam and sulphonamide antibiotics in the production chain of improved breed poultry production chain might have resulted in the non-development of resistance. Overall, results suggested there were no major MDR *Salmonella* spp. circulating in the improved native poultry breed production chain of Maharashtra, India. Judicial use of antimicrobials and biosecurity measures shall be implemented in small-scale farms, backyards, and hatcheries principally in countryside areas of Maharashtra, India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS’ CONTRIBUTIONS

All authors contributed to the study conception and design.Designed study: [Rupesh Waghamare, Radha Rasve]; Experiment: [Radha Rasve, Nandini Kuntawar, M.F.M.F. Siddiqui]; Supervision: [Rupesh Waghamare]; Data Analysis: [KakasahebKhose, Nandini Kuntawar]; Statistical analysis: [Radha Rasve, Nandini Kuntawar]; Manuscript: [Rupesh Waghamare, Radha Rasve]; Manuscript review: [KakasahebKhose, M.F.M.F. Siddiqui].All authors read and approved the final manuscript.

CONSENT (WHEREEVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Not applicable

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