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Research Article

DETECTION OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA ENTERICA* ISOLATED FROM DUCKS AND THEIR ENVIRONMENTS IN WEST BENGAL, INDIA

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ABSTRACT: *Salmonella* infection in Poultry birds may lead to food poisoning in human beings. *Salmonella*e are pathogenic, causing enteritis in birds, and others. This study aims to determine the presence of *Salmonella* spp. isolates with detailed characterization, in ducks and associated environments from a few districts of South Bengal (India). A total of 236 samples (cloacal swabs of ducks, samples from the environment, and duck eggs) were collected, and *Salmonella* spp. was isolated following standard methodology with molecular confirmation of the isolates done by PCR. Standard methods were followed in all steps of the study. An *in vitro* antibiogram of the isolates positive for ESBL and biofilm formation was done against 12 antibiotics commonly used in veterinary medicine. Twenty-seven (11.44%) *Salmonella enterica* strains were detected with environmental samples to show the highest prevalence (13.9%) among other sources. Out of 27, 18 (66.67%) *Salmonella* spp. isolates were positive for either ESBL or ACBL production *in vitro* in a double-disc diffusion assay. Twelve (44.44%) isolates showed the presence of two major ESBL genes (blaCTX-Mand blaTEM) but no blaSHV whereas the blaAmpC was found in 11 (40.74%) isolates. The virulence gene (invA) was noticed in 12 (44.44%) isolates, whereas 16 (59.25%) isolates were revealed to have at least one of the biofilm-associated protein-encoding genes. All 12 selected *Salmonella enterica* isolates were identified with 100% resistance to ampicillin, followed by ertapenem and cefotaxime (both 83%), ceftazidime (75%), etc. In contrast, drugs like chloramphenicol (67%), co-trimoxazole (50%), gentamicin (33%) etc. were sensitive against these isolates.

Keywords: ACBL, Antibiogram, Biofilm, Ducks, ESBL, *Salmonella enterica*.

INTRODUCTION

Indian agriculture depends on various components; duck farming occupies an important position in those. Ducks provide about 1/10th of the total Indian poultry population and supply about 7-8% of the total yearly eggs produced in India [1]. Duck rearing is commonly done in households and backyards. Ducks are mostly reared in their backyards by poultry farmers throughout the world for different perspectives, like a source of high protein diet (duck meat and eggs), raised as pets for young family members, nurtured in small ponds or lakes as natural decor, in conservation areas, hunted in game preserves, and also used for entertainment at some country fairs [2]. Co-evolution of ducks with Influenza A virus and polymorphism in duck MHC1 molecule were associated with resistance to avian influenza and many bacteria such as pathogenic *E. coli* but may get infected with *Salmonella* spp. leading to enteritis mainly with greenish-yellow faeces, weight loss, conjunctivitis, etc. [3].

Ducks may spread infections to human beings via direct contact and may cause adverse health effects in consumers via food. *Salmonella*e may cause food-borne infections, via infected eggs/meat, and a few non-typhoidal *Salmonella* (NTS) infections are also reported with day-old ducklings [4]. There is no reliable

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information on the relative presence of *Salmonella* organisms in different types of domestic poultry including ducks. It is avowed that the involvement of other species with human infection bears a positive correlation to the quantity of meat/ meat products consumed from those sources. Details are available on the pathogenesis as well as characteristics of *Salmonella* infections in commercial ducks with different serovars of *Salmonella enterica* (*S.* Typhimurium, *S. enteritidis*, *S. infantis*, etc.) isolated from ducks causing different infections [2, 5].

Salmonella spp. is generally pinkish short rods (on Gram's staining), facultative anaerobes included in the family *Enterobacteriaceae*. It most commonly causes foodborne illness and can be detected in the intestines of different animals like cattle, dogs, pigs, ducks, and other poultry birds, etc. [6]. *Salmonella enterica* is always pathogenic and may also show antimicrobial resistance (AMR) properties making their control very tough in recent times [7]. *Salmonellae* can yield both extended-spectrum β-lactamases (ESBL) and Ampicillinase C β-lactamase (ACBL) and are the common associations of dreadful human infections like cancer, diabetes, etc. [8]. These pathogens may also possess several virulence and biofilm-forming properties, making them the most dangerous food-borne human pathogens. So, in this background, this study was focused on the detection of *Salmonella* spp. from duck, their environments, and eggs followed by their detailed characterization and antibiogram to assess the depth of the risk towards human beings.

MATERIALS AND METHODS

Sampling background

Two hundred and thirty-six different samples (154 cloacal swabs, 43 environmental samples, and 39 eggs) were collected from ducks and their environments from March 2020 to April 2021. The samples (including eggs) were collected mostly from apparently healthy backyard ducks (174) and the rest (62) from organized duck farms without any typical clinical symptoms. The environmental samples include soil samples (n=19), water collected from duck environment/sheds (n=13) and duck feed (n=11). Out of 154 cloacal swabs, 88 samples were collected from Indigenous or 'Deshi' ducks, 56 samples were clutched from Khaki Campbell ducks and the rest were from Pekin duck breeds; and out of 39 eggs, 25 were procured from Indigenous ducks, 12 were from Khaki Campbell ducks and 2 eggs were from Pekin ducks from a few districts of West Bengal, namely Purba Barddhaman, Hooghly, Kolkata, and South 24 Parganas. The eggs were collected in an egg tray and transported very carefully. After collection, all the cloacal swab samples were dipped in separate peptone water vials for transport to the laboratory. The egg samples (collected in the lab from the outer eggshell, inner shell wall, and egg yolks with swabs) were processed on the same day for better results.

Spotting and pinpointing the *Salmonella enterica* strains

The swab samples brooded into Selenite F broth (HiMedia, India) aseptically at 37°C for 24 hours. The eggshell swabs (from the outer eggshell, inner shell wall, and egg yolks) were collected in the laboratory and were enriched. Following enrichment in Selenite F broth, 1 loopful (1µl) of all samples was striped on both Xylose Lysine Deoxycholate (XLD) agar (HiMedia, India) and *Salmonella-Shigella* agar (SSA, HiMedia, India) plates followed by brooding at 37°C for 16-18 hrs. The red color colonies with black centers from the XLD agar and the black round colonies on the SSA agar plates were tentatively positive and were preserved on nutrient agar (HiMedia, India) slants for further depiction morphologically and biochemically.

Morphological and biochemical characterization

The *Salmonella enterica* isolates (preserved in nutrients agar slants) were stained with Gram's stain for their morphological examination and biochemical identifications (IMViC tests) were performed as per standard protocols narrated by Quinn *et al.* [9] and Edwards and Ewing [10] with slight modifications.

Molecular characterization/confirmation

The morphologically and biochemically confirmed (tentative) *Salmonella enterica* strains were screened by PCR for molecular validation as per Pradhap *et al.* [11] with minute modifications.

For DNA extraction, *Salmonella* spp. samples were enriched into 2ml Nutrient broth and nurtured at 37°C, overnight. Organisms were pelleted by centrifugation at 8000 rpm for 10 minutes. The obtained pellet was further suspended in 150µl nuclease-free water and lysed by boiling it in a water bath for 10 minutes escorted by immediate chilling. Removal of cell debris was done by high-speed centrifugation (@2000 rpm for 5 mins) and the supernatant was used as template DNA for PCRs [12].

PCR was done using 5µl of obtained bacterial DNA samples with 20µl PCR mixture having 2 mM $MgCl_2$, 0.2 mM of each dNTP, 15pmol of each primer (Table 1), 0.5U of Taq DNA polymerase, 10x Taq buffer, and 0.05% Tween 20. The test conditions were: denaturation (1st) at 94°C for 5 mins followed by 35 cycles of denaturation at 92°C for 45s, 50°C for 15s for annealing, elongation at 72°C for 2 mins, and final extension at 72°C for 2 mins. The PCR product was visualized by gel doc. system (UVP, UK) next to electrophoresis in 1.5% (w/v) agarose (SRL, India) gel containing ethidium bromide (0.5µg/ml) (SRL, India). Positive controls were supplied by the department only.

Phenotypical detection of ESBL and ACBL production

Antibiotic discs, *viz.* cefotaxime (30µg, Hi-Media) and ceftazidime (30µg, Hi-Media) with and without clavulanate (10µg, Hi-Media) were used in double-disc diffusion assay (DDSA) [13] to confirm the presence of ESBLs in all *Salmonella enterica* isolates *in vitro*. The standard results were considered for phenotypic confirmation of ESBL production [14]. Phenotypic confirmation of AmpC β-lactamase production in all the isolates was done by a cefoxitin-cloxacillin double disc synergy (CC-DDS) test as per Tan *et al.* [15, 16].

PCR diagnosis of ESBL and ACBL genes

All the positive ESBL and ACBL *Salmonella enterica* strains were screened for detection of major antibiotic-resistant genes namely, blaTEM, blaSHV, blaCTX-M, and blaAmpC (Table 1) [17, 18, 19]. The PCR tests were performed as per the conditions detailed in Table 2. The PCR products were visualized after gel electrophoresis by a gel doc. system (UVP, UK).

Detection of virulence gene

All positive *Salmonella enterica* strains were checked by PCR for the presence of the invA gene [20] with some modifications. All details of PCR conditions are given in Tables 1 and 2.

Detection of biofilm-associated protein-encoding genes

The presence of biofilm-associated protein-encoding genes (csgA, rpoS, rcsA, and sdiA) was confirmed in all positive strains by PCR as per Silva *et al.* [21] and Adamus-Bialek *et al.* [22]. All details of PCR conditions are given in Tables 1 and 2.

Antibiotic sensitivity assay

The ESBL and biofilm-associated protein-encoding genes possessing *Salmonella enterica* isolates were screened by the standard method of disc diffusion [13], and tested for the detection of their sensitivity and resistance patterns [14] using 12 different antibiotics commonly used in veterinary practice in India. The antibiotics used were amikacin (30µg), ciprofloxacin (5µg), ampicillin (10µg), norfloxacin (5µg), chloramphenicol (30µg), ertapenem (30µg), tetracycline (30µg), gentamicin (10µg), cefotaxime (30µg), co-trimoxazole (25µg), ceftazidime (30µg), and ticarcillin/ clavulanic acid (30/10µg).

RESULTS AND DISCUSSION

Isolation and identification

Twenty-seven (11.44%, 27/236) bacterial isolates were primarily identified (based on their morphological, cultural, and biochemical traits) as *Salmonella enterica* of which 16 (10.39%, 16/154) were from cloacal swabs of duck, 6 (13.95%, 6/43) were from environmental samples and 5 (12.82%, 5/39) were isolated from outer eggshell of ducks. None of the isolates were detected from the inner eggshell or the egg contents. In cultural studies, all the *Salmonella* isolates showed brick red color in Selenite F Broth, red color colonies with black centers on XLD agar, and black round colonies in SSA agar plates due to the growth of this pathogen. Morphological studies showed all the isolates to be pink color bacilli under the light microscope (100x). Ducks are quite resistant to common bacterial infections like pathogenic *E. coli*. This study revealed 11.44% (27) positivity of *Salmonella enterica*, out of 236 duck samples tested, which is quite a significantly higher prevalence rate of *Salmonella enterica* in West Bengal than the previous study made by Banerjee *et al.* [23].

All 27 isolates were detected tentatively to be *Salmonella enterica* by biochemical characterizations, with IMViC tests. All of them were found to be negative to Indole and VP, but positive to MR and Citrate utilization tests. All the isolates showed typical results in cultural and biochemical characterization [10, 24]. Molecular confirmation of the isolates revealed all 27 had the 16S rRNA gene (1428bp) specific to this genus [23] (Fig. 1). They found 6.44% positive *Salmonella* spp. isolates from ducks, mostly from the unorganized sectors (7.27%). The environmental samples showed the highest prevalence rate (13.95%) in this study in comparison to other types of samples,

Table 1. Features of Primers used in this study.

Sl. No.	Target Gene	Sequence of Primer (5'→3')	Predicted Product length (bp)	References
1	*Salmonella* 16S *rRNA*	F: AGAGTTTGATCMTGGCTCAG R: TACGGYTACCTTGTTACGACTT	1428	[11]
2	*bla*TEM	F: ATGAGTATTCAACATTTCCG R: CGTACAGTTACCAATGCTTA	867	[16]
3	*bla*CTX-M	F: CAATGTGCAGCACCAAGTAA R: CGCGATATATCGTTGGTGGTTGGTG	540	[18]
4	*bla*SHV	F: AGGATTGACTGCCTTTTTG R: ATTTGCTGATTTCGCTCG	392	[17]
5	*bla*AmpC	F: CCCCGCTTATAGAGCAACAA R: TCAATGGTCGACTTCACACC	634	[19]
6	*Salmonella invA*	F: GTGAAATTATCGCCACGTTCG GGCAA R: TCATCGCACCGTCAAAGGAACC	284	[20]
7	*csgA*	F: ATCTGACCCAACGTGGCTTCG R: GATGAGCGGTCGCGTTGTTACC	178	[21]
8	*sdiA*	F: TCGCTATCTCTGCTGATGTC R: TTTAATGCTGCCAAATCGGG	239	[22]
9	*rpoS*	F: GCAGAGCATCGTCAAATGGCTGTT R: ATCTTCCAGTGTTGCCGCTTCGTA	120	[22]
10	*rcsA*	F: GTGATTCACAGCGCCCTTCA R: TACTCGATTCGGTTCGGCTC	306	

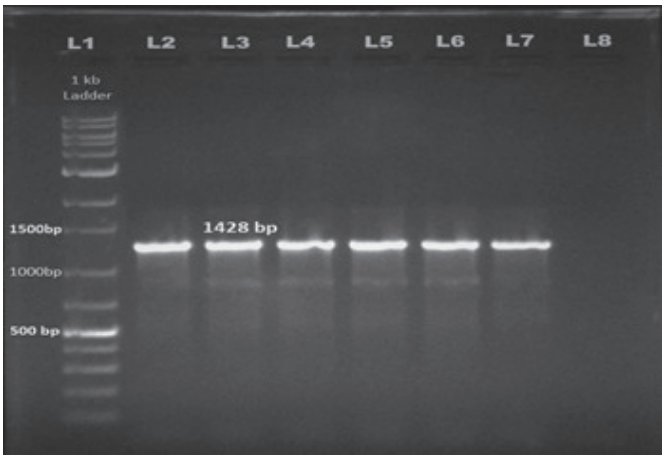

Fig. 1. Confirmation of *Salmonella enterica* strains with PCR detection of specific 16S rRNA gene (1428 bp). [L1: 100 bp DNA Ladder, L2: Positive control, L3-L7: Test samples, L8: Blank Negative control].

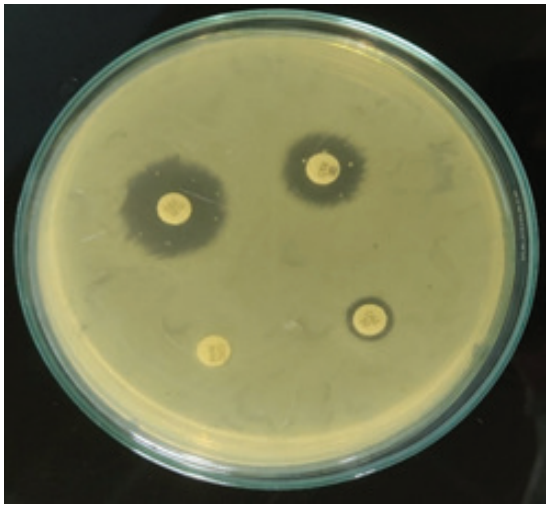

Fig. 2. Phenotypic assay of ESBL-producing *Salmonella enterica* isolates.

which is also quite higher (61.3%) than this one as reported by Mir *et al.* [25], from fecal and caecal contents of duck, chicken, and emu.

Out of 16 *Salmonella enterica* isolates of cloacal swab origin, 10 (11.36%) were obtained from Indigenous duck, 6 (10.71%) were from Khaki Campbell duck and none of the isolates were found in Pekin duck breeds. Among the environmental samples, 4 (21.05%) were obtained from soil samples, 2 (15.38%) were from water collected from duck environments/sheds and none was detected in feed samples. Most of the *Salmonella enterica* isolates of duck eggs were detected from Khaki Campbell duck eggs (16.67%, 2/12), while 12% (3/25) were obtained from Indigenous duck eggs

Table 2. Details of PCR conditions followed in this study.

Target gene	Reaction Mixtures (25µl)	PCR Conditions
*bla*TEM genes [16]	5µl of DNA templates, mixed with 2.5mM $MgCl_2$, 20 picomol of each primer, 1U Taq polymerase, 0.2 mM of each dNTPs, and 5x Taq buffer (Promega, USA)	Initial reaction for 5mins at 95°C, the 35cycles of 1min at 95°C (denaturation), annealing for 1min at 55°C, then extension for 1min at 72°C with the final extension at 72°C for 10mins
*bla*SHV genes [17]	Mix 5X Taq buffer (Promega, USA), with 200 µM dNTPs, 1.5 mM $MgCl_2$, 20 picomol of each primer, 1U Taq DNA polymerase, and 5µl DNA template	Denaturation (initial stage) of 3mins at 94°C, then 35cycles of 94°C for 30secs, 54°C for 30 secs (annealing), extension at 72°C for 1min and final extension of 10 mins at 72°C
*bla*CTX-M gene [18]	Five µl DNA templates mixed with 1U Taq DNA polymerase, 50pmol of each primer, 200mM dNTPs (Promega, USA), 2mM $MgC1_2$, and 10% dimethyl sulfoxide	Primary denaturation at 94°C, 30s of denaturation at 94°C (10mins), 30s of annealing at 53°C, 1min of extension at 72°C for 35 cycles, and extension (final) at 72°C for 10 mins.
*bla*AmpC gene [19]	Mixture contains 2mM $MgC1_2$, with 1U Taq DNA Polymerase (Promega, USA), 200 mM of each deoxynucleoside triphosphates, 100 pmol of each primer, 10% dimethyl sulfoxide & 5µl of DNA both templates	Denaturation (primary one) at 94°C for 5mins; then 30 cycles of amplification consisting of 30s of denaturation at 94°C, annealing of 30s at 57°C, elongation of 1min at 72°C and 10 min of final extension at 72°C.
Salmonella invA [20]	0.8 pmol of each primer mixed with 5µl of DNA template, 2.5mM $MgCl_2$, 0.2mM of deoxynucleoside triphosphate, 5µl of 10x buffer, and finally 1U Taq DNA Polymerase (Promega, USA)	Denaturation done at 94°C for 1min, further 35 cycles of denaturation at 94°C for 60s, annealing at 64°C for 30s, 30s of elongation at 72°C and final extension at 72°C for 7 mins.
csgA gene [21]	50pmol of each primer mixed with 5µl DNA templates,200mM dNTPs, 2mM $MgC1_2$, 1U Taq DNA polymerase (Promega, USA), and 10% DMSO	Four mins of denaturation (primary) at 94°C followed by 30s of denaturation at 94°C, annealing at 55°C for 30s and extension for 30s at 72°C for 30 cycles and 4 mins of final extension at 72°C.
sdiA / rpoS / rcsA genes [22]	5µl bacterial DNA templates, 2 mM $MgC1_2$, 10 pmol of each primer, 200mM deoxy-nucleoside triphosphate, 1U Taq DNA polymerase (Promega, USA), and 5 µl of Taq buffer	2 mins of primary denaturation at 95°C then 1min of denaturation at 94°C, 1min of annealing at 52°C (*sdiA*) / 54°C (*rcsA*)/ 60°C (*rpoS*) and 35 cycles of 1 min extension and 5 mins of final extension at 72°C.

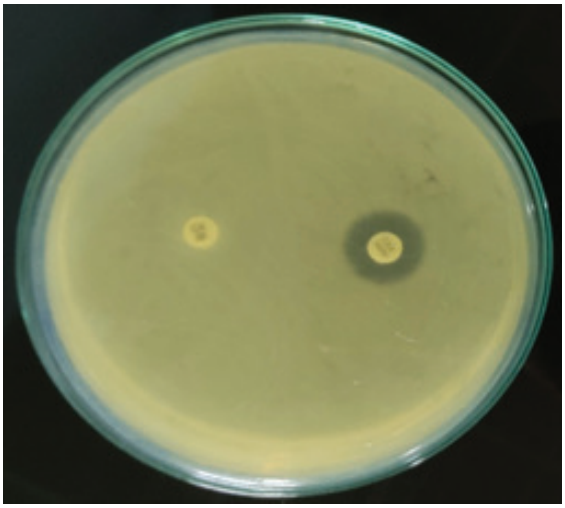

Fig. 3. Phenotypic assay of ACBL-producing *Salmonella enterica* isolates.

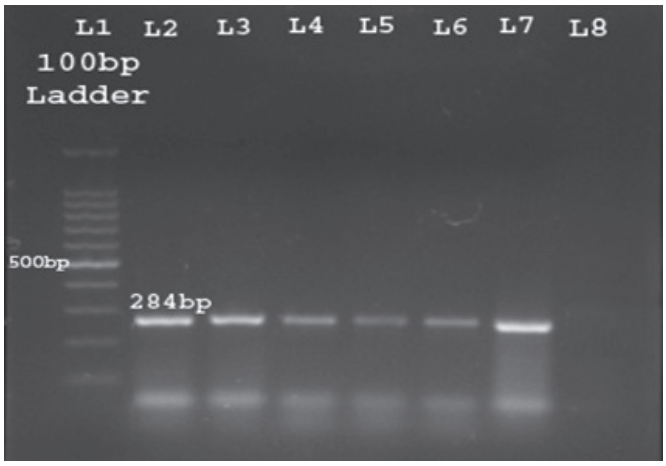

Fig. 4. Detection of the invA gene (284 bp) in *Salmonella enterica* strains. [L1: 100 bp Ladder, L2: Positive control, L3 -L7: Test samples, L8: Blank Negative control].

Table 3. Frequency of the ESBLs and ACBL genes in different isolates.

Types of bacteria	Positive in DDSA	PCR positive		
		Name of genes tested	No. of isolates detected	Total
Salmonella enterica Isolates (27)	ESBL (8)	*bla*CTX-M	8	12 (44.4%)
		*bla*TEM	4	
		*bla*SHV	0	
	ACBL (10)	*bla*AmpC	11	11 (40.7%)

Table 5. Distribution of the biofilm-associated protein-encoding genes in *Salmonella enterica* isolates.

Bacterial isolates tested	No. of biofilm gene-positive isolates	PCR detection of genes			
		rpoS	*csgA*	*sdiA*	*rcsA*
Salmonella enterica (27)	16	8	9	7	8
Percentages (%)		29.62	33.33	25.92	29.62

and no isolates were found in Pekin duck eggs. A 6% *Salmonella* prevalence from egg shells and 51.33% in egg samples were reported by Harsha *et al.* [26]. *Salmonella* spp. was reported to be a quite significant bacterial pathogen of ducks by Mondal *et al.* [2], who found 13.07% positive *Salmonella* spp. isolates the duck cloacal swabs from different farms in Bangladesh. Olaitan *et al.* [27] and Adzitey *et al.* [28] showed 30.5% and 23.54%, prevalence rates of *Salmonella* spp. isolates which might be due to differences in sampling patterns and other factors. A much higher prevalence rate of *Salmonella* spp. isolates (39.58%) from ducks were reported by Rahman *et al.* [29]. The Indigenous ducks showed the highest *Salmonella enterica* positivity (11.36%) in cloacal samples in comparison to Khaki Campbell and Pekin ducks, whereas Khaki Campbell duck eggs showed the highest (16.67%) positivity among the egg samples. Ema *et al.* [30] reported 12.5% positivity of *Salmonella* spp. from duck eggs but Zubair *et al.* [31] showed very little positivity (4.85%) from duck eggs which might be due to the differences in vaccination protocols and environmental factors. Differences in sampling pattern, period of sampling, and geographical locations might be the reason for the differences in the prevalence rate.

ESBL and ACBL production

Out of 27, 18 (66.67%) *Salmonella enterica* isolates were phenotypically detected as either ESBL (8) or ACBL (10) producers based on the double disc synergy

Table 4. Distribution of invA gene in *Salmonella enterica* isolates.

Type of specimens tested	No. of *Salmonella enterica* (16S *rRNA* +ve)	No. of *Salmonella enterica* with *invA* gene
Cloacal	16	9
Environmental	6	2
Egg (outer shell)	5	1
Total	27	12

assay (Fig. 2 and Fig. 3). During molecular detection of the resistance genes, 8(29.63%) isolates harbored the blaCTX-M gene (540 bp), 04(14.81%) isolates had the blaTEM gene (867 bp), whereas 11 (40.74%) positivity for blaAmpC gene (634 bp) was reported here. No blaSHV gene was found here (Table 3). Significant drug resistance properties (50% and 46.15%) in *Salmonella* spp. isolates were revealed by researchers like Bialvaei *et al.* [32], and Banerjee *et al.* [23]. This type of *Salmonella enterica* isolates have any of the beta-lactamase-producing genes and thus were quite capable of blocking several antibiotics both *in vivo* and *in vitro* [33].

Detection of the virulence gene

Here, 12 (44.44%) *Salmonella* spp. isolates showed positivity for the invA gene (284bp) in PCR (Fig. 4, Table 4), of which 9 (56.25%) were from cloacal swab isolated *Salmonella* spp., 2 (33.33%) isolates were from environmental samples (soil) and only one (20%) was from egg (outer shell) isolated *Salmonella* spp. isolates. Though this gene is considered to be very common across all *Salmonella* strains several reports are showing variable positivity of the gene in *Salmonella* spp. isolates in different studies [34, 35].

Invasion protein invA helps in the penetration of cells of the intestinal epithelium. This gene is invariably seen in *Salmonella* Pathogenicity Island 1 (SPI-1) and is responsible for virulence in cells [36]. This study reported approximately 44.44% of *Salmonella* spp. isolates to be positive for virulence property which is quite a significant one. In a similar study, Salehi *et al.* [20] reported 30 (15.6%) virulent *Salmonella* strains by visualizing specific DNA products of the invA gene from 192 samples of poultry carcasses which were quite lower than the present study. Again, Osman *et al.* [4] detected 18.5% *Salmonella* isolates from imported ducklings, and 14 types of serovars were identified from imported ducklings which were further screened for 11 virulence genes, and all serovars were found positive for the invA gene. Staji *et al.* [37] isolated

Table 6. Antibiotic Sensitivity Test of ESBL and biofilm-associated protein-encoding gene possessing *Salmonella enterica* isolates (n = 12).

Sl. No.	Used Antibiotics (Concentration)	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Ampicillin (10µg)	00	00	100
2	Chloramphenicol (30µg)	66.67	00	33.33
3	Co-Trimoxazole (25µg)	50	8.33	41.67
4	Tetracycline (30µg)	25	16.66	58.33
5	Gentamicin (10µg)	33.33	41.67	25
6	Amikacin (30µg)	8.33	66.67	25
7	Norfloxacin (10µg)	25	00	75
8	Ciprofloxacin (5µg)	25	8.33	66.67
9	Cefotaxime (30µg)	16.66	00	83.33
10	Ceftazidime (30µg)	00	25	75
11	Ticarcillin/Clavulanic acid (75/10µg)	00	33.33	66.67
12	Ertapenem (10µg)	00	16.66	83.33

eighteen (7.2%) *Salmonella enterica* strains from fecal samples of a mallard duck, among them, nine (50%) isolates possessed the invA gene which was quite similar to the present study. Mir *et al.* [38], Krawiec *et al.* [39], and Ammar *et al.* [40], showed7-17%invA gene positivity in *Salmonella* spp. isolates that were quite significant findings too.

Detection of biofilm-associated protein-encoding genes

All the isolated *Salmonella* spp. were checked molecularly to observe biofilm-associated protein-encoding genes *i.e.,* rpoS, csgA, sdiA and rcsA with amplified product size 120bp, 178bp, 239bp and 306bp respectively, and found that 16 (59.26%) *Salmonella* spp. harbored at least one of these genes. The prevalence of rpoS, csgA, sdiA, and rcsA genes in isolated *Salmonella* spp. was 29.62%, 33.33%, 25.92%, and 29.62% respectively (Table 5). Out of 16 biofilm-associated protein-encoding genes possessing *Salmonella* spp. isolates 12 (75%) isolates had ESBL positivity and among the 12 *Salmonella* spp. isolates with virulence genes, 4 (33.33%) isolates were detected for ESBL genes which showed a positive correlation between biofilm-producing isolates with ESBL-producing and virulence gene-carrying isolates. Biofilms are produced on the surface with aggregates of cells covered by a self-produced extracellular polymeric substance (EPS). Bacteria develop these structures often in adverse environments and changes in cell temperature, oxygen, pH, and nutrient availability. Biofilms allow the bacteria to survive well in target cells [41]. Biofilm production is quite a common trait of *Salmonella enterica* ssp. *enterica*. This property helps *Salmonella* spp. to form colonies persistently both inside and outside of the animal host and increase bacterial survival and transmission [42]. The present study also found approx. 59.26% of bacterial isolates are positive to harbor biofilm-producing genes among which csgA has the highest frequency (33.33%). Again 12 (44.4%) *Salmonella* spp. isolates showed both ESBL and biofilm production which is quite alarming as detected in this study [23, 32].

Antibiotic resistance patterns

The ESBL and biofilm-associated protein-encoding genes possessing *Salmonella* spp. isolates (12), showed total resistance to ampicillin (Table 6). The *Salmonella* spp. isolates also showed higher insensitivity to antibiotics, such as cefotaxime and ertapenem (83.33%) followed by norfloxacin (75%), ceftazidime (75%), and ticarcillin/clavulanic acid (66.67%), ciprofloxacin (66.67%) and tetracycline (58.33%), respectively. Chloramphenicol and co-trimoxazole showed higher sensitivity i.e., 66.67% and 50% respectively towards ESBL and biofilm-associated protein-encoding genes possessing *Salmonella enterica* isolates (Table 6). Over the past few years, several strains (types) of *Salmonella* spp. have become resistant to multiple antibiotics. A few strains have also emerged to gain extensively drug-resistant (XDR) status. The ESBL, ACBL, and biofilm-associated protein-encoding gene possessing *Salmonella*

enterica isolates are quite capable of resisting many commonly used antibiotics [43]. The ESBL and biofilm-producing *Salmonella* spp., isolates showed high to moderate antibiotic resistance in this study, against drugs like cefotaxime, ertapenem, norfloxacin, ceftazidime, ticarcillin/clavulanic acid, and ciprofloxacin. Chloramphenicol and co-trimoxazole showed significant sensitivity against the bacterial isolates in this study. Such antibiotic resistance in *Salmonella enterica* strains were also shown by Mir *et al.* [25] *i.e.* approx. 100% resistance to penicillin, clindamycin, and oxacillin, followed by tetracycline (65.62%), ampicillin (68.75%), nalidixic acid (56.25%), colistin (46.87%) and highly sensitive to chloramphenicol (96.87%) followed by meropenem (84.37%). Zhang *et al.* [44] reported such resistance patterns in *Salmonella* towards different antibiotics like sulfisoxazole (76.1%), tetracycline (75.3%), ampicillin, and ofloxacin, etc. which supports the present reports too.

CONCLUSION

Salmonellosis is quite a significant problem in ducks causing dreadful illness in ducks and also plays an alarming zoonotic role. A higher prevalence of *Salmonella enterica* was observed in the environmental samples of ducks and also in Deshi ducks. The duck *Salmonella enterica* isolates were positive for beta-lactamases and biofilm production to a significant level. The isolates were pathogenic too possessing the virulent invA gene. The ESBL and biofilm-production positive strains were resistant to several commonly used antimicrobials which is a major point of concern.

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