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VETERINARY MICROBIOLOGY - RESEARCH PAPER

Salmonella enterica serovars linked with poultry in India: antibiotic resistance profiles and carriage of virulence genes

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Abstract

Salmonella is an important poultry pathogen with zoonotic potential. Being a foodborne pathogen, *Salmonella*-contaminated poultry products can act as the major source of infection in humans. In India, limited studies have addressed the diversity of *Salmonella* strains of poultry origin. This study represented 26 strains belonging to *Salmonella* serovars Typhimurium, Infantis, Virchow, Kentucky, and Agona. The strains were tested for resistance to 14 different antimicrobial agents using the Kirby-Bauer disk-diffusion assay. The presence of the *invA*, *hilA*, *agfA*, *lpfA*, *sopE*, and *spvC* virulence genes was assessed by polymerase chain reaction (PCR), and the genetic diversity was assessed by Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR). The highest resistance to tetracycline ($n = 17$; 65.38%) followed by nalidixic acid ($n = 16$; 61.53%) was detected among the strains. Among the strains ($n = 17$) phenotypically resistant to tetracycline, 94% ($n = 16$) were also positive for the *tetA* gene. Based on the presence of virulence genes, the strains were characterized into three virulence profiles (PI, P2, and P3). Among the investigated virulence genes, *invA*, *hilA*, *agfA*, and *lpfA* were present in all strains. The *sopE* gene was mostly associated with serovars Virchow ($n = 3$; 100%) and Typhimurium ($n = 8$; 80%), whereas *spvC* gene was exclusive for two Typhimurium strains that lacked *sopE* gene. ERIC-PCR profiling indicated clusters correlating their serovar, geographical, and farm origins. These results demonstrate that *Salmonella* isolates with a wide genetic range, antibiotic resistance, and virulence characteristics can colonize poultry. The presence of such strains is crucial for both food safety and public health.

Keywords *Salmonella* · Virulence · Serovar · Antimicrobial resistance · Poultry · India

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Introduction

Each year, diarrheal diseases impact an estimated 550 million people worldwide and are thought to be the cause of 33 million fatalities. In India, *Salmonella* is one of the most frequent pathogens for gastrointestinal and systemic diseases [1, 2]. Approximately 2600 serovars of *Salmonella enterica* subsp. enterica (*Salmonella enterica*) have been reported, which cause enteric infections in both animals and humans [3, 4]. Non-typhoidal *Salmonella* (NTS) associated with many food products, poultry meat and eggs, is recognized as a potent zoonotic pathogen linked with foodborne transmission [5–7]. *Salmonella* spp. serovars are equipped with various mechanisms to invade and counter the oxidative stress within the cells. They also harbor diverse virulence factors resulting in enteric and systemic clinical manifestations in the host [8–11]. In the recent years, emergence of virulent multidrug-resistant (MDR) isolates of *Salmonella* has become a matter of serious public health concern [11].

A recent study reported the predominance of serovars Typhimurium, Infantis, Kentucky, Gallinarum, and Virchow in Indian poultry (2005–2019) [12]. A comparative study on poultry *Salmonella* strains representing 17 states (2011–2016) also involving the major egg-producing and consumer states reported Typhimurium, Gallinarum, and Enteritidis as the predominant serovars [13]. With an overall *Salmonella* prevalence of 3.5%, the predominant serovars reported were Enteritidis (68.1%) and Typhimurium (31.8%) from poultry and poultry products from Karnataka state. The study also reported 72.7% isolates as MDR (≥3 antimicrobial class) with the highest resistance was observed for polymyxin-B (81.8%) followed by nalidixic acid (72.7%) [14]. The emergence of serovar Agona among poultry farms in addition to commonly reported serovars was recently reported from the union territory of Jammu and Kashmir [15]. The use of antibiotic growth promoters (AGP) in poultry feed is unregulated in India. Testing of antimicrobial residues in 70 chicken meat samples meant for human consumption in New Delhi, India, indicated 40% positivity, with the predominant ones indicated were norfloxacin (20%), ciprofloxacin (14.3%), doxycycline (14.3%), oxytetracycline (11.4%), and chlortetracycline (1.4%) [16].

DNA-based fingerprinting techniques such as Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) are rapid and sensitive assays and can be easily performed in small-scale laboratories [17]. Hence, many researchers have attempted genotyping of *Salmonella* isolates to infer their persistence and spread in different geographical regions [18, 19]. Thus, continuous monitoring of the emergence and spread of virulent AMR *Salmonella* serovars is essential to prepare for establishing preventive and control strategies. Despite the high prevalence of salmonellosis in India, there are limited reports on the detailed characterization of *Salmonella* strains associated with poultry. Considering these facts, the present study was envisaged to study AMR, virulence, and genotypic characteristics of *Salmonella* strains recovered from poultry in India during the last four years.

Materials and methods

Bacterial strains and serotyping

Twenty-six (n=26) *Salmonella* strains maintained at the repository of National *Salmonella* Centre-Veterinary (NSC-Vet), Indian Veterinary Research Institute were used in the present study. The strains were recovered from poultry (broilers) during a period ranging from 2019 to 2022 recovered from four different states viz., Uttar Pradesh, Uttarakhand, Andaman and Nicobar Islands, and Jammu and Kashmir, India. Among these, n = 12 strains were also reported in a previous study from Jammu and Kashmir, India [15]. The strains were revived using brain heart infusion (BHI) broth after incubation for 12–18 h at 37 °C following the sub-culturing on Hektoen Enteric (HE) agar. Green-colored colonies with black centers were presumptively identified as *Salmonella* strains and were subsequently sub-cultured on nutrient agar for downstream analysis. Further, biochemical characterization was carried out using catalase, triple sugar iron, urease, citrate, and motility indole lysine (MIL) tests. *Salmonella* serotyping was performed using agglutination test and specific antisera (SSI Diagnostica A/S, Denmark) according to the White-Kauffmann Le-minor (WKL) scheme [20]. Molecular serotyping of the strains was also performed using serovar-specific PCR as described earlier [12]. The genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen, USA). All PCR assays in the present study were carried out in 25 µL reaction mixture containing 2.5 µL of DNA template, 12.5 µL of 2x master mix (DreamTaq Green PCR Master Mix, Thermo Scientific™), 0.5 µL each of forward and reverse primers (10 pmol/µL), and nuclease-free water to make up the volume. The primers, amplicon lengths, and annealing temperatures used for molecular serotyping PCR are mentioned in Supplementary Table 1. The amplified PCR products were electrophoresed on 2% agarose gels containing ethidium bromide and visualized using the UV gel documentation system (Alpha Imager, Germany).

Antimicrobial susceptibility testing

The antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method [21] as per the Clinical

and Laboratory Standards Institute guidelines [22]. Fourteen (*n* = 14) antimicrobial agents (Himedia, India) were tested at the following concentrations: cefotaxime + clavulanic acid (CEC, 30/10 µg), ceftazidime (CAZ, 10 µg), ceftazidime + clavulanic acid (CAC, 30/10 µg), streptomycin (S, 10 µg), levofloxacin (LE, 5 µg), ciprofloxacin (CIP, 5 µg), nitrofurantoin (NIT, 300 µg), tetracycline (TE, 30 µg), doripenem (DOR, 10 µg), ertapenem (ERT, 10 µg), meropenem (MER, 10 µg), imipenem (IMP, 10 µg), nalidixic acid (NA, 30 µg), kanamycin (K, 30 µg), amoxicillin/clavulanic acid (AMC, 30, 20/10), aztreonam (AT, 30 µg), trimethoprim-sulphathoxazole/co-trimoxazole (COT, 25 µg), and trimethoprim (TR, 5 µg). The extended spectrum beta-lactamase (ESBL) production in the *Salmonella* strains was determined by double disk diffusion test [23]. *Escherichia coli* strain ATCC 25922 and *Klebsiella pneumoniae* strain ATCC 700603 were used as the quality control strains. The zone of inhibition around the antibiotic disks was measured in mm and compared with the CLSI clinical break points (CLSI, 2018).

For genotypic resistance profiling, resistance genes were selected against antibiotics for which maximum strains were showing resistance. Molecular detection was carried out using polymerase chain reaction targeting the resistance genes like *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *Ib-cr*, and *qepA* for quinolones [24–28], *tetA*, *tetB*, and *tetC* genes for tetracycline [29], and *dfrA* gene for trimethoprim [30]. The genomic DNA extracted using QIAamp DNA Mini Kit (Qiagen, USA) was used as the template DNA. PCR conditions and product visualization were carried out as mentioned in earlier section. The primers, amplicon lengths, and annealing temperatures used for PCR are mentioned in Supplementary Table 1.

Virulence profiling

The *Salmonella* strains were investigated by PCR for the presence of virulence genes using standard PCR protocols. Uniplex PCR targeting six virulence genes *invA*, *agfA*, *lpfA*, *hilA*, *sopE*, and *spvC* were performed. [31–35]. PCR conditions and product visualization were carried out as mentioned in the earlier section. The primers, amplicon lengths, and annealing temperatures used for PCR are mentioned in Supplementary Table 1.

ERIC profiling

Genotyping of the *Salmonella* strains was performed by Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR using the primer pairs ERIC-F (5′-ATG TAA GCT CCT GGG GAT TCA C-3′) and ERIC-R (5′-AAG TAA GTG ACT GGG GTG AGC G-3′) [36]. The cycling conditions were as follows: initial denaturation at 95 °C for 7 min, followed by 30 cycles of denaturation at 90 °C for 30 s, annealing at 52 °C for 1 min, and extension at 65 °C for 8 min, and a final extension at 65 °C for 16 min [37]. The PCR products were separated using 2% agarose containing ethidium bromide. After electrophoresis, the gel images were captured and profiles were assigned manually.

Results

Bacterial strains and serotyping

Based on both the conventional and molecular serotyping, the strains were confirmed as belonging to the serovars Typhimurium (10/26), Infantis (5/26), Virchow (3/26), Kentucky (4/26), and Agona (4/26). The details of strains with respect to their serovar, host, source, farm, year of isolation, and geographical locations are shown in Supplementary Table 2.

Antimicrobial susceptibility testing

Twenty out of 26 strains (76.9%) were resistant to ≥1 of the tested antimicrobial drugs. The antimicrobial resistance profiles obtained for the strains are shown in Table 1. The antibiotic sensitivity testing (ABST) results revealed most of the strains resistant to tetracycline (TE) (*n* = 17/26; 65.38%) followed by nalidixic acid (NA) (*n* = 16/26; 61.53%) and trimethoprim (TR) (*n* = 8/26; 30.76%). In the present study, 61.53% (*n* = 16/26) of the strains were MDR isolates. None of the strains (0%; *n* = 26/26) were susceptible for all antibiotics, whereas 15.38% (*n* = 4/26) was resistant to one antibiotic, and another 15.38% (*n* = 4/26) was resistant to two different antibiotic classes. All the strains belonging to the serovars Infantis (5/5) and Virchow (3/3) were 100% MDR, whereas 75% (3/4) among Kentucky strains was MDR. However, only one *Salmonella* Typhimurium strain (*n* = 1/10; 10%) was identified as MDR. The two strains representing the serovar Infantis and one Typhimurium strain exhibited resistance to maximum numbers [6] of antimicrobial drugs, whereas the remaining Typhimurium strains were susceptible to most of the antimicrobial drugs. All strains except one representing serovars Kentucky (*n* = 3) and all Agona strains (*n* = 4) were resistant to levofloxacin, whereas among them 6 were resistant to ciprofloxacin. None of the *Salmonella* strains were identified as ESBL producers based on the double disk diffusion assay. Other than this, amoxicillin/clavulanic acid (AMC) resistance was exclusive to serovar Kentucky strains, and CIP resistance was exclusive to serovar Agona and Kentucky strains, whereas NIT resistance was predominant for serovar Infantis strains and K resistance predominant for serovar Infantis and Kentucky strains. All the above mentioned resistance profiles were also observed within strains belonging to the same geographical location

Table 1 Antimicrobial resistance profile of *Salmonella* strains. Among all strains (n = 26), 20 showed resistance to at least one antimicrobial agent tested. The highest number of strains showed resistance to tetracycline followed by nalidixic acid and trimethoprim

Sl no	Strain ID	Serovar	Antibiotic-resistant pattern
1	21JK01	*Salmonella* Infantis	TE, NIT, COT, TR, NA, K, CTX
2	21JK02	*Salmonella* Infantis	TE, NIT, COT, TR, NA, K
3	21JK03	*Salmonella* Infantis	TE, NIT, COT, TR, NA, K
4	21JK04	*Salmonella* Infantis	TE, NIT, COT, TR, NA, K
5	21JK05	*Salmonella* Infantis	TE, NIT, COT, TR, NA, K, CTX
6	21JK06	*Salmonella* Agona	TE, CIP, LE, NA
7	21JK07	*Salmonella* Agona	TE, CIP, LE, NA
8	21JK08	*Salmonella* Agona	TE, CIP, LE, NA
9	21JK09	*Salmonella* Agona	TE, CIP, LE, NA
10	21JK10	*Salmonella* Kentucky	TE, CIP, LE, NA, AMC, K
11	21JK11	*Salmonella* Kentucky	TE, CIP, LE,NA, AMC, K
12	21JK12	*Salmonella* Kentucky	TE, LE, NA, AMC, K
13	21UK05	*Salmonella* Virchow	TE, NIT, COT, TR, NA
14	21UK08	*Salmonella* Virchow	TE, COT, TR, NA
15	21UK10	*Salmonella* Typhimurium	TE
16	21UK11	*Salmonella* Typhimurium	CTX
17	21UK14	*Salmonella* Virchow	TE, COT, TR, NA, CTX
18	22NSC003	*Salmonella* Typhimurium	TE, COT, TR, K, NA, C, CTX
19	22NSC004	*Salmonella* Typhimurium	NA
20	20AN60	*Salmonella* Kentucky	CTX

(Jammu and Kashmir) and in most cases represented the same farms. On the other hand, the two Typhimurium strains representing two different farms from the same region differed for their AMR profiles. The serovar-wise resistance profiles are shown in Fig. 1A.

Out of the 26 *Salmonella* strains, 17 were resistant to TE in the disk diffusion assay. In accordance with the phenotypic assay, 16 out of the 17 tetracycline-resistant *Salmonella* strains (94.44%) were positive for the *tetA* (tetracycline efflux pump) gene. Thus, a good correlation between the phenotypic and genotypic expression of TE resistance was observed among the *Salmonella* strains. On the other hand, among the quinolone (levofloxacin and ciprofloxacin) (7/26; 26.92%) and trimethoprim (n = 8; 30.76%) resistant strains, corresponding resistant genes were not detected based on the genes targeted.

Virulence profiling

All the *Salmonella* strains were screened for the presence of virulence-associated genes, namely, *invA*, *hilA*, *agfA*, *lpfA*, *sopE*, and *spvC*. All the strains were found to carry a minimum of 3 virulence genes. The prevalence of virulence genes varied among strains. The highest prevalence was observed for *invA*, *hilA*, *agfA*, and *lpfA* (100%; n = 26/26). Strain wise distribution of virulence genes is shown in Table 2. Serovar wise distribution of virulence genes is shown in Fig. 1B. The strains were categorized into 3 virulence profiles based on the presence of virulence genes. Strains of profile P1 were having genes representing *invA*, *agfA*, *hilA*, *and lpfA*; P2 had virulence genes *invA*, *agfA*, *hilA*, *lpfA*, and *sopE*; P3 had virulence genes *inv A*, *agfA*, *hilA*, *lpfA*, and *spvC*. The frequency of occurrence of the virulence profile among the strains was P1 (50%), P2 (42.3%), and P3 (7.6%). The strains of the serovars Infantis (n = 5), Agona (n = 4), and Kentucky (n = 4) exhibited P1 virulence profile, whereas *Salmonella* Virchow (n = 3) showed P2 virulence profile. On the other hand, the strains of Typhimurium serovars (n = 10) manifested both P2 and P3 virulence profile. Out of the 10 *Salmonella* Typhimurium strains, 8 showed P2 profile and the 2 exhibited P3 profile. *Salmonella* serovars Infantis and Agona having the same geographical origin (Jammu and Kashmir) revealed similarity in virulence profile. *Salmonella* Kentucky (n = 4) strains from two different origins (Uttarakhand and Andaman and Nicobar Island) exhibited similar virulence profile. All the strains representing serovars Infantis, Kentucky, and Agona did not show the presence of *spvC* and *sopE* genes. Eight strains representing serovar Typhimuirum and three strains representing serovar Virchow harbored *sopE* gene, whereas *spvC* gene was unique to two Typhimurium strains isolated from suspected clinical cases from poultry.

ERIC profiling

PCR fingerprints of 26 *Salmonella* strains were obtained by ERIC-PCR. The ERIC-PCR profile was used to infer the genetic relatedness among isolates and to correlate with their

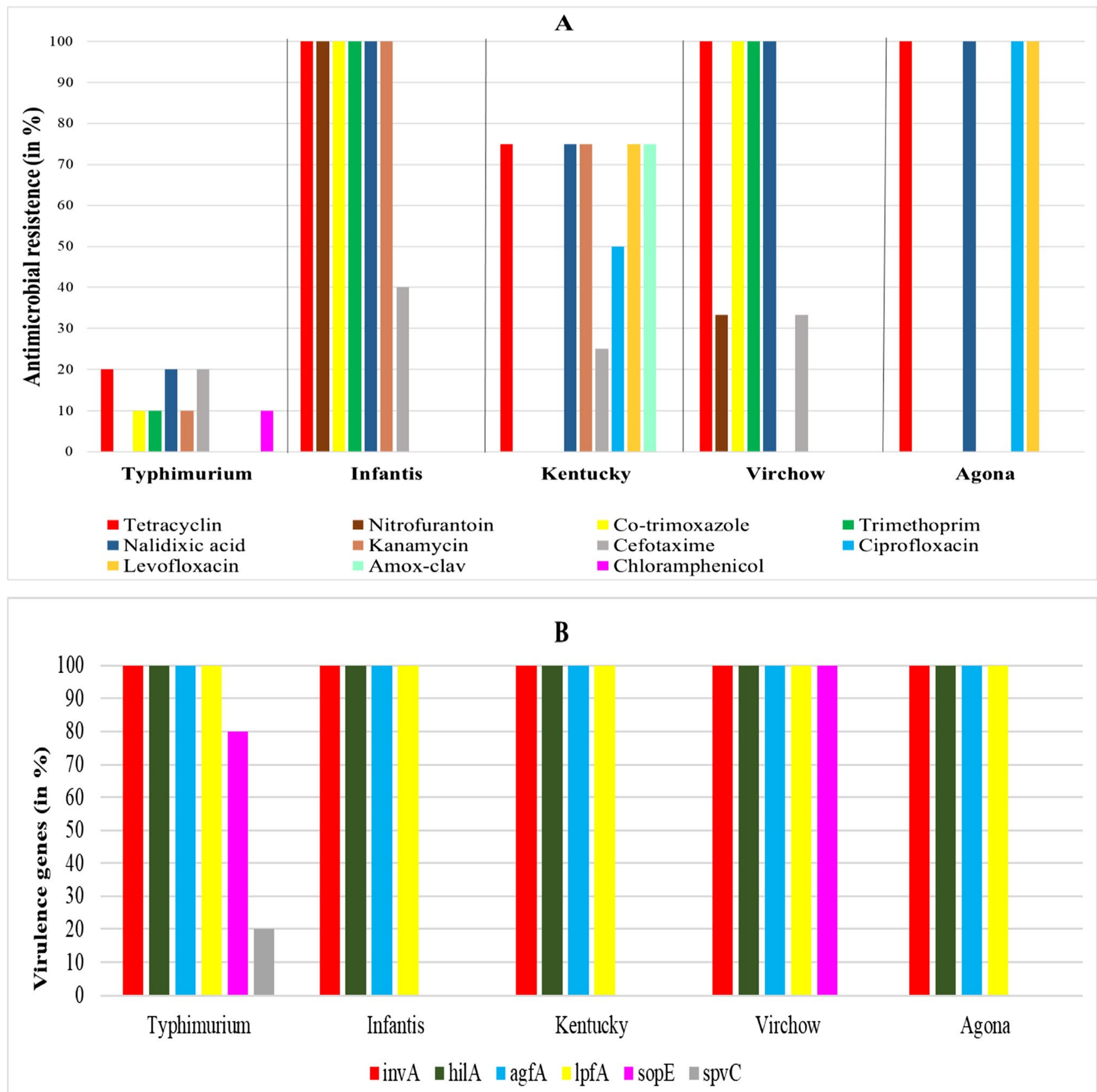

Fig. 1 Antimicrobial resistance and virulence pattern among *Salmonella* strains: serovar wise antimicrobial resistance **(A)** and virulence genes **(B)** are shown in the form of a bar diagram. The occurrence of viulence and AMR is represented as percentage calculated by considering each serovar seperately The number of strains respresenting each serovar was Typhimurium - 10, Infantis - 5, Kentucky - 4, Virchow - 3 and Agona - 4

serovar and geographical/farm origins. Strains with similar ERIC PCR fingerprints were assigned unique profile numbers (P1 to P10). In general, the strains representing the same serovar and belonging to same farm clustered together. The profile P1 represented Agona, P2 and P3 represented Kentucky, P4 and P5 represented Virchow and Infantis, P7–P10 represented Typhimurium strains. Two strains originating from the same geographical region (Jammu and Kashmir) within the serovar Kentucky formed a closely related cluster, whereas another strain belonging to a different geographical origin (Andaman and Nicobar Islands) formed separate cluster. Strains representing serovar Typhimurium represented 4 profiles, with two profiles (P9 and P10) representing strains from the same geographical origins (Uttarakhand). The

Table 2 Virulence profiles among *Salmonella* strains. The strains represented 3 virulence profiles based on the presence of the virulence genes. The strains ($n = 10$), representing serovar Typhimurium indicated P2 and P3 virulence profiles

Sl no.	Strain	Serovar	Virulence profile
1	20AN60	*Salmonella* Kentucky	**P1** (*invA*, *agfA*, *hilA*, *lpfA*)
2	21JK01	*Salmonell*a Infantis	
3	21JK02	*Salmonell*a Infantis	
4	21JK03	*Salmonell*a Infantis	
5	21JK04	*Salmonell*a Infantis	
6	21JK05	*Salmonell*a Infantis	
7	21JK06	*Salmonella* Agona	
8	21JK07	*Salmonella* Agona	
9	21JK08	*Salmonella* Agona	
10	21JK09	*Salmonella* Agona	
11	21JK10	*Salmonella* Kentucky	
12	21JK11	*Salmonella* Kentucky	
13	21JK12	*Salmonella* Kentucky	
14	21UK04	*Salmonella* Typhimurium	**P2** (*invA*, *agfA*, *hilA*, *lpfA*, *sopE)*
15	21UK05	*Salmonella* Virchow	
16	21UK06	*Salmonella* Typhimurium	
17	21UK07	*Salmonella* Typhimurium	
18	21UK08	*Salmonella* Virchow	
19	21UK09	*Salmonella* Typhimurium	
20	21UK10	*Salmonella* Typhimurium	
21	21UK11	*Salmonella* Typhimurium	
22	21UK12	*Salmonella* Typhimurium	
23	21UK13	*Salmonella* Typhimurium	
24	21UK14	*Salmonella* Virchow	
25	22NSC003	*Salmonella* Typhimurium	**P3** (*invA*, *agfA*, *hilA*, *lpfA*, *spvC*)
26	22NSC004	*Salmonella* Typhimurium	

remaining two strains from the same geographical origins (Uttar Pradesh) represented unique profiles (P7 and P8), but were isolated from different farms. The genetic diversity analysis of *Salmonella* strains using ERIC-PCR fingerprints along with the serovar information and geographical locations is depicted in Fig. 2.

Discussion

Salmonellosis is a significant threat to public health worldwide. Globally, an estimated 93 million enteric infections occur annually because of non-typhoidal *Salmonella* (NTS) infections, causing 155,000 deaths [38]. NTS-associated infection is considered to be a neglected emerging enteric infection in India [39]. Unhygienic practices during the slaughtering of animals, improper handling, and transportation of raw meat all increase the likelihood of contamination of meat products with NTS [40]. The pathogenic potential and AMR of *Salmonella* can change over time and hence need to be routinely investigated for implementing appropriate control measures. Considering these facts, in the present study, a total of twenty-six *Salmonella* strains of poultry origin were subjected to antimicrobial susceptibility testing, virulence profiling, and strain typing based on ERIC-PCR profiling.

Among all, 20 strains (76.92%) were found to be resistant to at least one antimicrobial drug. The highest number of strains was resistant to tetracycline (TE) (65.38%). Earlier studies reported TE resistance ranging from 36.3 to 100% for *Salmonella* strains associated with poultry in India [41–44]. Resistance to tetracycline associated with broiler chicken from other countries like South Africa (93%) and Brazil (83%) was also reported in similar ranges [45]. Among all tetracycline resistance mechanisms, efflux pump *tet* gene classes A and B are recognized as the most common genes associated with resistance in *Salmonella* [46]. In the present study, *tetA* was found to be present in 94% of the resistant isolates. Significant association of *tetA* gene (56% to 100%) for tetracycline resistance in poultry *Salmonella* strains has been reported in India [41, 43, 44].

Treatment regime using fluoroquinolones may fail in patients infected with *Salmonella* spp. resistant to nalidixic acid (NA) [47, 48]. The present study identified nalidixic acid resistance among 61.53% of the isolates. Corroborating resistance percentage (56.25%) was reported by an earlier

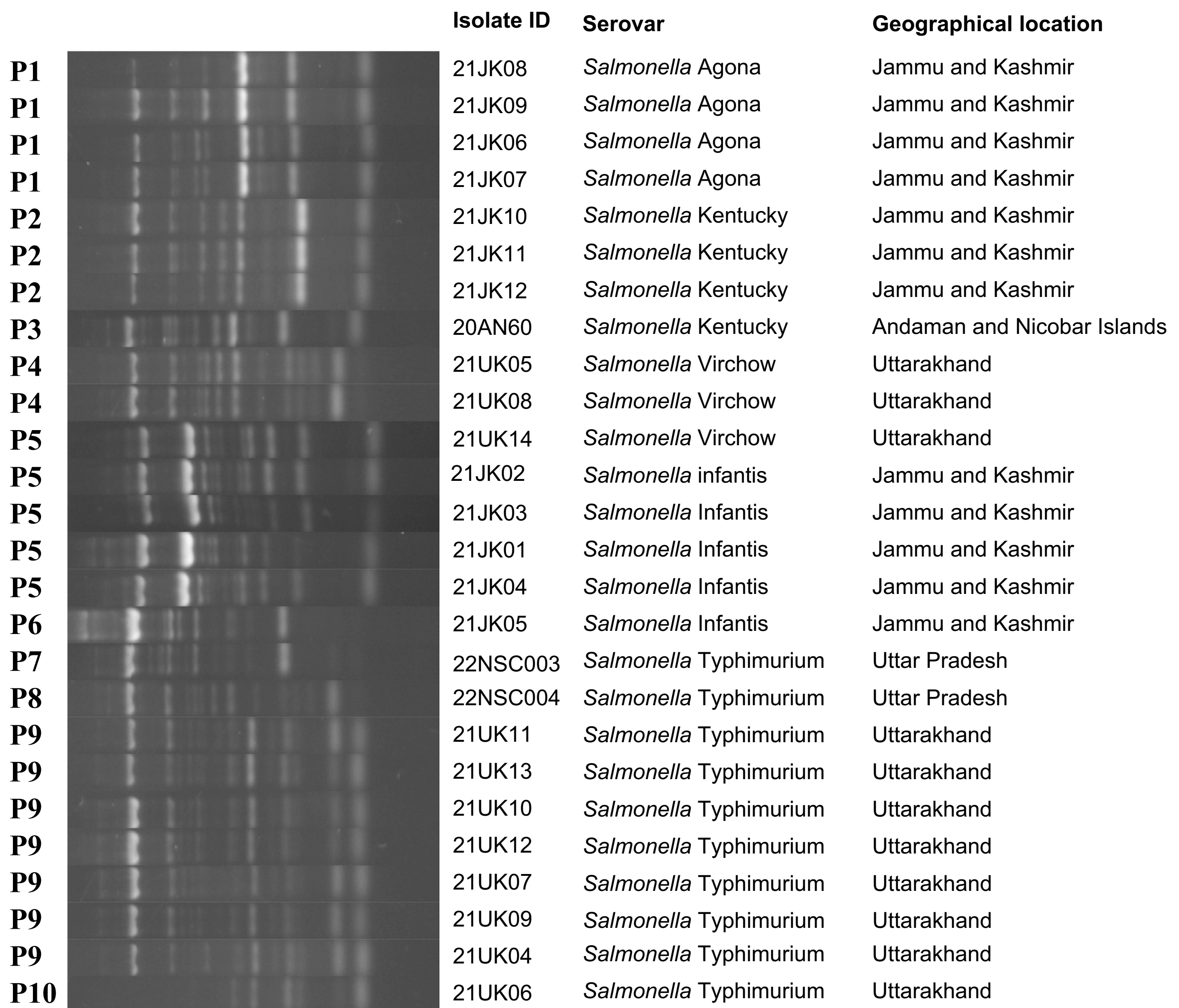

Fig. 2 ERIC PCR profiling of *Salmonella* strains. This figure depicts the ERIC-PCR profiles of *Salmonella* strains correlating with their serovars and geographical origins

study on poultry associated *Salmonella* in Rajasthan, India [42]. Strains representing different geographical origin strains were also indicated with time-dependent changes (90.63 to 46.43% from 1990 to 2017) in their susceptibility pattern for NA [41].

The current study detected both trimethoprim and co-trimoxazole (COT) resistance in 34 % and ciprofloxacin (CIP) resistance in 23.07% strains. An earlier study reported 25.00% and 15.62% strains resistant to trimethoprim and ciprofloxacin, respectively, among a total of 32 strains [42]. However, none of the resistant strains were positive for respective AMR genes targeted by PCR in the study. Thus, it may be assumed that the phenotypic resistance may be mediated by point mutations or other mechanisms that are not explored in the study.

Presently, there is no national database or surveillance data for estimating the use of antimicrobials in health and veterinary sectors in India [49]. The antimicrobial drugs of choice for the treatment of human NTS infections are chloramphenicol, ampicillin, trimethoprim-sulphamethoxazole (cotrimoxazole), fluoroquinolones, and extended spectrum cephalosporins [41, 50]. However, antibiotic growth promoters (AGP) often included in animal feeds to promote growth can contribute to development of AMR bacteria. The

AGPs reported for common use in Indian poultry sector are oxytetracycline, chlortetracycline, bacitracin, furazolidone, enrofloxacin, cephalosporins, ciprofloxacin, and tylosin [51]. Thus, the higher resistance for TE, NA, and CIP observed in the study correlates the possibility of high AGP usage in chicken. A high probability of resistance to NA, CIP, and TE in bacteria associated with Indian poultry sector was reported earlier based on studies in *E. coli* [52].

With respect to serovar association to any particular antibiotic resistance, we observed K, NIT, CIP, and AMC resistance associated to certain serovars such as Infantis, Agona, and Kentucky. In most cases, the strain represented the same farms and hence indicates these AMR profiles can be influenced by farm level managements including the source of poultry stock procurement to the antimicrobial usages within farm. Antimicrobial usage (AMU) pattern at farm levels correlating with high levels of resistance to the same antibiotic in *Salmonella* was reported in a recent study [53].

A 19-year spanning research study (2000 to 2018) conducted in India unveiled the slow emerging trends of antimicrobial resistance patterns associated with NTS serovars isolated from human feces [50]. A study involving 271 *Salmonella* isolates representing the period 1990 to 2017 from poultry, farm animals, and environmental sources similarly indicated a rise in antibiotic resistance for most of the tested antibiotics except cephalosporins and carbapenems [41]. All these factors emphasize the importance of NTS serovars and associated AMR risks in all sectors.

Salmonella serovars during the course of time can lose or acquire virulence factors as a result of adaptation to new hosts or environments [54]. In the present study, the presence of six virulence genes was investigated. None of the *Salmonella* strains was found to harbor all 6 virulence genes, whereas all the strains harbored the *invA*, *hilA*, *agfA*, and *lpfA* genes. Among these, *invA a*nd *hilA* genes are part of the *Salmonella* pathogenicity island 1 (SPI-1) and hence are required for host epithelial cell invasion in pathogenic *Salmonella*. On the other hand, aggregative fimbriae (*agfA*) and long polar fimbriae (*lpf*) are reported for involvement in colonization and virulence [55]. The *agfA* gene is associated with adhesion and biofilm formation [56], whereas *lpfA* gene is involved in adhesion to surfaces and epithelial cells, an essential prior stage of biofilm formation [57]. This hence indicates the essentiality of these genes for the survival of *Salmonella* in poultry gastrointestinal tracts and associated environments. Similar ubiquitous presence of *invA*, *hilA*, *agfA*, and *lpf* genes among all the involved poultry strains representing serovar Typhimurium and Enteritidis was reported by earlier studies [11, 54, 57].

In the present study, the *sopE* gene was detected in 11 (42%) and the *spvC* gene was detected in two (6.79%) strains. The *spv* (*Salmonella* plasmid virulence) operon is a highly conserved region that attenuates intestinal inflammation, promotes bacterial dissemination, and results in systemic infection [58]. In the present study, the presence of these genes in serovar Typhimurium strains isolated from clinical cases probably indicates their role in the septicemic manifestation of disease. *Salmonella* outer proteins (Sop) encoded by *sop* genes are the effector molecules of type-III secretion system (TTSS) which are involved in the early stages of *Salmonella* infection. Several isoforms of the *sop* genes have been identified (*sopA–sopE*) [59]. For the study strains, *sopE* gene was unique to all the *Salmonella* Virchow strains [3] and to those *Salmonella* Typhimurium strains [5] lacking the *spvC* gene. Similar variation among *Salmonella* Typhimurium strains with respect to carriage of *spvC and spoE* genes was reported from poultry strains in earlier studies [60]. The possible reason could be that the association of these genes differs within the genome as the *spvC* gene is a plasmid-borne virulence gene, whereas *spoE* gene is a prophage-related virulence gene [61].

In the present study, ERIC profiles indicated strain clustering correlating their serovar and geographical/farm origins. A unique profile mostly involved strains representing a particular serovar. Exceptions were observed for serovar Typhimurium and Kentucky were strains represented multiple profiles. In most cases, a different profile was observed when the strain had a different geographical or farm origin. Multiple ERIC profiles [5] within a single serovar are reported while involving 22 strains of serovar Gallinarum. The strains involved in a single profile were also belonging to different geographical locations and years of isolation [62]. Similarly, ERIC profiling of 45 poultry *Salmonella* isolates representing six serovars was indicated with 8 major profiles correlating their serovar and host origins, whereas 6 strains had unique profiles and remained unclustered in phylogeny. Among these, serovars Kentucky and Enteritidis strains were also involved in unrelated clusters [63]. This hence indicates strains representing a particular serovar usually cluster together, but often can also be represented in multiple clusters or form unique clusters.

Conclusion

To conclude, the present study characterized 26 strains of *Salmonella* belonging to serovars Typhimurium, Virchow, Kentucky, Infantis, and Agona. The strains exhibiting both MDR phenotype and maximum virulence genes may have the potential to evolve into a dominant clone with high zoonotic potential. Salmonellosis in humans due to such strain may overwhelm the current therapeutic regimes resulting in high treatment costs and fatality. The genetic diversity among the strains regardless of the serovars further necessitates continuous monitoring and surveillance of *Salmonella* strains among the poultry industry**.**

Abbreviations NTS: Non-typhoidal *Salmonella*; ERIC-PCR: Enterobacterial Repetitive Intergenic Consensus PCR; AMR: Antimicrobial resistance; MDR: Multidrug-resistant

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Code availability Not applicable.

Author contributions DG and PT conceptualized and designed the study. DG, YMI, and AS carried out strain isolation and culturing. AKM carried out postmortem examination and sampling. DG, LP, SI and SN carried out AMR studies and virulence profiling. AB and PT carried out serotyping. SK generated ERIC profiles. PT and VC supervised the study. DG, LP, MS, PD, and PT wrote the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

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Data availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval Not applicable as animal experiments were not conducted for this research. All sample collections were made after approval and as per the guidelines of the Institutional Animal Ethics Committee, ICAR-IVRI.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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