



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2025; SP-11(7): 10-15

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www.veterinarypaper.com

Received: 19-04-2026

Accepted: 23-05-2026

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Phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli* and salmonella isolated across the production cycle of integrated broiler chicken farms in Karnataka, India

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i7Sa.3597>

Abstract

Antimicrobial resistance (AMR) in *Escherichia coli* and *Salmonella* from broiler production systems is a growing One Health concern, yet longitudinal surveillance across an entire production cycle remains limited in India. This study characterized the phenotypic and genotypic AMR profiles of *E. coli* and *Salmonella* recovered from two integrated broiler farms and one franchisee farm in Bengaluru Rural district, Karnataka, tracking boot socks, water, feed, faecal swabs and ileal/caecal contents at six time points (days 0, 7, 14, 21, 28 and 35) of a single crop cycle. Of 101 samples processed, culture and *uspA/hilA*-PCR confirmed 342 *E. coli* and 10 *Salmonella* isolates, corresponding to farm-level recoveries of 76.2% and 9.9%, respectively; no *Salmonella* was recovered from the newly established Farm 1. Disc diffusion testing of a representative isolate subset (117 *E. coli*; 10 *Salmonella*) showed that every isolate was resistant to at least one antimicrobial. *E. coli* resistance was highest to ampicillin (91.3%) and doxycycline (89.3%) and lowest to ceftiofur (13.3%); colistin resistance by broth microdilution was 42.3%. *Salmonella* resistance followed a similar pattern, with doxycycline (92%) and colistin sulphate (76–79%) predominating. Screening for 22 resistance determinants by PCR identified *qnrB* (96.9%), *qepA* (92.7%) and *blaTEM* (72.0%) as the most frequent genes in *E. coli*, while *blaTEM* and *blaSHV* were universal (100%) in *Salmonella*; *mcr-3* was the only mobile colistin-resistance gene detected, and only in *E. coli*. Chi-square analysis confirmed significant phenotype–genotype concordance for quinolones and β -lactams but not for colistin. Detectable resistance genes on day zero of the cycle indicate that AMR is seeded before farm-level antimicrobial exposure, most plausibly through vertical transmission from breeder stock, underscoring the need for hatchery-level surveillance alongside farm biosecurity.

Keywords: Antimicrobial resistance, broiler chicken, *Escherichia coli*, *Salmonella*, *qnr* genes, *blaTEM*, *mcr-3*, production cycle, One Health

1. Introduction

Broiler production is among the fastest-expanding segments of Indian agriculture, and antimicrobials continue to be used therapeutically, prophylactically and, in some systems, for growth promotion. This reliance has coincided with a global rise in antimicrobial resistance (AMR), which the World Health Organization has identified as one of the leading public health threats of the current era ^[1–4]. *Escherichia coli* and *Salmonella* are widely used sentinel organisms for AMR surveillance in food animals because they are ubiquitous commensals or pathogens, are readily recovered from diverse sample matrices, and reflect the selective pressure exerted by antimicrobial use in the production environment ^[6, 7].

Cross-sectional surveys of AMR in broiler flocks are increasingly available from South Asia, but comparatively few studies have tracked resistance longitudinally across a full production cycle – from day-old chick placement to slaughter age – in a way that distinguishes environmental, feed- and water-borne sources of resistant bacteria from bird-associated ones. Understanding when, during the cycle, resistant organisms and resistance genes first appear, and where they persist, is central to designing targeted interventions, since resistance already

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present at placement points toward hatchery- or breeder-level transmission rather than on-farm antimicrobial pressure alone. This study was undertaken to (i) isolate and identify *E. coli* and *Salmonella* from two integrated broiler farms and one franchisee farm in Bengaluru Rural district, Karnataka, at defined intervals across a single crop cycle; (ii) determine the phenotypic antimicrobial susceptibility profiles of the recovered isolates; and (iii) screen isolates for 22 resistance genes spanning β -lactam, plasmid-mediated quinolone resistance (PMQR) and mobile colistin-resistance (*mcr*) determinants, so as to identify the principal drivers and reservoirs of AMR within the broiler production chain.

2. Materials and Methods

2.1 Study area, farms and sample collection

The study was based in the Department of Veterinary Microbiology, Veterinary College, Bengaluru (Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar). Three commercial broiler farms in Bengaluru Rural district – two integrated units and one franchisee farm affiliated to a commercial breeder company – were monitored through one complete crop cycle each, between September and October 2021, using proportionate random sampling. From each farm, five sample categories – boot socks (inside and outside the shed), water (drinkers, tank, borewell and puddle sources), feed (bags and feeders), pooled faecal swabs (inside and outside the shed) and gastrointestinal contents (ileum and caecum, collected on day 35) – were obtained on days 0, 7, 14, 21, 28 and 35, giving a total of 101 samples. Samples were transported to the laboratory under cold chain and processed within 24 h.

2.2 Isolation and molecular confirmation

Samples were pre-enriched in buffered peptone water; for *Salmonella*, pre-enrichment was followed by selective enrichment in tetrathionate brilliant green bile broth. Enriched cultures were streaked onto Eosin Methylene Blue agar (EMBA) and EMBA supplemented with cefotaxime (to enrich for cephalosporin-resistant/ESBL-producing *E. coli*), and onto Xylose Lysine Deoxycholate (XLD) and Brilliant Green agar (BGA) for *Salmonella*. Presumptive colonies were purified on nutrient agar and confirmed by colony PCR targeting the *E. coli*-specific *uspA* gene (884 bp) [9] and the *Salmonella*-specific *hlyA* gene (784 bp) [10]. Confirmed isolates were archived in duplicate as 30% glycerol stocks at –20 °C.

2.3 Antimicrobial susceptibility testing

A representative subset of isolates (one *E. coli* and one cefotaxime-selected *E. coli* per positive sample, $n = 117$; and all 10 confirmed *Salmonella* isolates) was tested by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar against eight antimicrobials from six classes commonly used in poultry: ampicillin (10 μ g), cefotaxime/ceftiofur (30 μ g), doxycycline (30 μ g), gentamicin (10 μ g), amikacin (30 μ g), enrofloxacin (5 μ g) and trimethoprim–sulfamethoxazole (1.25/23.75 μ g). Zone diameters were interpreted against standard breakpoints.

2.4 Minimum inhibitory concentration for colistin

Because colistin susceptibility is not reliably assessed by disc diffusion, resistance to colistin sulphate was determined for all 117 *E. coli* and 10 *Salmonella* isolates by broth microdilution, with an MIC greater than 2 μ g/mL classified as

resistant.

2.5 Creening for antimicrobial resistance genes

Genomic DNA extracted by boiling lysis was screened by multiplex/simplex PCR for 22 resistance genes spanning three antimicrobial classes: nine β -lactamase genes (*bla*TEM, *bla*SHV, *bla*OXA, *bla*NDM, and *bla*CTX-M universal and groups 1, 2, 8/25 and 9) [11], eight PMQR/quinolone-resistance genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *oqxAB*, *qepA* and *aac*(6')-Ib-cr), and five mobile colistin-resistance genes (*mcr*-1 to *mcr*-5) [12]. Each run included positive, negative and no-template controls, and amplicons were resolved on 2% agarose gels against a 100 bp DNA ladder.

2.6 Statistical analysis

Data were analysed in R (RStudio). The association between phenotypic resistance and the presence of the corresponding resistance gene(s) was assessed for enrofloxacin, ampicillin, ceftiofur and colistin sulphate using Pearson's chi-square test, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Isolation of *E. coli* and *Salmonella*

Of 101 samples processed, 342 *E. coli* isolates were recovered (287 on EMBA and 55 on EMBA + cefotaxime) and confirmed by *uspA* PCR, corresponding to an overall culture-based prevalence of 76.2%. Recovery was highest (100%) from feed-feeder swabs, externally collected faecal swabs and ileal/caecal contents, and lowest from water tank (12%) and borewell/puddle sources (33.3%); *E. coli* was not recovered from boot socks collected inside the shed or from borewell water in Farm 1. Ten *hlyA*-PCR-confirmed *Salmonella* isolates were obtained, an overall prevalence of 9.9%. *Salmonella* was not detected in Farm 1, a newly established unit, while boot-sock swabs yielded the highest recovery (27.7%) in Farms 2 and 3, followed by ileal/caecal contents (6.6%). Neither organism was recovered from feed bags or from water sources other than drinkers (Table 1).

Table 1: Recovery of *E. coli* and *Salmonella* from different sample categories across the three farms.

Sample category	<i>E. coli</i> recovery (%)	<i>Salmonella</i> recovery (%)
Feed (feeders)	100.0	0.0
External faecal swabs	100.0	–
Ileal and caecal contents	100.0	6.6
Faecal swabs (in-shed, pooled)	94.0	11.1
Boot socks (outside)	88.0	27.7
Water drinkers	66.6	0.0
Water – borewell / puddle (outside)	33.3	0.0
Feed bags	16.0	0.0
Water tank	12.0	0.0
Boot socks (inside shed)	–	0.0

3.2 Antibigram of *E. coli*

All 117 *E. coli* isolates tested were resistant to at least one antimicrobial. Resistance was highest to ampicillin (91.3%) and doxycycline (89.3%), followed by gentamicin (74.0%), enrofloxacin (72.0%), trimethoprim–sulfamethoxazole (56.0%) and amikacin (48.7%); ceftiofur retained the greatest activity, with only 13.3% resistance. The pattern was broadly consistent across the three farms, although Farm 1 recorded the highest resistance to gentamicin, enrofloxacin and amikacin (Table 2). Notably, resistance to most agents was already substantial in isolates recovered on day 0, before birds

had received any farm-level antimicrobial treatment.

Table 2: Farm-wise antibiogram of *E. coli* isolates by disc diffusion / broth microdilution (% resistant, n = 117)

Antimicrobial agent	Farm 1	Farm 2	Farm 3	Overall
Ampicillin	92.0	92.0	83.0	91.33
Doxycycline	86.0	92.0	90.0	89.33
Gentamicin	84.0	61.0	77.0	74.00
Enrofloxacin	84.0	61.0	71.0	72.00
Trimethoprim-sulfamethoxazole	59.0	53.0	56.0	56.00
Amikacin	57.0	37.0	52.0	48.66
Colistin sulphate (MIC)	57.0	32.0	38.0	42.33
Ceftiofur	19.0	11.0	10.0	13.33

3.3 Colistin resistance by minimum inhibitory concentration

Colistin sulphate resistance (MIC > 2 µg/mL) was recorded in 42.3% of *E. coli* isolates overall, ranging from 32% (Farm 2) to 57% (Farm 1). Among *Salmonella* isolates, colistin resistance was markedly higher, at 79% overall (75% in Farm 2 and 83% in Farm 3).

3.4 Antibiogram of *Salmonella*

All ten *Salmonella* isolates were resistant to at least one antimicrobial. Resistance was highest to doxycycline (92%) and colistin sulphate (76% by disc diffusion; 79% by MIC), followed by ampicillin and enrofloxacin (64% each), gentamicin (44%) and trimethoprim-sulfamethoxazole/amikacin (24% each); all isolates remained sensitive to ceftiofur (Table 2b).

Table 2b: Overall antibiogram of *Salmonella* isolates (% resistant, n = 10).

Antimicrobial agent	Resistance (%) (n = 10)
Doxycycline	92.0
Colistin sulphate (MIC)	76.0 / 79.0*
Ampicillin	64.0
Enrofloxacin	64.0
Gentamicin	44.0
Trimethoprim-sulfamethoxazole	24.0
Amikacin	24.0
Ceftiofur	0.0

*Disc diffusion / broth microdilution (MIC) values for colistin sulphate.

3.5 Prevalence of antimicrobial resistance genes

Sixteen of the 22 screened genes were detected among the *E. coli* isolates. *qnrB* (96.9%) and *qepA* (92.7%) were near-ubiquitous, followed by *blaTEM* (72.0%), *blaCTX-M* universal (29.6%), *qnrS* (28.9%) and *qnrD* (28.6%); individual *blaCTX-M* groups, *oqxAB*, *blaSHV*, *blaOXA*, *aac(6')-Ib-cr* and *qnrA* were present at lower frequencies (0.5–10.8%). Among the *mcr* genes, only *mcr-3* was detected, at 6.3% overall (farm range 4.2–10.5%); *mcr-1*, -2, -4 and -5 were not found in any isolate. Gene positivity was already 45.5% among isolates recovered on day 0, declining marginally to 37.9% by day 35, and was concentrated in boot-sock, faecal-swab and ileal/caecal isolates.

In *Salmonella*, only β -lactamase and PMQR genes were detected; no *mcr* gene was found. *blaTEM* and *blaSHV* were present in all ten isolates (100%), while *qnrD* (68.3%), *qnrB* (61.6%), *qnrA* (15%) and *oqxAB* (5%) were the predominant quinolone-resistance determinants. None of the *blaOXA*, *blaNDM* or *blaCTX-M* variants, nor *qnrC*, *qnrS* or *aac(6')-Ib-cr*, were detected (Table 3).

Table 3: Prevalence of the 22 screened antimicrobial resistance genes in *E. coli* and *Salmonella* isolates

Resistance gene	<i>E. coli</i> (%) (n = 117)	<i>Salmonella</i> (%) (n = 10)
β-lactamase genes		
<i>blaTEM</i>	71.96	100.0
<i>blaSHV</i>	4.00	100.0
<i>blaOXA</i>	2.50	0.0
<i>blaNDM</i>	0.0	0.0
<i>blaCTX-M</i> (universal)	29.56	0.0
<i>blaCTX-M</i> group 1	10.80	0.0
<i>blaCTX-M</i> group 2	5.64	0.0
<i>blaCTX-M</i> group 9	7.85	0.0
<i>blaCTX-M</i> group 8/25	1.85	0.0
Quinolone / PMQR genes		
<i>qnrA</i>	0.50	15.0
<i>qnrB</i>	96.87	61.6
<i>qnrC</i>	0.0	0.0
<i>qnrD</i>	28.62	68.3
<i>qnrS</i>	28.93	0.0
<i>oqxAB</i>	5.50	5.0
<i>qepA</i>	92.70	0.0
<i>aac(6')-Ib-cr</i>	1.50	0.0
Mobile colistin-resistance genes		
<i>mcr-1</i>	0.0	0.0
<i>mcr-2</i>	0.0	0.0
<i>mcr-3</i>	6.27	0.0
<i>mcr-4</i>	0.0	0.0
<i>mcr-5</i>	0.0	0.0

3.6 Phenotype-genotype correlation

Chi-square analysis showed a significant association between phenotypic resistance and the presence of the corresponding resistance gene(s) for enrofloxacin ($\chi^2 = 54.576$, $p = 3.99 \times 10^{-11}$), ampicillin ($\chi^2 = 124.71$, $p < 2.2 \times 10^{-16}$) and ceftiofur

($\chi^2 = 106.78$, $p < 2.2 \times 10^{-16}$). No significant association was found for colistin sulphate ($\chi^2 = 3.723$, $p = 0.0537$), indicating that a negative *mcr* genotype does not reliably predict phenotypic colistin susceptibility (Table 4).

Table 4: Association between phenotypic resistance and corresponding resistance genes in *E. coli* (Pearson's chi-square test)

Antimicrobial class	Agent tested	χ^2	p-value	Association
Fluoroquinolones (PMQR)	Enrofloxacin	54.576	3.99×10^{-11}	Significant
β -lactams	Ampicillin	124.71	$< 2.2 \times 10^{-16}$	Significant
β -lactams	Ceftiofur	106.78	$< 2.2 \times 10^{-16}$	Significant
Polymyxins	Colistin sulphate	3.723	0.0537	Not significant

4. Discussion

The 76.2% culture-based prevalence of *E. coli* recorded here is broadly consistent with earlier broiler-associated surveys, in which recovery has typically ranged between about 62% and 72% [13, 15]. *Salmonella* prevalence (9.9%) was comparatively low, sitting within the wide range (roughly 10–50%) reported elsewhere depending on flock age, biosecurity practice and sample matrix [24, 31]. The absence of *Salmonella* from Farm 1 – a newly commissioned unit – together with its recovery predominantly from boot-sock swabs in the other two farms, agrees with reports identifying boot-sock or environmental sampling as a sensitive indicator of farm-level *Salmonella* contamination [25].

Resistance to ampicillin and doxycycline dominated the phenotypic profile of both organisms, echoing regional surveys that report similarly high resistance to these older, widely used agents alongside comparatively preserved susceptibility to extended-spectrum cephalosporins such as ceftiofur. Sarker *et al.* [16] reported an overall *E. coli* resistance of 61.7% in Bangladeshi broilers, with near-complete resistance to ampicillin and tetracycline and colistin resistance of 48.7% – figures that align closely with the present findings. A notable observation was the substantial phenotypic and genotypic resistance already present on day 0, before birds had received any farm-level antimicrobial treatment. This points to sources of AMR that precede on-farm antimicrobial exposure: vertical transmission of resistant organisms and resistance genes from breeder flocks through the hatchery to newly placed chicks have been repeatedly implicated in comparable studies [27, 28]. This finding reframes farm-level antimicrobial stewardship as necessary but not, on its own, sufficient; hatchery- and breeder-level surveillance is likely required to meaningfully reduce the resistance burden entering grow-out farms.

The near-universal presence of *qnrB* and *qepA* in *E. coli*, and of *bla*TEM/*bla*SHV in *Salmonella*, mirrors the wide dissemination of plasmid-mediated quinolone resistance and TEM/SHV β -lactamases among poultry-associated Enterobacteriaceae, both of which are frequently carried on mobile genetic elements that facilitate horizontal transfer [18, 19]. The comparatively restricted *mcr* profile – only *mcr-3* detected, and only in *E. coli* – is noteworthy given that colistin use in animal feed has been prohibited in India. Persistence of *mcr-3*-positive isolates and of phenotypic colistin resistance (42.3% in *E. coli*, 79% in *Salmonella*) despite this restriction suggests either residual off-label use, environmental persistence of resistant clones, or resistance maintained through co-selection by other antimicrobials, consistent with observations from other South Asian settings where regulatory bans have not eliminated colistin-resistant isolates [21, 22].

The strong phenotype–genotype concordance observed for quinolones and β -lactams, contrasted with its absence for colistin, is consistent with the more complex and still incompletely catalogued genetic basis of colistin resistance, which includes chromosomal mechanisms not captured by an *mcr*-targeted panel. This underscores that phenotypic MIC testing, rather than genotypic screening alone, remains

essential for reliable colistin-resistance surveillance.

Taken together, these findings identify boot socks, faecal material and gastrointestinal contents – rather than feed or water – as the principal reservoirs of resistant *E. coli* and *Salmonella* within the farm environment, consistent with previous reports implicating litter and faecal material as major AMR reservoirs capable of subsequently contaminating agricultural land through litter reuse [17].

5. Conclusion

This production-cycle-wide survey shows that resistant *E. coli* and *Salmonella*, together with a substantial burden of transferable resistance genes, are already present in broiler flocks at placement and persist throughout the grow-out period, implicating vertical and environmental transmission alongside farm-level antimicrobial use as drivers of AMR. Boot-sock, faecal and gastrointestinal samples consistently carried the highest resistance-gene loads, making them practical sentinel matrices for routine surveillance. Given the near-universal presence of β -lactam and PMQR determinants, and continuing phenotypic colistin resistance despite regulatory restrictions, integrated interventions – hatchery-level monitoring, stricter farm biosecurity, prudent antimicrobial stewardship and continued genomic surveillance – are needed to curb the spread of AMR along the broiler production chain.

6. Acknowledgements

The authors thank the management and staff of the participating broiler farms for permitting access for sample collection, and the Department of Veterinary Microbiology, Veterinary College, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, for providing the laboratory facilities used in this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. [Edit to reflect actual funding source, if any.]

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Conceived and designed the study: R.S. Conducted sample collection, laboratory analysis and data curation: A.A.H.J. Supervised molecular and microbiological analyses: D.R., S.I. Contributed reagents/materials/analysis tools: K.M.C., W.R., K.R.A.K. Wrote and revised the manuscript: A.A.H.J. and R.S. All authors read and approved the final manuscript. [Verify and edit contributions as appropriate.]

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How to Cite This Article

Joannas AAH. Phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli* and *salmonella* isolated across the production cycle of integrated broiler chicken farms in Karnataka, India. International Journal of Veterinary Sciences and Animal Husbandry. 2026;SP-11(7):10-15.

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