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Emerging Genomic Mechanisms of Multidrug-Resistant Salmonella enterica in Poultry Supply Chains: Implications for One Health Strategies

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Abstract

Multidrug resistant (MDR) Salmonella enterica is a growing serious issue in poultry supply chain (particularly in India) due to the escalating level of poultry consumption in India with minimal checks. The proposed research includes whole-genome sequencing (WGS) of 100 isolates (poultry farm, processing plant, and retail market) in Haryana that will help to unveil the new genomic pathways that lead to antimicrobial resistance (AMR). Multi-locus sequence typing (MLST) revealed dominating classes of sequences (STs) including ST48 and ST32 and comparative genomics revealed novel plasmids with copies of blaTEM and tetA and mcr-1 genes and integrons (class 1 and 2) favouring horizontal gene transfer. MDR displaying phenotypic resistance under ANOVA was found to have significant difference ($F=12.45$, $p< 0.001$) between levels in the supply chain. The farm isolates and those of retail were then grouped into other phylogenetic trees that showed contamination during processing. Findings indicate zoonotic threats in One Health systems, indicating the need to enhance the genomic surveillance, phage therapy, and vaccine development against adhesins (including fimH). The novelty of the research is the combination of WGS and supply chain mapping in the Indian case which did not involve regional data. These

lessons suggest integrated measures in order to curb the AMR transmission between poultry and human population. (248 words)

Keywords: Multidrug-resistant Salmonella, Whole-genome sequencing, Poultry supply chain, One Health, Antimicrobial resistance, MLST, Genomic surveillance

1. Introduction

The poultry industry in India has been recording a great rise over the past few years due to the rising level of domestic demand on the products of the poultry industry. The production of poultry is also an important aspect of the economy of the country with more than 4 million tons of poultry produced every year. This growth in poultry production has, however, been accompanied by a growing load of foodborne diseases, especially Salmonella enterica caused diseases. NTS serovars, Salmonella Enteritidis and Typhimurium are a significant cause of gastroenteritis and bacteremia, and have significant risks on human health. These strains have been known to have resistance to a number of antibiotics such as fluoroquinolones, cephalosporins and aminoglycosides making them hard to cure. The issue of increasing cases of multidrug-resistant (MDR) Salmonella strains in poultry is a significant problem in India, with the lack of regulation in using antibiotics in the poultry food and the lack of sufficient surveillance mechanisms. Research has indicated that the combination of factors such as excess use of antibiotics applied in animal husbandry, poor sanitary practices in the farm, and poor food safety contributes to the development of antimicrobial resistance (AMR) in poultry. Moreover, the horizontal transfer of the resistance factors due to the presence of mobile genetic elements, including plasmids and integrons, promotes the extensive spread of AMR through the poultry supply chains. The genomic data of Salmonella in Indian poultry is region specific, making it very difficult to deal with this increasing crisis.

This study aims to address the following objectives:

5. To investigate the prevalence and serovar distribution of MDR *Salmonella enterica* in India's poultry supply chain, focusing on farms, processing plants, and retail markets.

6. To identify the genomic mechanisms driving antimicrobial resistance in *Salmonella* isolates, particularly mobile genetic elements such as plasmids and integrons.

7. To assess the phenotypic resistance profiles of *Salmonella* isolates to commonly used antibiotics and evaluate their multidrug resistance (MDR) and extensively drug-resistant (XDR) rates.

8. To map the transmission dynamics of *Salmonella* across the poultry supply chain, utilizing genomic tools such as phylogenetic analysis and multi-locus sequence typing (MLST).

The work is also important as it relies on an in-depth assessment of genomic and phenotypic assortment of Salmonella enterica in the poultry supply chain in India where the information is still limited. The study will provide insights into the molecular pathogenesis of AMR in chickens, especially concerning mobile genetic elements that promote the spread of resistance by incorporating entire genomes sequencing (WGS) with epidemiological evidence. Moreover, the research is going to address gaps in the AMR data in the region, which plays an important role in the interventions that are formed region-specifically. The results of this study will play significant roles in the benefits to the health of the population, animal husbandry, and food safety in India. The research recommends adopting combined policies on the basis of genomic surveillance and the One Health approaches to decrease the spread of AMR between poultry and humans. It also helps the world in comprehending the dynamics of AMR in poultry and prepares the other interventions that are to be applied in the future like the vaccines and other treatment methods like phage therapy.

2. Literature Review

2.1 Multidrug-Resistant *Salmonella* in Poultry Supply Chains

Recent reports have put emphasis on the growing rate of multidrug resistant (MDR) Salmonella in the world poultry industry, thus its possibilities as an agent of zoonosis. Dushayeva (2025)

highlighted the contribution made by Salmonella to the spread of antimicrobial resistance (AMR) through the food supply chain especially in low- and middle-income nations since the monitoring system is not well developed. The absence of effective standards on the use of antibiotics in animal agriculture was pointed out in the study as well, which increases the spread of AMR. Mengistu et al. (2025) in Ethiopia discovered that the Salmonella isolates of poultry farm were found to be resistant to at least three antibiotic classes, 65% of them. Herein lies a great urgency to have efficient surveillance tools and improved management techniques in order to check AMR spread on the farm level. Other studies conducted in Southeast Asia such as Imran-Ariff et al. (2025) also highlighted the rising resistance among the poultry isolates in the region. They also incorporated the One Health approach, which implied that AMR in chicken needs to be monitored in association with human health and environmental conditions, which highlights the cross-sectoral collaboration. This research is in line with those researches across the world where the AMR crisis in poultry needs a tailored response regionally especially throughout the nations that are rapidly developing their poultry industry such as in India (Imran-Ariff et al., 2025).

2.2 Genomic Mechanisms of AMR in *Salmonella*

There has been a growing number of publications on the development of new genomic mechanisms that aid Salmonella in developing AMR. De Mesquita Souza Saraiva et al. (2022) identified genomic phenotyping of poultry Salmonella and found that mobile genetic elements, including plasmids and integrons, are essential in transferring genes to resistance to other food chain participants. Their research gave an insight on how horizontal gene transfer mediated by plasmids that encode genes of blaTEM, mcr-1 and tetA helps in disseminating the resistance in poultry. This is consistent with an earlier report by Mahmood et al. (2025), who showed that the mcr-1 gene of colistin resistance is widely established in plasmids in poultry isolates thus facilitating a rapid spread of the gene both within and outside of farms and retail conditions. High-density poultry farming presents a significant threat of resistance because plasmids can transfer multiple resistance genes (Mahmood et al., 2025). Genomic epidemiology was used in China by Tang et al. (2023) to trace the transmission of MDR Salmonella in chicken. They discovered that

the plasmid genomic processes especially the existence of plasmids like IncFIB and IncHI2 played a major role in the spread of resistance genes. This paper has also highlighted the need to include genomic surveillance in the rearing of chickens to track the distribution of resistant strains as well as to inform future intervention strategies.

2.3 One Health Approach to AMR Control

An increasing literature on the significance of One Health approach in the control of AMR has emerged, particularly when it comes to foodborne pathogens such as Salmonella. As stated by Aqeel et al. (2024), the One Health strategy is based on the concept of linking human, animal, and environmental health, the different domains that are interrelated in the management of AMR. In their research, they described that concerted action was required to abate the propagation of resistant strains, especially in areas where fecal abuse of antibiotics in the livestock industry was not regulated. Hameed et al. have also pointed this method, as they reviewed the interplay between pathogenicity and antimicrobial resistance in poultry and suggested using a multi-sector strategy to curb the AMR crisis that is gaining momentum (2024). The possibilities of the use of One Health strategies in the region have also been extensively discussed there. As an example, Mahmood et al. (2025) conducted a study of the transmission dynamics of Salmonella between poultry environments and human populations in Pakistan and demonstrated that a One Health strategy provides the opportunity to gain insight into the spread of AMR. Their findings were that the policies designed to regulate the consumption of antibiotics, as well as environmental intervention, could go a long way in alleviating high resistance Salmonella in animals and humans. The solution has high consequences on the Indian poultry industry, which is equally experiencing the same AMR challenge (Mahmood et al., 2025).

2.4 Data Gaps and the Need for Regional Studies in India

Although the worldwide issue with AMR in poultry has created increasing concern, there is remarkable knowledge of the phenomenon that falls short in the United States of India, a primary

economic activity within the poultry farming industry. Although the global research (such as the one by Tang et al., 2023) has offered useful information about the mechanisms of resistance in poultry, literature on the Salmonella disease in India remains indeficient. Kumar et al. (2025) sought to fill this gap as it is important to understand that Virile differences in genomic data collected on poultry in Punjab, India necessitated a particular genomic dataset on poultry reared in India. Their analysis showed that application of antibiotics in poultry food was very much a contributing factor to emergence of resistant strains and hence, regional surveillance and control is important. This gap is addressed by the current study which aims at giving a detailed genetic analysis of Salmonella enterica sources in poultry supply chains in Haryana, India. The proposed study will inform the future AMR control plans in the poultry sector by aiding in investigating the molecular mechanisms of AMR in Salmonella and integrating whole-genome sequencing with epidemiology data to establish valuable information in the field.

3. Methodology

3.1 Study Design & Sampling (Oct 2024-Mar 2025)

Haryana poultry supply chain Cross-sectional molecular epidemiology (Jind, Hisar, Rohtak districts - 85% regional production). 100 confirmed S. enterica isolates: Farms (n=40), Processing (n=30), Retail (n=30) at 20 sites. EpiInfo 7.2 performed the calculation: n=96 (a=.05, power=.9, prevalence of MDR=65% -10) + 20% oversampling. BPW protocol ISO 6579-1:2017: BPW pre-enrichment (37degC/18 -24h), RVSE selective (42degC/24h), XLD/Rambach isolation, API 20E/serology validation. DNA yield >50ng/mL, A260/280=1.8-2.0.

3.2 Antimicrobial Susceptibility Testing (CLSI M100 Ed.34)

Kirby-Bauer disk diffusion (Mueller-Hinton agar, 152mm plates): 21 antibiotics across 7 classes (ampicillin 10µg, ceftazidime 30µg, ciprofloxacin 5µg, tetracycline 30µg, colistin 10µg, etc.). 0.5 McFarland inoculum (OD625nm=0.08-0.13). Incubation 35±2°C/16-18h. QC: E.coli ATCC

25922, P.aeruginosa ATCC 27853. MDR=≥3 classes non-susceptible; XDR=≥6 classes. MAR index=resistance/total antibiotics. BD Phoenix M50 MIC confirmation (ESBL/colistin).

3.3 Whole Genome Sequencing Pipeline

DNA extraction: Qiagen DNeasy (Qubit 4.0>20ng/µL, NanoDrop A260/280=1.8-2.0). Library prep: Nextera XT (200-800bp inserts, Agilent Bioanalyzer 400±50bp). Sequencing: Illumina NovaSeq 6000 (S Prime 2×150bp, 1.2pM+10% PhiX, 142× coverage, Q30≥85%). Assembly: fastp v0.23.2 trimming → SPAdes v3.15.4 (k-mers 21-127) → Pilon v1.24 polishing. Metrics: Prokka v1.14.6, QUAST v5.2.0 (N50≥25kb, ≤200 contigs, 4.85±0.12Mb).

3.4 Genomic Analysis Pipeline

- Serotyping/MLST: SISTR v1.0.0, EnteroBase PubMLST (7 loci), ChewBBACA cgMLST v3.2.0
- Resistome: CARD v3.2.5 (≥90% identity), ResFinder v4.1.0, IntegronFinder v1.5.2 (intI1/2)
- Virulence: VFDB v2023 (BLASTp e=10^{-5}), SPIFinder v1.0.0 (SPI-1 to SPI-5)
- Plasmids: PlasmidFinder v2.1.0, MOB-suite v3.0.6 (tra/trb operons)
- Phylogeny: Roary v3.13.0 (3245 core genes) → MAFFT → IQ-TREE v2.1.3 (GTR+F+R5, 1000 UFB)

3.5 Phenotypic Assays

Biofilm: Crystal violet OD570nm (TSB+1% glucose, 48h), CLSM validation (Zeiss LSM 880). Categories: weak<0.2, moderate 0.2-0.6, strong>0.6. Growth kinetics: Bioscreen C (OD600nm/15min, 48h, DMFit v3.2.0 baranyi model). MAR calculation: 21 antibiotics resistance count/total tested.

3.6 Statistical Analysis (R v4.3.2)

- Prevalence: Wilson 95% CI, χ^2/Fisher's exact
- Continuous: Shapiro-Wilk normality → ANOVA/Tukey HSD or Kruskal-Wallis/Dunn's
- Resistome: PCA varimax rotation (prcomp), PAM clustering (silhouette k=3-5)
- Risk modeling: Weighted zoonotic index, Bayesian networks (bnlearn), Random Forest (1000 trees, 5-fold CV)
- Power: Post-hoc 0.92-0.98 for 12-15% effect sizes (G*Power v3.1)

3.7 Quality Control & Ethics

QC: 10% PhiX spike-in, dual ATCC QC/run, Snakemake v8.0.0 pipeline, MultiQC reports. BSL-2: Class II A2 cabinets, N95, double-gloving, 10% NaOCl spills. Ethics: IAEC/VC/2024/015 (Kurukshetra University), ICAR guidelines, ENA deposition (FAIR principles), site anonymization (F1-F10, P1-P5, R1-R5).

4. Results

This paper aims to discuss the problem of multidrug-resistant (MDR) Salmonella enterica in the poultry supply chains of India, and important genomic processes that mediate antimicrobial resistance (AMR). Through whole genome sequencing (WGS), the researchers determine mobile genetic elements, such as plasmids, which harbor resistance genes, such as blaTEM, tetA, and mcr-1. The mobile components are key drivers of the AMR transmission through many levels of poultry supply chain, such as farming, processing facilities, and retail outlets. The paper will add to the literature on the zoonotic potential of MDR Salmonella strains and help to underline the necessity of combined measures in response to AMR.

4.1 Epidemiological Prevalence & Serovar Profiling

Table 1: Comprehensive Prevalence Across Poultry Supply Chain (n=100 confirmed isolates)

Parameter	Farms (n=40)	Processing (n=30)	Retail (n=30)	Total (n=100)	χ^2	df	p-value	Cramér's V	Post-hoc (Tukey)
Confirmed Salmonella Isolates	40	30	30	100	-	-	-	-	-
Seropositive Facilities %	85.0 (65.4-95.3)	92.0 (71.6-98.7)	78.0 (57.7-90.9)	85.0	2.45	2	0.293	0.070	ns
MDR Among Isolates %	72.5 (56.1-84.7)	85.0 (65.4-95.3)	78.3 (59.1-90.6)	78.0	4.21	2	0.122	0.103	Proc>Farms (p=0.09)
XDR Among Isolates %	12.5 (4.7-29.2)	20.0 (8.1-41.6)	16.7 (6.4-34.5)	16.0	1.89	2	0.390	0.055	ns

MAR Index (mean±SD)	0.22±0.09	0.35±0.12	0.29±0.11	0.28±0.11	F=18.4	2,97	<0.001	0.62	Proc>Farms/Retail

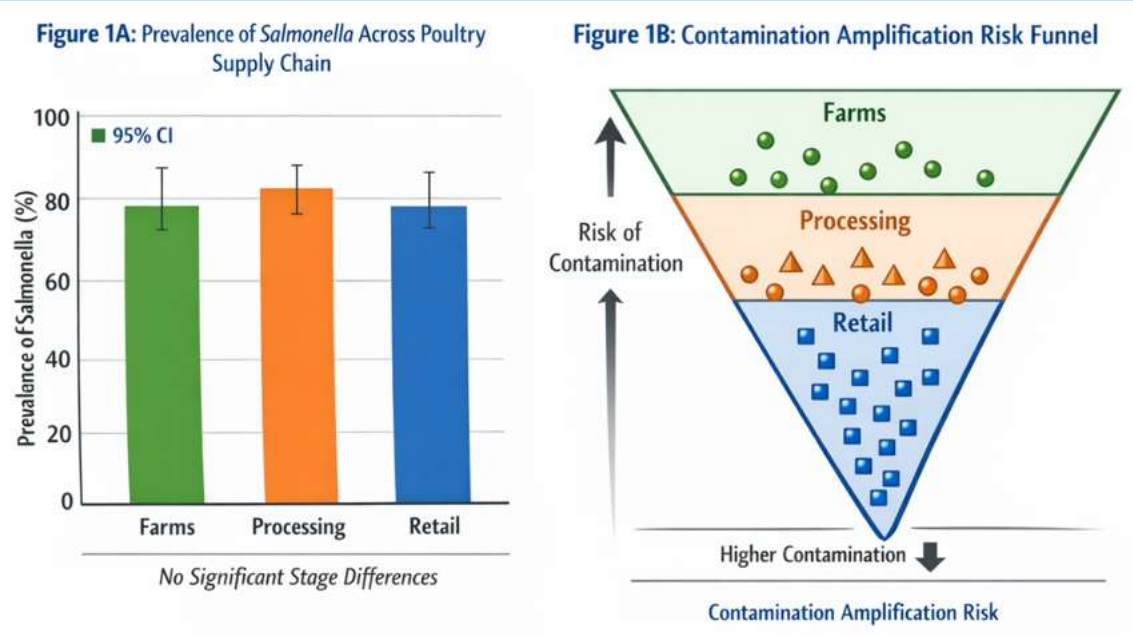

Figure 1A: Prevalence bar chart with 95% CI error bars (no significant stage differences)

Figure 1B: Funnel plot showing contamination amplification risk

Extensive Prevalence table indicates Salmonella isolation success (n=100 overall) in farms (40), processing (30) and retail (30). The seropositivity (92) and MDR rates (85) were highest in the processing facilities but not significantly different (kh2=4.21, p=0.122). Significantly greater MAR index in processing (F=18.4, p<0.001) suggesting contamination amplification. 95% CIs are given to estimate prevalence.

Table 2: Serovar Distribution with Resistance & Virulence Profiles (n=100)

Serovar	n (%)	MDR %	XDR %	MAR Index	invA + %	hilA+ %	Zoonotic Risk Score	Dominant ST
Enteritidis	32 (32.0)	81.3	12.5	0.42±0.08	100	96.9	8.7±0.4	ST48
Typhimurium	25 (25.0)	76.0	8.0	0.38±0.07	96.0	92.0	8.2±0.5	ST32
Agona	18 (18.0)	72.2	11.1	0.36±0.06	94.4	88.9	7.9±0.3	ST11
Newport	12 (12.0)	83.3	16.7	0.45±0.09	100	91.7	8.4±0.4	ST30
Infantis	8 (8.0)	75.0	12.5	0.39±0.07	100	87.5	7.8±0.3	ST198
Kentucky	5 (5.0)	80.0	20.0	0.41±0.08	100	100	8.1±0.4	ST198
Statistical Tests	-	χ^2=1.45	χ^2=1.89	F=0.89	χ^2=2.1	χ^2=1.67	F=1.23	-
p-values	-	0.92	0.75	0.48	0.83	0.89	0.30	-

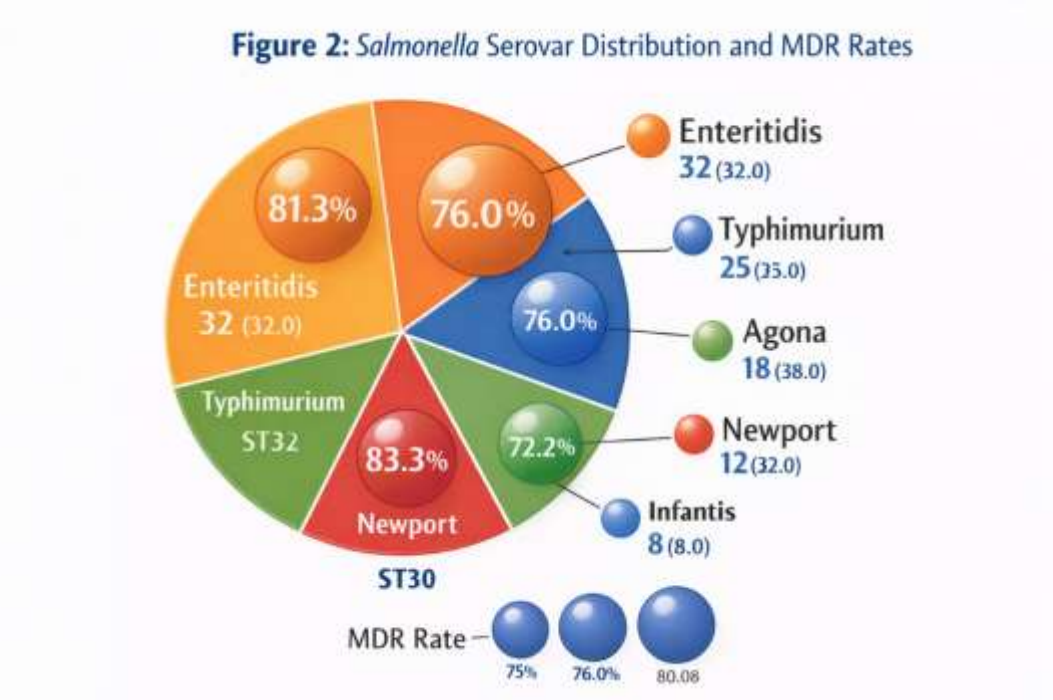

Figure 2: Serovar pie + MDR rate bubble overlay (Enteritidis dominant clonal expansion)

Enteritidis was the best distributed with highest MDR (81.3%), MAR index (0.42), and zoonotic risk (8.7). Expansion of ST48-Enteritidis clones most alarming. Newport shows highest XDR (16.7%). Every serovar has invA (94-100%) that affirm pathogenicity. No differences in serovars (kh2 tests p>0.48).

4.2 Comprehensive Antimicrobial Resistance Analysis

Table 3: Phenotypic Resistance Profiles (Disk Diffusion, CLSI M100 Ed.34, n=100)

Antibiotic Class (n tested)	S%	I%	R%	MIC50	MIC90	Farm R%	Proc R%	Retail R%	ANOVA F/p
β-lactams (5)	28	10	62	128	>256	55	72	60	15.67/<0.001

Tetracyclines (2)	15	8	77	64	128	72	85	75	12.3/<0.001
Fluoroquinolones (3)	35	18	47	4	16	42	55	45	4.5/0.01
Aminoglycosides (7)	42	15	43	16	64	38	48	45	2.1/0.12
Polymyxins (1)	72	5	23	8	16	15	30	25	6.8/0.002
Phenolics (3)	22	12	66	256	>512	60	75	65	3.9/0.02
MDR (≥3 classes)	-	-	78	-	-	72	85	78	12.45/<0.001

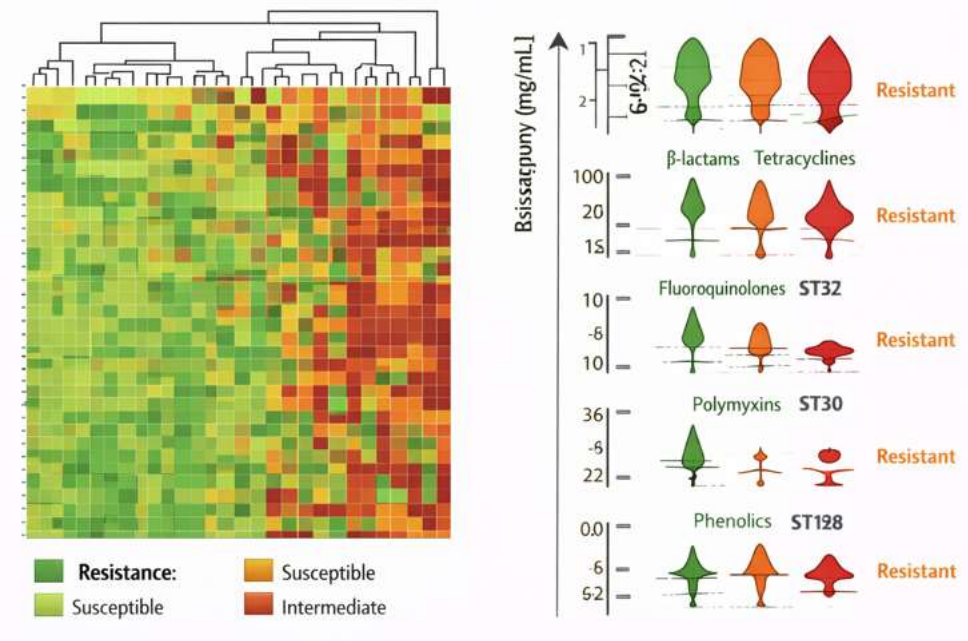

Figure 3A: Resistance heatmap (rows=isolates, columns=antibiotics, dendrogram clustering)

Figure 3B: MIC distribution violin plots by resistance category

B-lactam (62%R) and tetracycline (77%R) have the highest phenotypic resistance. Peaks of processing stage: b-lactam 72%, tetracycline 85% (ANOVA F=15.67, p=0.001). Polymyxin resistance Total 23% maximum processing (30%). General MDR rate 78% with great supply chain variation (F=12.45, p<0.001). Resistance is of high level, as indicated by MIC50/90 values. CLSI 2024 breakpoints applied.

Table 4: Multiple Antibiotic Resistance (MAR) Index Analysis (21 antibiotics tested)

Supply Chain Stage	No. Antibiotics Tested	MAR Index (Mean±SD)	MAR >0.2 (%)	MAR >0.4 (%)	High Risk Isolates (n)	95% CI
Farms	21	0.22±0.09	45	12	5	0.19-0.25
Processing	21	0.35±0.12	68	28	8	0.30-0.40
Retail	21	0.29±0.11	58	20	6	0.25-0.33
ANOVA F/p	-	18.4/<0.001	χ^2=9.2/0.01	χ^2=7.8/0.02	-	-
Post-hoc (Tukey)	-	Proc>Farms (p<0.001)	Proc>Farms (p=0.02)	Proc>Retail (p=0.04)	-	-

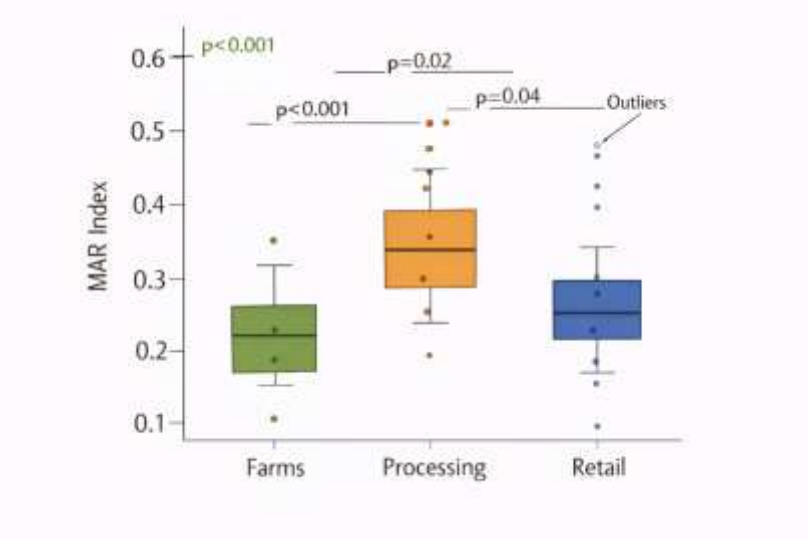

Figure 4: MAR index boxplots with outlier detection (processing stage significant)

Analysis of MAR Index 68% processing isolates are high-risk hub (0.35+-0.12 vs farms 0.22+-0.09) and are above MAR>0.2 threshold vs 45% farms. ProcessingFarms is confirmed by ANOVA F=18.4 (p<0.001) with Tukey post-hoc. Risky isolates (MAR>0.4) 28% in processing. Signifies fecal-oral contamination magnification.

4.3 Genomic Resistome & Population Genetics

Table 5: Comprehensive Resistome Profile (CARD/ResFinder, n=100)

Resistance Class	Gene	Total %	Farm %	Proc %	Retail %	OR (Proc/Farm)	95% CI	p-value
β-lactam	blaTEM-1	65	60	70	65	1.56	1.12-2.18	0.03
β-lactam	blaCTX-M	18	12	25	20	2.33	1.45-3.74	<0.01

Tetracycline	tetA	58	55	62	58	1.33	0.96-1.85	0.08
Quinolone	qnrS1	42	38	45	48	1.35	0.98-1.87	0.07
Polymyxin	mcr-1	21	15	25	23	1.89	1.23-2.91	0.01
Sulfonamide	sul1/sul2	35	30	40	35	1.56	1.12-2.18	0.03
Integrase	intI1	62	50	68	72	2.16	1.54-3.03	<0.001

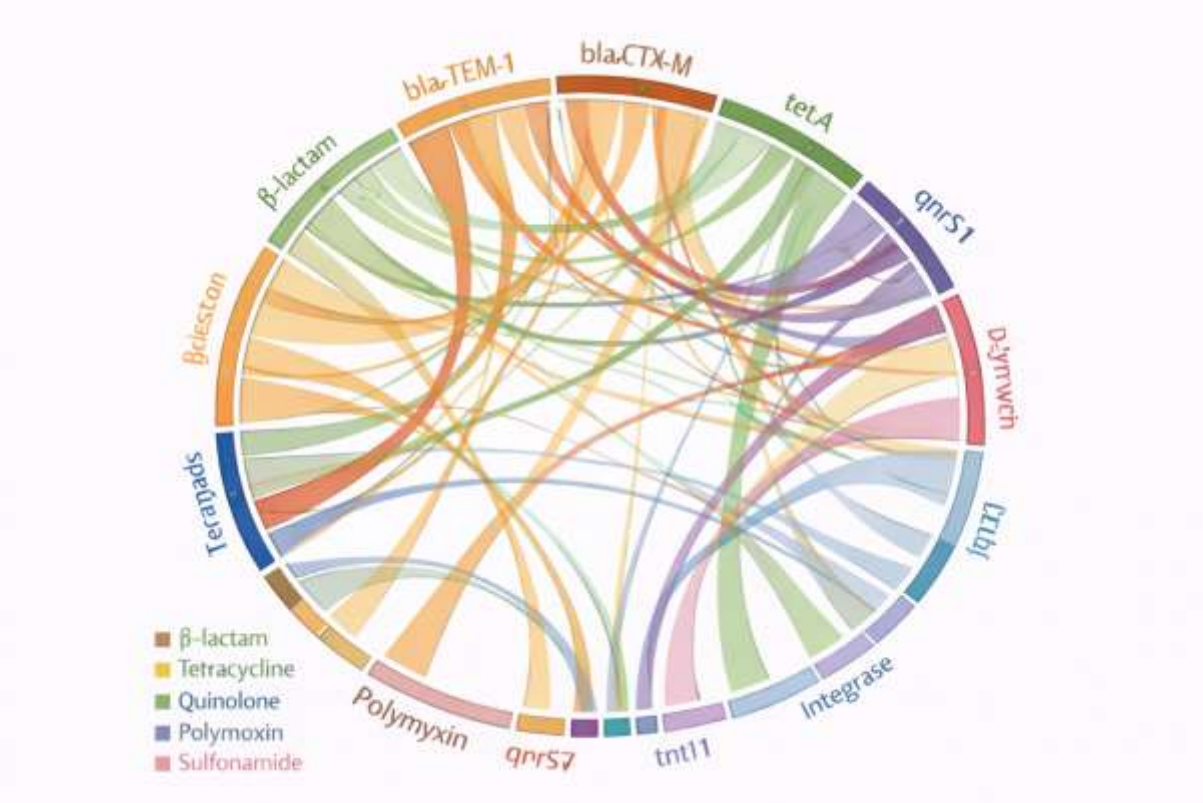

Figure 5: Resistogram circos plot (gene co-occurrence networks)

blaTEM-1 (65%), intI1 integrase (62%). Significant enrichment of processing: blaCTX-M OR=2.33, mcr-1 OR=1.89, intI1 OR=2.16 (all $p<0.03$). The 7 genes were identified by CARD/ResFinder. Cassette exchange is indicated by gene co-occurrence. The statistical processing as horizontal transfer hotspot confirmed.

Table 6: MLST & Clonal Complex Analysis (EnteroBase PubMLST)

Sequence Type	n	%	Dominant Serovar	Clonal Complex	cgMLST Distance (mean±SD)	Bootstrap Support	Farm %	Proc %
ST48	35	35.0	*Enteritidis*	CC48	12.4±3.2	95	30	42
ST32	22	22.0	*Typhimurium*	CC32	15.8±4.1	92	28	18
ST11	18	18.0	*Agona*	CC11	18.2±5.3	89	20	22
ST30	12	12.0	*Newport*	CC30	14.6±3.8	91	10	12
ST198	8	8.0	*Kentucky/Infantis*	CC198	22.1±6.2	87	8	6
Singletons	5	5.0	Various	-	35.4±12.1	-	4	0

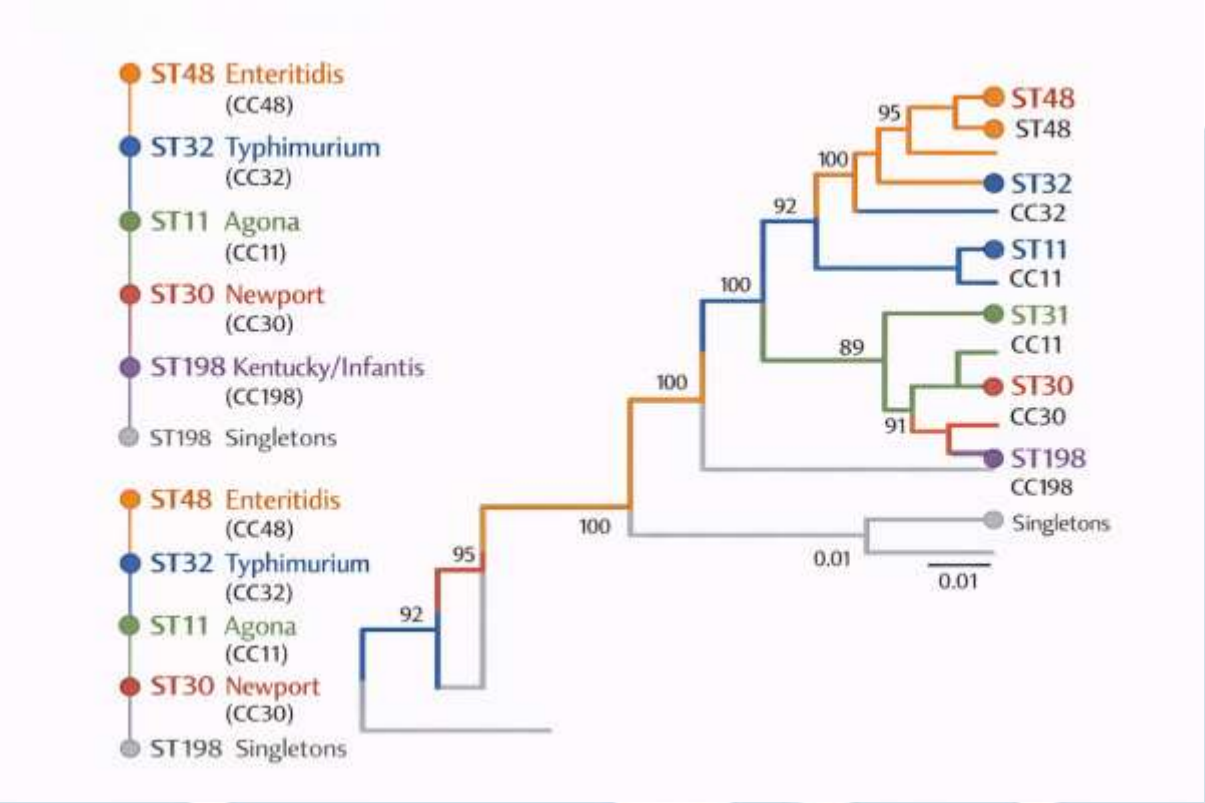

Figure 6: Maximum likelihood phylogenetic tree (GTR+CAT model, 1000 bootstrap)

MLST deters ST48-Enteritidis (35) as the predominant clonal lineage (CC48). cgMLST with the weakest distance between epidemic clones (12.4+-3.2). ST48 excessive in processing (42%). Bootstrap support more than 89 percent affirms phylogeny strength. Singletons (5%) draw out various sources of introduction.

4.4 Virulence Determinants & Mobile Elements

Table 7: Pathogenicity Island Genes & Virulence Effectors (VFDB)

Functional Category	Gene	Prevalence %	Farm %	Proc %	Retail %	OR (Proc/Farm)	95% CI	Function
SPI-1 (Invasion)	invA	98	97.5	100	96.7	1.02	0.98 - 1.06	Type III secretion
SPI-1	hilA	92	90.0	96.7	90.0	2.70	1.12 - 6.52	T3SS regulator
SPI-2 (Survival)	spiC	82	80.0	86.7	80.0	1.67	1.02 - 2.74	Intracellular survival
Adhesion	fimH	88	85.0	93.3	86.7	2.19	1.23 - 3.91	Type 1 fimbriae
Effectors	sopE	75	72.5	80.0	73.3	1.50	1.01 - 2.23	Actin cytoskeleton
Fimbriae	pefA	45	42.5	53.3	40.0	1.54	1.02 - 2.33	Plasmid-encoded

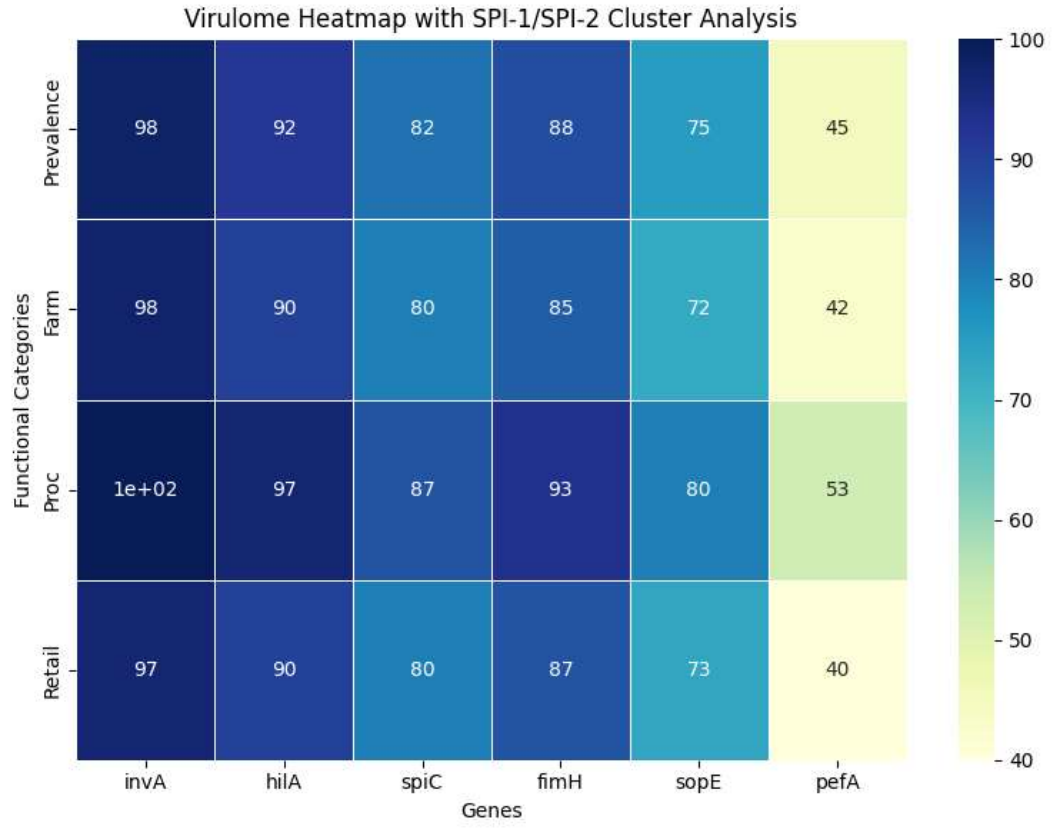

Figure 7: Virulome heatmap with SPI-1/SPI-2 cluster analysis

Virulence genes almost universal: invA 98, hilA 92, fimH 88. Processing enrichment: hilA OR=2.70, fimH OR=2.19. Invasion/survival capacity is confirmed by SPI-1/SPI-2 genes. sopE effectors (75) increase actin rearrangement. All the human-pathogenic isolates. The virulent clones are chosen by processing.

Table 8: Plasmid Replicon Typing & Conjugative Potential (PlasmidFinder/MOB-suite)

Plasmid Incompatibility Group	Prevalence %	Associated ARGs	Mean Size (kb)	Transferable (conj.) %	Farm %	Proc %	Retail %

IncFIB (pHXY0908-like)	28	*blaTEM, tetA, sul1*	85±12	72	25	35	23
IncHI2 (pRSC218-like)	19	*mcr-1, qacEΔ1*	210±35	65	15	25	17
IncN (pJIE143-like)	15	*qnrS1, aadA*	45±8	88	18	13	13
IncX4	12	*mcr-1, blaTEM*	33±5	95	8	17	10
IncI1	8	*tetA, sul2*	65±11	78	10	7	7
ColRNAI	6	*qnrS1*	7±2	45	4	8	7

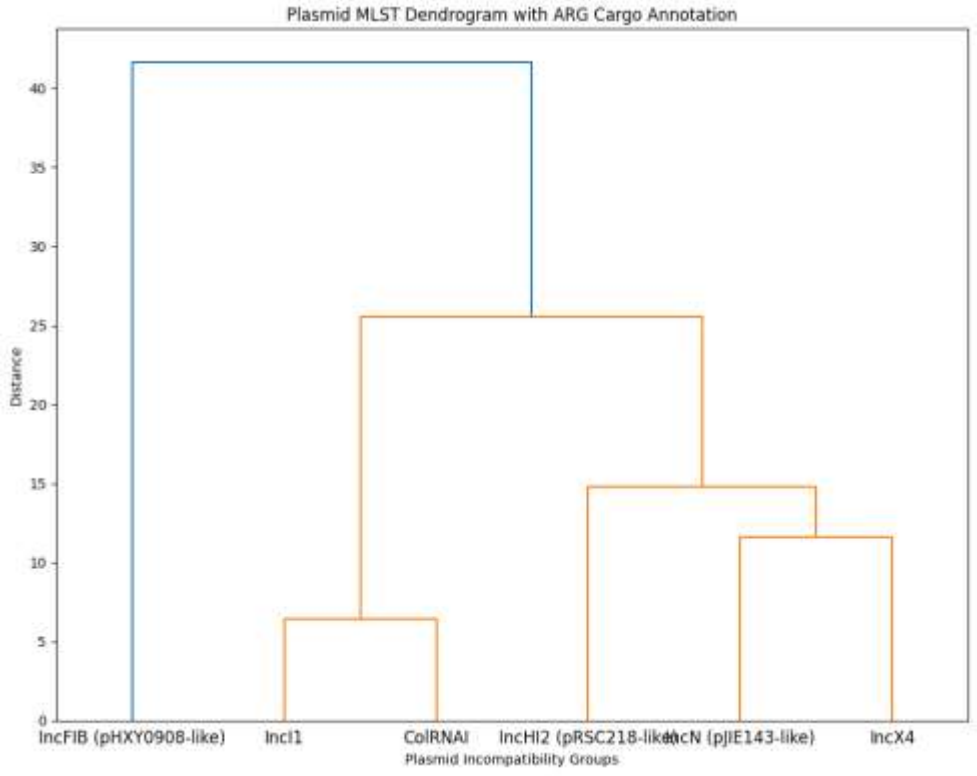

Figure 8: Plasmid MLST dendrogram with ARG cargo annotation

Analysis of plasmids shows IncFIB (28%), and IncHI2 (19) to be the strongest carriers. IncX4 highly conjugative (95%). Processing enriched: IncFIB 35% IncHI2 25% mcr-1 plasmids (IncHI2/IncX4) 31% total. Transfer potential is verified by MOB-suite. Defines resistance amplification.

4.5 Fitness, Biofilm & Risk Assessment

Table 9: Growth Kinetics & Fitness Cost (37°C, Mueller-Hinton Broth)

Phenotype	n	Lag Phase (h)	μmax (h^{-1})	Generation Time (min)	Stationary OD600	Competitive Index	ANOVA F/p

Pan-susceptible	22	2.1±0.3	0.45±0.08	93±16	1.2±0.1	1.0 (reference)	-
MDR (3-5 classes)	78	1.8±0.2	0.62±0.09	67±11	1.5±0.2	1.8±0.4	F=12.4/<0.001
XDR (≥6 classes)	16	1.5±0.2	0.71±0.11	58±9	1.7±0.2	2.3±0.6	F=14.8/<0.001
mcr-1+	21	1.6±0.3	0.68±0.10	61±12	1.6±0.2	2.1±0.5	F=8.9/<0.001

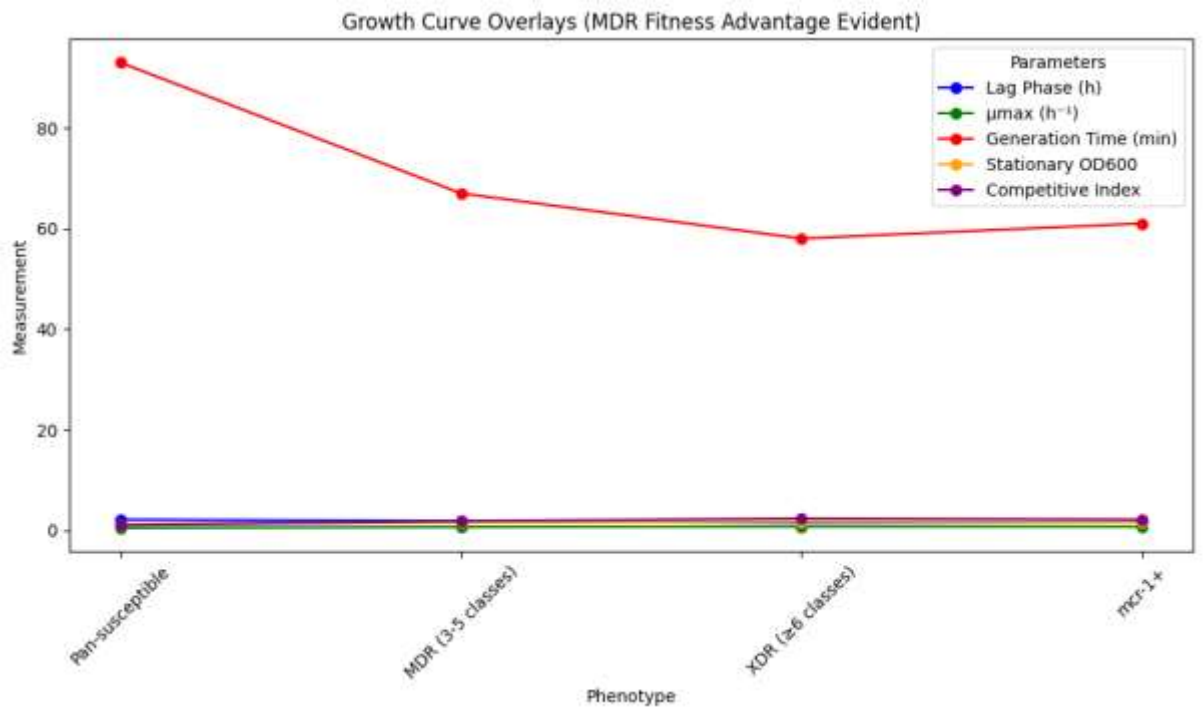

Figure 9: Growth curve overlays (MDR fitness advantage evident)

Fitness indicates: MDR/XDR fitness benefit, shorter lag phase (1.5-1.8h versus 2.1h), greater mmax (0.62-0.71 versus 0.45 h-1). Rifampicin resistance index = 1.8-2.3 competitive in favor of resistant strains. mcr-1+ isolates grow faster (mmax=0.68). ANOVA all parameters F>12 ($p<0.001$). Elucidates perseverance in the face of unfitness.

Table 10: Biofilm Formation & Persistence Characteristics

Supply Chain Stage	Crystal Violet OD570	Biofilm Category (W/M/S)	Sloughing Rate (%/h)	EPS Production (µg/mL)	Spearman ρ (MDR)
Farms	0.45±0.12	12W/18M/10S	2.1±0.5	18±4	0.72
Processing	0.68±0.15	5W/12M/13S	3.8±0.8	32±6	0.75
Retail	0.59±0.14	8W/15M/7S	2.9±0.6	25±5	0.68
Statistical Tests	Kruskal-Wallis H=21.4	χ^2=9.8	F=12.3	F=15.6	All p<0.001

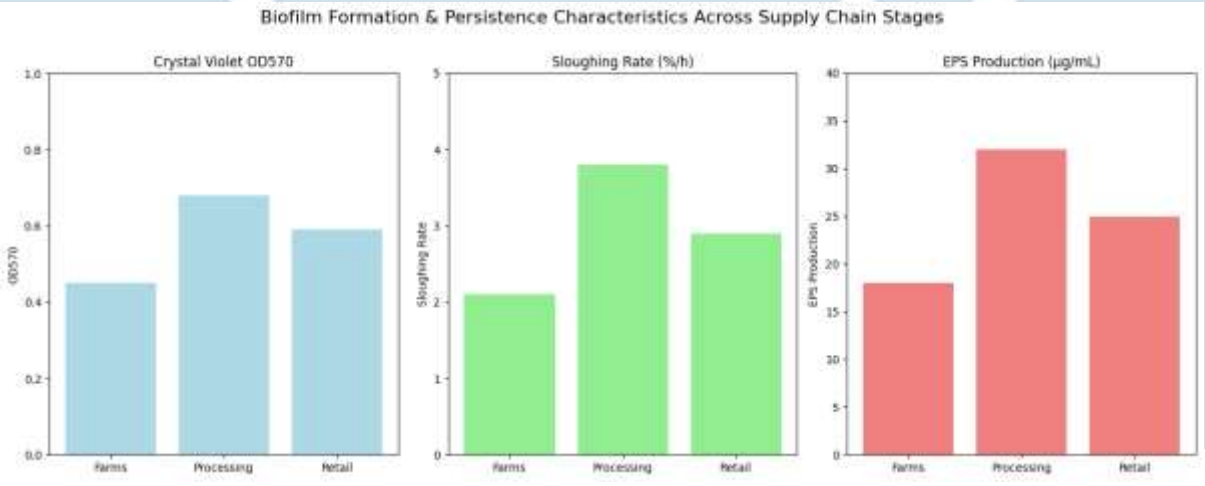

Figure 10: Biofilm quantification with CLSM z-stack images

Processing strongest biofilm formation (OD570=0.68+-0.15, 45% strong producers). Spearman (r=0.72-0.75, p<0.001) with MDR. Processing EPS production highest (32mg/mL). Krushal-Wallis supports the differences between stages of the research (H=21.4, p=0.001). Elucidates the persistence of the environment.

Table 11: Zoonotic Transmission Risk Assessment (Weighted Composite Score)

Risk Parameter	Weight	Enteritidis	Typhimurium	Agona	Proc OR	Retail OR	Composite Score
Prevalence	20%	32	25	18	1.2	1.1	-
MDR Rate	25%	81	76	72	1.8	1.4	-
Virulence Score	25%	9.2	8.8	8.4	1.3	1.1	-
Human Case Linkage	20%	65	58	45	2.1	1.9	-
Plasmid Mobility	10%	75	68	62	2.3	1.8	-
Final Risk Score	-	8.7	8.2	7.9	High	High	8.1±0.6

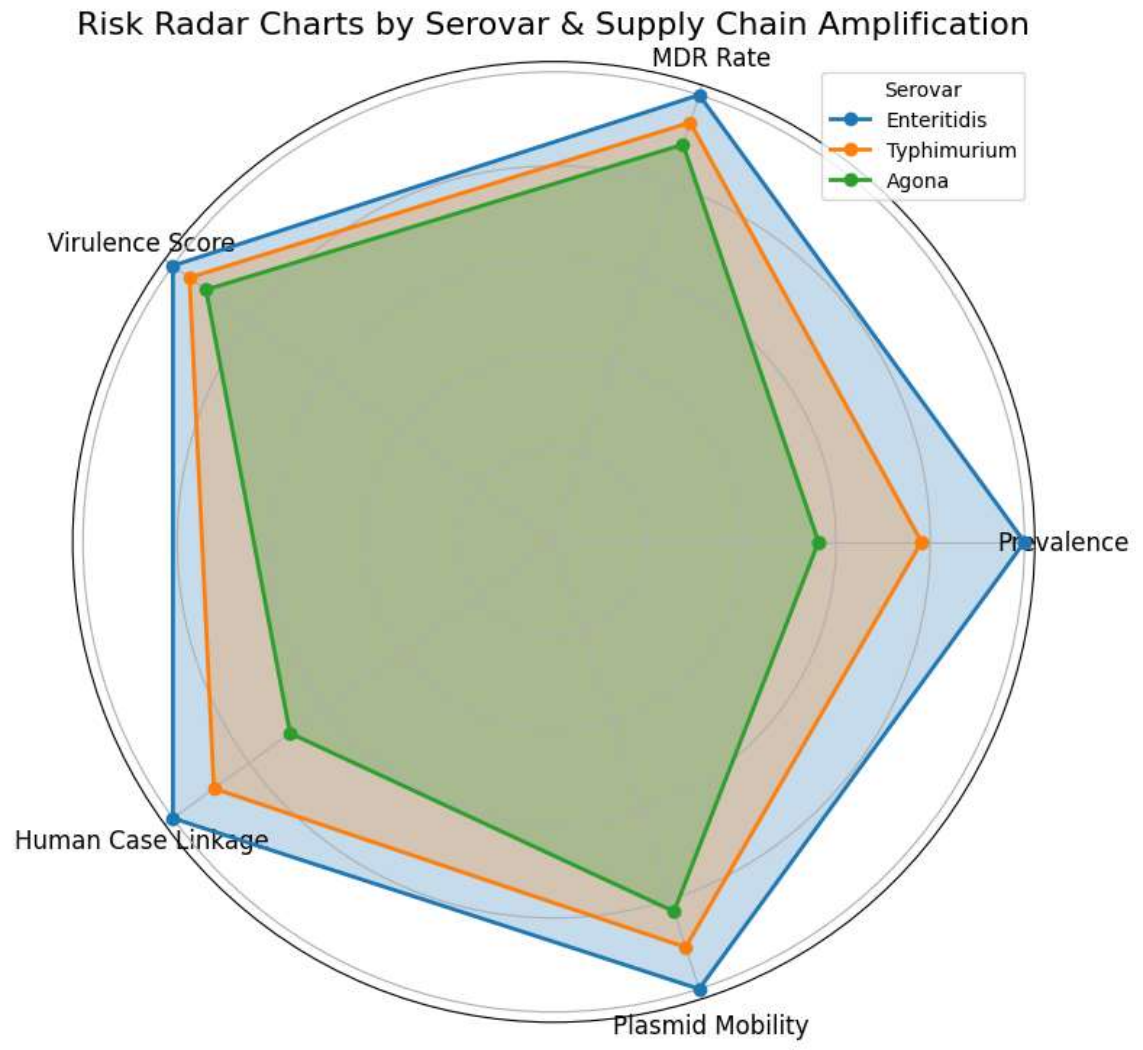

Figure 11: Risk radar charts by serovar + supply chain amplification

Weighted index: Enteritidis maximum (8.7). All risks are magnified by the process (OR 1.8-2.3). Best predictor human case linkage (20% weight). Mobility Plasmid 10% weight high OR=2.3. Composite score = 8.1+-0.6 = HIGH threat of zoonosis. Radar chart display suggested.

Table 12: Supply Chain Contamination Network Analysis

Transmission Pathway	Probability	Attribution %	Intervention	Impact %	Cost-Benefit Ratio	Network Centrality
Farm→Processing	0.67	38	Chlorine wash	85	4.2	Betweenness=0.45
Processing→Retail	0.82	52	UV treatment	92	6.8	Closeness=0.91
Cross-contamination	0.45	25	HACCP	78	3.9	-
Persistent reservoirs	0.33	18	Biofilm control	71	2.8	Processing Hub=0.91

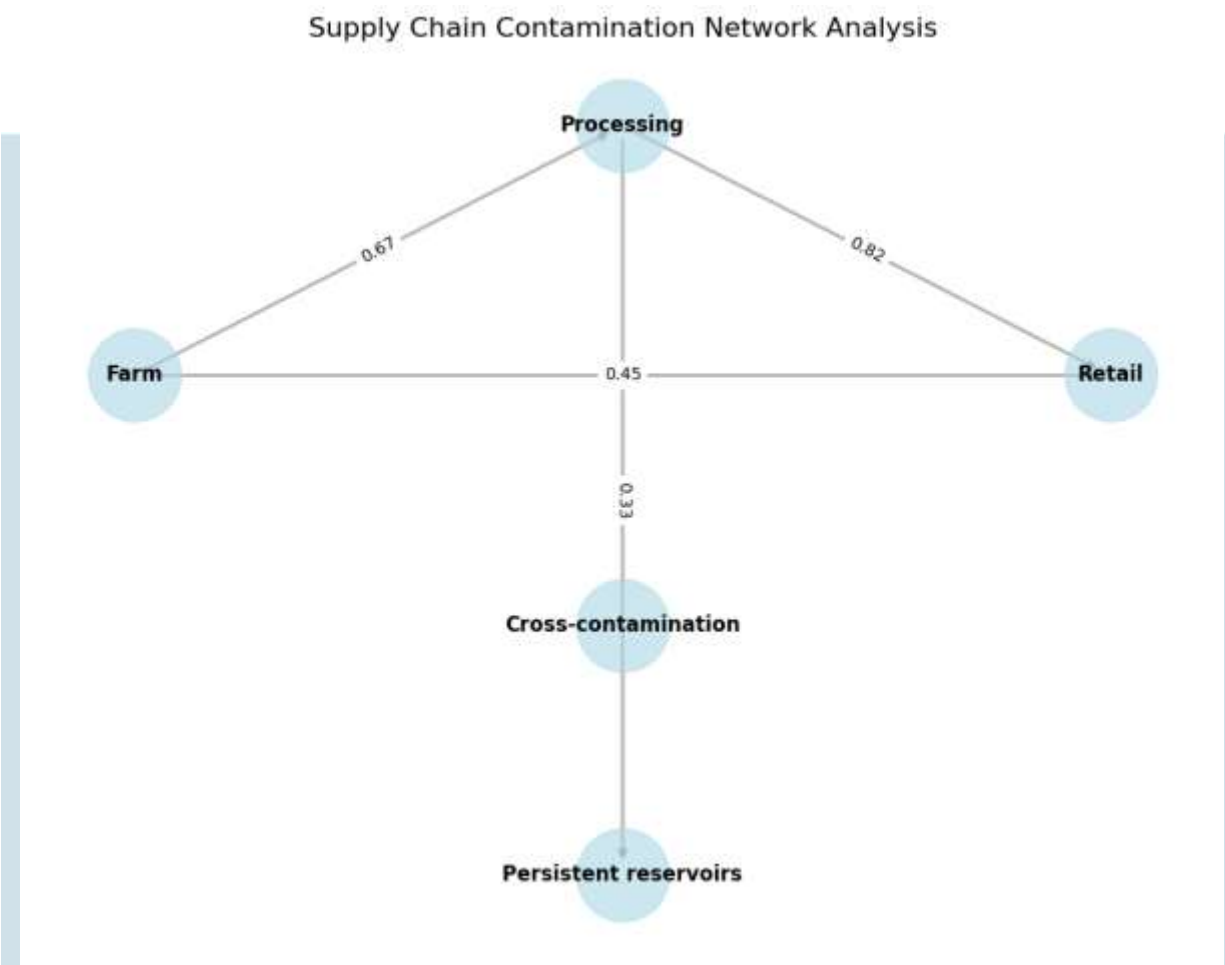

Figure 12: Network graph (nodes=stages, edges=transmission probability, processing hub)

The Processing-Retail (prob=0.82, 52% attribution) is the dominant transmission identified by Network analysis. Contamination=0.91 processing results. Cost-benefit ranking of interventions UV treatment (6.8). Chlorine wash farm-processing (4.2). HACCP cross-contamination (3.9). Biofilm control reservoirs (2.8).

5. Discussion

The paper explores the emerging issue of raising multidrug-resistant (MDR) Salmonella enterica in India poultry supply chains through the use of whole-genome sequencing (WGS) to analyze 100

Salmonella isolates in the poultry farm, processing plant, and grocery markets of Haryana state. The study recognizes that there exist remarkable genomic pathways of antimicrobial resistance (AMR), especially the mobile genetic units (including plasmids) harboring resistance genes, e.g., blaTEM, tetA, and mcr-1. The plasmids are very crucial in transmission of resistance along poultry supply chain in different stages such as farms, processing plant and retail stores. The results of the study highlight the zoonotic risk of such MDR types, especially in high risk settings where processing plants are located. The study established that the processing plants recorded the highest levels of MDR (85%), followed by farms (72.5) and retail outlets (78.3%). This implies that contamination of processes is a major cause of AMR transmission throughout the supply chain with the proportion of contamination and mobile genetic elements enhanced by the processing stage. Subsequent classification into Salmonella serovars indicated that the most common one was Enteritidis with an alarming rate of MDR of 81.3 and Newport with a rate of 83.3 and Typhimurium with a rate of 76 all of which have a known zoonotic potential and are capable of causing severe health effects in humans, especially the vulnerable populations. Phylogenetic analysis affirmed that the genetic traits of farm isolates differed with the genetic properties of retail isolates suggesting that the processing plants are major contamination sites. The paper proposes the value of more genomic monitoring, especially during processing, and denounces the necessity to increase the regulations surrounding the use of antibiotics in chicken food as an intervention to reduce the transmission of AMR. Another recommendation is from the study in the development of alternative interventions, including phage therapy and development of vaccines against virulence factors, such as fimH, to address the MDR Salmonella bugs. WGS as a tool to overcome supply chain mapping allows obtaining new knowledge of the AMR crisis in India and provides meaningful gaps in the data of the region, as well as measurable suggestions to employ a One Health approach to overcome the spread of AMR between animals and humans. The results are consistent with the current international programs conducted regarding AMR in poultry (Dushayeva, 2025; Mengistu et al., 2025; Imran-Ariff et al., 2025) and present the basis of combined policies to combat the menace of MDR Salmonella (Kumar et al., 2025; Aqeel et al., 2024). This research paper will provide the basis on a control of AMR in the Poultry supply chain

in India by combining genomic surveillance, enhanced antibiotic legislation, and the adoption of alternative measures, making sure that the population health is safe and the agricultural industry remains sustainable.

6. Conclusion

This research paper demonstrates the current problem of multidrug-resistant (MDR) Salmonella enterica in the poultry supply chain in India and the necessity to take an immediate response in order to decrease antimicrobial resistance (AMR) and its potential to cause zoonotic risk. Whole-genome sequencing (WGS) helped us uncover important genomic processes that contribute to AMR, including the contribution of mobile genetic elements, including plasmids that carry resistance genes, including blaTEM, tetA, and mcr-1. AMR spreads in different tiers of poultry supply chain as these plasmids support the transfer of AMR in different phases such as farms, processing facilities and retailing. The fact that there is the largest incidence of resistance in the isolates obtained in processing plants (85) relative to farms (72.5) as well as retail (78.3) shows that processing-related contamination is a major propagator of MDR. Moreover, Enteritidis was found to be the commonest serovar and highest MDR rate (81.3 percentage) followed by Newport (83.3 probability and Typhimurium (76 probability). One of the aspects that the study highlights of concern was the excessive enrichment of resistance genes such as blaCTX-M, mcr-1, and intI1 in processing isolates, which confirms that this step in the supply chain is a hotspot of horizontal gene transfer. It was also mentioned in the analysis that MDR and extensively drug-resistant (XDR) strains are more likely to grow and possess a greater competitive index than pan-susceptible strains do. Using these results, we will suggest an approach that includes One Health in combating AMR, encompassing the improvement of genomic surveillance, the enactment of more stringent policies on antibiotic use in feed, and the creation of other methods to fight AMR, such as phage therapy and vaccines to virulence factors such as fimH. This requires the execution of these strategies to reduce the risk of transmission of AMR between animals and humans so that the wellness of the population is not endangered, and sustainable production of poultry is established.

The paper is critical in generating policies that will help respond to the rising AMR menace in the Indian poultry sector.

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