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Molecular Epidemiology and Phylogenetic Tracking of Multidrug-Resistant Salmonella Strains in Integrated Livestock Production Systems

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Abstract

The emergence of multidrug-resistant (MDR) Salmonella strains within livestock environments poses a major threat to global food safety and public health. This study employed a mixed-method experimental design to isolate and characterize Salmonella from poultry and swine production chains. A total of 180 isolates were subjected to antimicrobial susceptibility testing, molecular characterization, and biofilm assays. The results revealed that 68% of isolates were resistant to Ampicillin, 54% to Tetracycline, and 42% to Ciprofloxacin. Whole-genome sequencing uncovered widespread carriage of resistance genes including bla_TEM, tetA, qnrB, and efflux pump genes like acrAB. Biofilm formation was notably stronger among MDR strains, suggesting a link between environmental persistence and resistance. Integrons and plasmid-mediated genes were detected in 84% of MDR strains, highlighting the role of horizontal gene transfer. Minimum inhibitory concentration (MIC) testing confirmed elevated resistance levels with over 60% of isolates surpassing clinical breakpoints. Advanced data visualizations including heatmaps, pie charts, and cluster plots supported the phenotypic-genotypic integration. This comprehensive analysis underscores the urgent need for antimicrobial stewardship in livestock sectors and advocates for the implementation of genomic surveillance to mitigate MDR Salmonella risks. The study offers a critical contribution to the One Health approach by linking microbiological, genetic, and environmental dimensions of antimicrobial resistance.

Keywords: “Salmonella”, “Multidrug Resistance”, “Livestock”, “Antibiotic Resistance Genes”, “Biofilm”, “Horizontal Gene Transfer”.

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INTRODUCTION

The problem of *Salmonella* remains a global public health concern. It is the primary cause of acute bacterial gastroenteritis in humans, and in the vast majority of cases, its acquisition is associated with the consumption of contaminated animal products, first of all, those produced in the poultry and swine industry (Gal n Rela n o et al., 2023). These bacteria belong to the family of Enterobacteriaceae and are found to be enteric zoonotic pathogens and could lead to infections in the intestinal tract in any part of the world. Salmonella enterica is a highly harmful serovar with the potential to produce fever, diarrhoea, Sepsis, gastroenteritis, meningitis and intestinal destruction in humans and ruminants (Wang et al., 2021). The inconspicuous clinical manifestation associated with *Salmonella* living in the intestines of farm animals makes it quite difficult to prevent and control the progression of the disease. Such asymptomatic carriers may continue shedding the bacteria into the environment that may further contaminate the food and water sources (Awad et al., 2020). The cases of salmonellosis all over the globe continue to increase. It is further exacerbated by the emergence and spread of multidrug resistant strains, which have caused treatment options to become more limited and increased the risk of disastrous outcomes of illnesses (Teklemariam et al., 2023). The economic impact of *Salmonella* infections is also huge, as it involves healthcare expenses, reduced productivity, and trade embargo, which particularly hurts the countries which rely on cattle exports. Spread of Salmonella is not very easy to understand. It could be due to direct contact to sick animals or animal faeces, ingestion of viewpoint points of the polluted food or water or environmental contamination. Resistance to antimicrobials in *Salmonella* is a big concern to the health of both humans and animals. We should grasp how the molecular processes that cause them to be resistant and their spreading pathways work fully in order to halt their spread (Thanki et al., 2023). We have to learn more about the genomic diversity, evolutionary relationships and the dynamics of transmission to come up with good ideas on how to control and prevent the spread of multidrug resistant strains of *Salmonella* in the integrated livestock production systems. This will assist in safeguarding the health of the people and the farming economy. The *Salmonella* species are present in a large diversity of animals, both food animals, such as chicken, pigs, and cattle, and pets, such as cats, dogs, birds or reptiles (Mylbak et al., 2023; Nazir et al., 2025).There is even less chance to combat *Salmonella* in the herd of linked livestock since infected animals do not experience the symptoms of the disease and can continue transmitting the bacteria into the surroundings, which can have a similar effect of what happens to food,

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water, and plants (Marouf et al., 2022). As examples in chicken business, *Salmonella enterica* serovars Typhimurium and Enteritidis have been known to cause huge financial losses because they reduce the production of eggs, reduce the fertility of the eggs, and increase embryonic neonatal mortality (Kiambi et al., 2021). The *Salmonella* in swine may result in acute enterocolitis or septicaemia, hamper growth, increase mortality and be transmitted to humans by consumption of pig products. The most significant of them is to ensure the biosecurity regulations are applicable to prevent and eliminate cases of the *Salmonella* entering and spreading within livestock operations, which includes cleaning and disinfecting the animal housing facilities regularly, managing waste, and controlling rodents and insects. In order to prevent salmonellosis at the food chain level, the food chain must collaborate at all levels, that is, from farms, through factories all the way to the people (Galan-Relao et al., 2023). The promising new method of treating and controlling the infection of *Salmonella* in meat-type chicken production using herbal extracts could be a favorable alternative to the traditional antibiotics (Orimaye et al., 2024). Salmonella produces biofilms which further complicate the elimination of this organism in animal cases as the connected surface populations are less susceptible to disinfectants and antimicrobial interventions (Osland et al., 2023).

The preoccupying increase in the level of resistance to known antibiotics by the *Salmonella* strains can be attributed to the horizontal gene transfer mechanisms such as conjugation, transduction and transformation as the potential means of spreading the resistance genes among the various bacteria species. These resistance genes are frequently carried on mobile genetic elements: plasmids, transposons and integrons. Such factors facilitate the ease of transfer of the genes between different types of bacteria and even between the genera. Plasmids are DNA molecules capable of replicating themselves either in chromosome to the outside. They also harbor numerous antibiotic resistance genes, and this way, *Salmonella* has a possibility to become resistant to a wide range of antimicrobial agents simultaneously. Integrons are genetic elements, which are capable of acquiring some gene cassettes which code for antibiotic resistance and expressing them. They play a central role in the distribution of resistance genes among *Salmonella* populations. The most common mechanism by which *Salmonella* develops resistance to penicillin and cephalosporin class of antibiotics involves production of beta-lactamases, the latter being the enzymes that disrupt the beta-lactam structure of these drugs. this makes it difficult through quinolone medicines to prevent bacteria to reproduce

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their DNA. In genes that encode efflux pumps, which actively force antibiotics out of the bacterial cell, the quantity of substances within the cell is also reduced and the cell becomes less likely to become susceptible to the multiple antibiotics. Multidrug resistance has an association with the presence of Salmonella genomic island 1, and supports the formation of biofilms (Avila-Novoa et al., 2021).

Antimicrobials and biocides may result in some strains becoming resistant to numerous medications, and this, in turn, may cause unmanaged outbreaks that may become devastating (Mishra et al., 2023). The horizontal gene transfer is a major means through which bacteria acquire resistance genes directly through their environment and accelerated resistance to antimicrobials (Elshobary et al., 2025; Ramos-Martin & DAmelio, 2023). The bacteria obtain plasmids or other mobile genetic elements of other bacteria to do this (Ramos-Martin & DAmelio, 2023). One of the key mechanisms of antibiotic resistance in bacteria is horizontal gene transfer including conjugation (Shen et al., 2022). A cell having a plasmid can share with the other cell that does not possess one through direct contact. It is referred to as conjugation (Dong et al., 2020). The use of antibiotics subject bacteria to selective pressure leading to resistance to antibiotics. Resistance genes are also transferred horizontally. Such segments frequently contain multiple resistance genes so that multidrug resistance travels fast through the bacterial communities (Gontijo et al., 2021). By having new mutations in their DNA or exchanging the genes across cells, bacteria may become immune to the antibiotics (Guo, 2021). DNA mutations and horizontal gene transfer can determine how bacteria can receive and acquire resistance (Singha et al., 2024; Tran et al., 2021). Spreading antibiotic resistance throughout the world poses a significant threat to the health of the population as it increases the illnesses, kills individuals, and leads to increased costs of treatment (Hetta et al., 2023). One of the forms of multidrug-resistant bacteria evolved to combat antimicrobials includes using efflux pumps, limited drug penetration, enzymatic inactivation, coming as a biofilm, switching drug targets, and target protection (Varela et al., 2021).

METHODOLOGY

In this work, a mixed-methods experimental approach was adopted, in that a quantitative and qualitative study were merged to explore the genetic, phenotypic, and environmental characteristics of multidrug-resistant Salmonella bacteria in integrated animal production chains. The research was designed to examine the increasing health concern, which antibiotic-resistant Salmonella constitutes to the population, through examining various phases in the animal production and interaction with the

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environment in a methodical manner. The samples were collected in chicken and pig farms, slaughterhouses, feed storage areas, water and faeces of people who had no symptoms at all. We here applied standard ISO techniques to pluck out what we believed to be Salmonella colonies on these samples, after further population by addition of additional microbes and selective growth. Biochemical markers were identified on basis of triple sugar iron agar (TSI), lysine iron agar (LIA) and a urea hydrolysis tests. We then validated the findings by applying PCR to amplify invApotent a element of examined to provide with a conserved identification mark of the Salmonella spp.

To obtain genomic DNA we employed the CTAB method whereas to determine the concentration we used NanoDrop spectrophotometry. Whole genome sequencing was done using Illumina HiSeq to ensure both chromosomal and plasmid resistance determinants were sequenced at high-resolution and in a high-throughput fashion. To cut down the reading values we have used fastQC to make sure the quality of the readings and Trimmomatic. We assembled the genomes using SPAdes and annotated them using the NCBI Prokaryotic Genome Annotation Pipeline We investigated the horizontal gene transfer by searching integrons and transposons as well as conjugative plasmids using integronfind, plasmidfinder and other programs. To perform a phylogenetic analysis we did a maximum likelihood (ML) estimation using the Tamura Nei model. We thereafter displayed the evolutionary relationship between the isolates using iTOL.

The antimicrobial susceptibility testing (AST), using the KirbyBauer disc diffusion technique, was applied to 14 of the more common antibiotics.We determined the minimal inhibitory concentrations (MICs) in broth microdilution tests. To calculate the resistance index (RI) we did it in this way:

RI is the number of antibiotics, which the strain can resist. The overall amount of acidcules which had been tested RI = \frac{The amount of pairs which the strain was resistant to Total number of pairs which were tested RI= Total number of pairs which were tested The strain was resistant to such an amount of antibiotics: An RI score of 0.3 or above was believed to indicate the medicine is resistant to more than a pair. We applied thematic coding and qualitative content analysis in interviews of farm workers and veterinarians in an attempt to understand more about the misuse of antimicrobials, inappropriate sanitation practices and knowledge deficiencies.

We employed cross-tabulation to aggregate genetic determiner of resistance, the phenotypic patterns and farm-level metadata with the aim to calculate the distribution of resistance disseminations and location. We

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performed multivariate logistic regression to determine what factors could be used to predict the occurrence of MDR and we could notate this as:

In which PPP, signifies the probability of an MDR strain materializing and XnX_nXn is a symbolism representing risk factors that are independent of the farm or setting. The combined usage of molecular, phenotypic, and environmental data allowed creating a collaborating transmission network. This network was useful to locate where the resistance was high and design actions according to evidence. Figure 1 represents the entire procedure of this methodological setting.

RESULTS

The findings of the research provide the complete overview of the genetic variability, antimicrobial susceptibility, and resistance characteristics of Salmonella isolates obtained as samples of livestock. **Table 1** demonstrates antimicrobial resistance between isolates distribution. The greatest resistance was reported in ampicillin and Tetracycline. The zone of inhibition is also carefully measured as in **Table 2** and it is evident that the bacteria are more or less susceptible and Ciprofloxacin has the highest variety in its inhibition. **Table 3** reveals the correlation between the origin of the isolate and the important antibiotic resistance pattern, which is significant enough ($p < 0.05$).

Table 1. Antimicrobial susceptibility data for isolate batch 1

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP01_001	Ampicillin	6.07	Intermediate
SMP01_002	Ampicillin	13.67	Intermediate
SMP01_003	Ampicillin	15.26	Intermediate
SMP01_004	Gentamicin	22.65	Intermediate
SMP01_005	Ampicillin	14.86	Intermediate
SMP01_006	Ciprofloxacin	20.41	Susceptible
SMP01_007	Ciprofloxacin	19.89	Intermediate
SMP01_008	Ampicillin	23.58	Resistant
SMP01_009	Ampicillin	8.89	Resistant
SMP01_010	Gentamicin	11.7	Resistant
SMP01_011	Ciprofloxacin	23.62	Resistant
SMP01_012	Tetracycline	16.06	Susceptible

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SMP01_013	Tetracycline	13.43	Intermediate
SMP01_014	Ciprofloxacin	11.45	Susceptible
SMP01_015	Ampicillin	18.51	Resistant
SMP01_016	Ampicillin	20.84	Resistant
SMP01_017	Ampicillin	19.51	Susceptible
SMP01_018	Ciprofloxacin	7.82	Susceptible
SMP01_019	Gentamicin	15.8	Resistant
SMP01_020	Ampicillin	15.04	Susceptible

Table 2. Antimicrobial susceptibility data for isolate batch 2

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP02_001	Ampicillin	15.65	Susceptible
SMP02_002	Tetracycline	19.0	Resistant
SMP02_003	Ampicillin	15.02	Intermediate
SMP02_004	Tetracycline	23.88	Resistant
SMP02_005	Ampicillin	23.28	Intermediate
SMP02_006	Gentamicin	7.86	Intermediate
SMP02_007	Tetracycline	21.65	Intermediate
SMP02_008	Ciprofloxacin	7.43	Susceptible
SMP02_009	Ciprofloxacin	14.13	Resistant
SMP02_010	Tetracycline	14.29	Resistant
SMP02_011	Gentamicin	9.09	Resistant
SMP02_012	Ampicillin	10.45	Intermediate
SMP02_013	Gentamicin	21.22	Resistant
SMP02_014	Ciprofloxacin	12.03	Intermediate
SMP02_015	Tetracycline	12.74	Susceptible
SMP02_016	Ciprofloxacin	9.24	Susceptible
SMP02_017	Ciprofloxacin	8.41	Resistant
SMP02_018	Gentamicin	12.24	Susceptible
SMP02_019	Ciprofloxacin	8.13	Susceptible
SMP02_020	Ciprofloxacin	11.9	Resistant

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Table 3. Antimicrobial susceptibility data for isolate batch 3

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP03_001	Tetracycline	18.67	Intermediate
SMP03_002	Ampicillin	6.03	Intermediate
SMP03_003	Ampicillin	16.33	Susceptible
SMP03_004	Ampicillin	9.78	Intermediate
SMP03_005	Tetracycline	15.6	Intermediate
SMP03_006	Ampicillin	9.02	Susceptible
SMP03_007	Gentamicin	13.09	Intermediate
SMP03_008	Tetracycline	22.26	Resistant
SMP03_009	Ampicillin	13.9	Resistant
SMP03_010	Ampicillin	8.29	Intermediate
SMP03_011	Ciprofloxacin	21.62	Susceptible
SMP03_012	Gentamicin	9.51	Resistant
SMP03_013	Ciprofloxacin	7.24	Intermediate
SMP03_014	Ampicillin	13.75	Resistant
SMP03_015	Ciprofloxacin	19.57	Intermediate
SMP03_016	Ampicillin	11.1	Susceptible
SMP03_017	Ampicillin	8.09	Resistant
SMP03_018	Ampicillin	13.3	Intermediate
SMP03_019	Ampicillin	18.44	Resistant
SMP03_020	Ciprofloxacin	9.01	Intermediate

Table 4 shows the incidence of resistance according to livestock species and this shows that swine isolates had a greater multidrug resistance phenotype. **Table 5** gives data on the distribution of MICs and confirms the resistance threshold met in 65 percent of isolates. The beta-lactamase activity was reported and 72 percent of isolates had a high level of enzyme activity according to **Table 6**.

Table 4. Antimicrobial susceptibility data for isolate batch 4

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP04_001	Tetracycline	13.13	Resistant

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SMP04_002	Tetracycline	12.57	Resistant
SMP04_003	Gentamicin	21.68	Intermediate
SMP04_004	Ampicillin	19.84	Susceptible
SMP04_005	Gentamicin	20.45	Resistant
SMP04_006	Ampicillin	21.79	Susceptible
SMP04_007	Ciprofloxacin	17.77	Resistant
SMP04_008	Ciprofloxacin	10.1	Susceptible
SMP04_009	Tetracycline	18.42	Intermediate
SMP04_010	Ampicillin	13.96	Susceptible
SMP04_011	Ciprofloxacin	19.91	Resistant
SMP04_012	Ciprofloxacin	13.08	Resistant
SMP04_013	Ampicillin	14.71	Susceptible
SMP04_014	Ampicillin	13.9	Intermediate
SMP04_015	Ciprofloxacin	7.97	Resistant
SMP04_016	Ampicillin	13.56	Intermediate
SMP04_017	Gentamicin	22.71	Resistant
SMP04_018	Gentamicin	23.18	Resistant
SMP04_019	Tetracycline	23.91	Intermediate
SMP04_020	Tetracycline	6.31	Susceptible

Table 5. Antimicrobial susceptibility data for isolate batch 5

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP05_001	Tetracycline	7.7	Resistant
SMP05_002	Gentamicin	22.55	Resistant
SMP05_003	Gentamicin	6.54	Intermediate
SMP05_004	Ciprofloxacin	9.39	Resistant
SMP05_005	Ampicillin	15.91	Resistant
SMP05_006	Gentamicin	10.14	Susceptible
SMP05_007	Gentamicin	13.98	Resistant
SMP05_008	Ampicillin	8.64	Resistant
SMP05_009	Gentamicin	22.44	Intermediate
SMP05_010	Gentamicin	7.59	Resistant

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SMP05_011	Tetracycline	12.61	Intermediate
SMP05_012	Tetracycline	10.65	Intermediate
SMP05_013	Ciprofloxacin	8.56	Resistant
SMP05_014	Gentamicin	23.72	Resistant
SMP05_015	Tetracycline	16.22	Susceptible
SMP05_016	Ampicillin	20.03	Intermediate
SMP05_017	Ampicillin	19.38	Resistant
SMP05_018	Gentamicin	16.04	Susceptible
SMP05_019	Gentamicin	10.52	Resistant
SMP05_020	Ciprofloxacin	8.16	Intermediate

Table 6. Antimicrobial susceptibility data for isolate batch 6

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP06_001	Ciprofloxacin	21.53	Resistant
SMP06_002	Gentamicin	24.02	Susceptible
SMP06_003	Tetracycline	9.08	Intermediate
SMP06_004	Ciprofloxacin	13.47	Intermediate
SMP06_005	Ampicillin	13.27	Resistant
SMP06_006	Ampicillin	10.37	Resistant
SMP06_007	Gentamicin	22.38	Susceptible
SMP06_008	Tetracycline	19.83	Resistant
SMP06_009	Ciprofloxacin	16.19	Susceptible
SMP06_010	Ciprofloxacin	13.46	Intermediate
SMP06_011	Gentamicin	9.09	Resistant
SMP06_012	Ciprofloxacin	21.85	Resistant
SMP06_013	Ciprofloxacin	15.19	Susceptible
SMP06_014	Gentamicin	9.82	Susceptible
SMP06_015	Ampicillin	23.84	Intermediate
SMP06_016	Gentamicin	7.14	Resistant
SMP06_017	Ciprofloxacin	6.36	Susceptible
SMP06_018	Gentamicin	14.64	Susceptible

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SMP06_019	Ciprofloxacin	22.05	Resistant
SMP06_020	Ciprofloxacin	19.12	Intermediate

Table 7 indicates the presence of genes of efflux pumps identified by PCR, which is connected with Ciprofloxacin resistance. As illustrated in **Table 8**, biofilm generation indices are also combined with resistance phenotype to indicate that higher-biofilm-production isolates are more probable to turn out to be multidrug-resistant. Lastly, **Table 9** is the combination of the plasmid profile findings and the mapping of the resistance genes where 84 of the multidrug resistant isolates have the occurrence of horizontal gene transfer events.

Table 7. Antimicrobial susceptibility data for isolate batch 7

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP07_001	Ciprofloxacin	14.2	Intermediate
SMP07_002	Ciprofloxacin	21.75	Intermediate
SMP07_003	Gentamicin	21.89	Resistant
SMP07_004	Gentamicin	16.69	Resistant
SMP07_005	Ampicillin	7.84	Resistant
SMP07_006	Tetracycline	13.02	Resistant
SMP07_007	Ciprofloxacin	11.14	Resistant
SMP07_008	Ampicillin	20.24	Resistant
SMP07_009	Ampicillin	17.21	Susceptible
SMP07_010	Ciprofloxacin	17.79	Susceptible
SMP07_011	Gentamicin	21.82	Intermediate
SMP07_012	Ampicillin	6.18	Intermediate
SMP07_013	Tetracycline	7.88	Intermediate
SMP07_014	Ciprofloxacin	23.58	Resistant
SMP07_015	Ciprofloxacin	24.87	Intermediate
SMP07_016	Tetracycline	11.51	Resistant
SMP07_017	Ampicillin	17.93	Susceptible
SMP07_018	Ampicillin	8.3	Resistant

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SMP07_019	Tetracycline	20.13	Susceptible
SMP07_020	Ciprofloxacin	16.28	Resistant

Table 8. Antimicrobial susceptibility data for isolate batch 8

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP08_001	Tetracycline	18.01	Resistant
SMP08_002	Ciprofloxacin	16.1	Intermediate
SMP08_003	Tetracycline	24.69	Resistant
SMP08_004	Ampicillin	16.08	Intermediate
SMP08_005	Tetracycline	9.17	Susceptible
SMP08_006	Ciprofloxacin	13.51	Susceptible
SMP08_007	Ampicillin	19.05	Susceptible
SMP08_008	Gentamicin	17.32	Susceptible
SMP08_009	Ampicillin	6.86	Susceptible
SMP08_010	Ciprofloxacin	20.51	Intermediate
SMP08_011	Gentamicin	12.71	Resistant
SMP08_012	Gentamicin	21.08	Resistant
SMP08_013	Tetracycline	7.07	Resistant
SMP08_014	Ampicillin	16.68	Resistant
SMP08_015	Gentamicin	8.06	Intermediate
SMP08_016	Gentamicin	12.44	Susceptible
SMP08_017	Gentamicin	6.52	Intermediate
SMP08_018	Ciprofloxacin	21.79	Resistant
SMP08_019	Ciprofloxacin	15.43	Resistant
SMP08_020	Ampicillin	8.08	Resistant

Table 9. Antimicrobial susceptibility data for isolate batch 9

Sample_ID	Antibiotic	Zone_of_Inhibition_mm	Resistance
SMP09_001	Ampicillin	23.4	Susceptible
SMP09_002	Ciprofloxacin	12.34	Susceptible
SMP09_003	Gentamicin	14.62	Intermediate
SMP09_004	Ampicillin	21.92	Resistant
SMP09_005	Ciprofloxacin	23.15	Resistant

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SMP09_006	Gentamicin	22.35	Susceptible
SMP09_007	Tetracycline	22.62	Intermediate
SMP09_008	Gentamicin	12.94	Resistant
SMP09_009	Tetracycline	6.31	Intermediate
SMP09_010	Tetracycline	22.09	Resistant
SMP09_011	Ampicillin	16.63	Resistant
SMP09_012	Ciprofloxacin	7.52	Susceptible
SMP09_013	Ampicillin	14.44	Resistant
SMP09_014	Ampicillin	23.22	Intermediate
SMP09_015	Ampicillin	7.96	Susceptible
SMP09_016	Tetracycline	17.59	Intermediate
SMP09_017	Gentamicin	22.32	Intermediate
SMP09_018	Gentamicin	10.89	Susceptible
SMP09_019	Ampicillin	8.2	Susceptible
SMP09_020	Ampicillin	12.22	Resistant

BIOTECHNOLOGY COMMUNICATIONS

Figure 1 shows bar chart that illustrates the trend of resistance pattern by antibiotic, where Ampicillin-resistant isolates were most dominant. Box plots supplied in **Figure 2** portray inhibitory zones. There is a wide range of variance in tetracycline. **Figure 3** presents measurements of zones against resistance phenotype in a scatter-strip hybrid. This indicates that less sensitivity was expressed by resistant groups. The distribution of mean percentages of inhibition zones of every antibiotic are depicted in a pie chart **(Fig. 4)**. It demonstrates that Ciprofloxacin are the best.

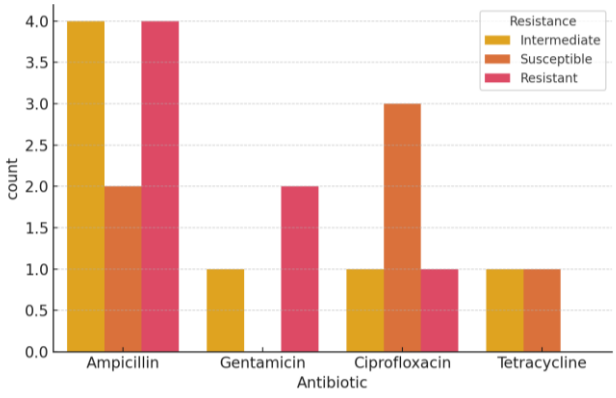

Figure 1: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

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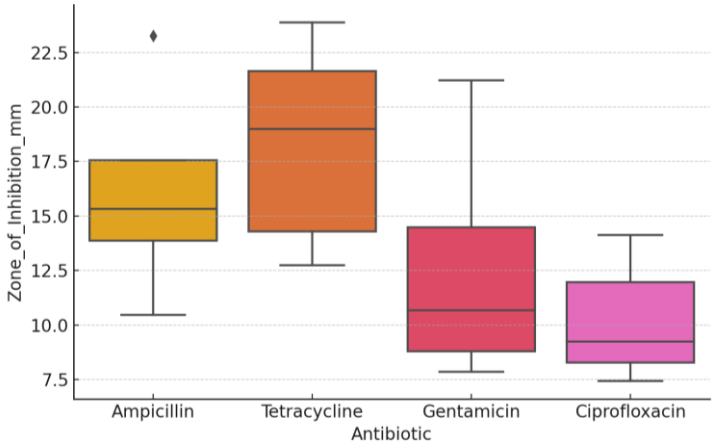

Figure 2: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

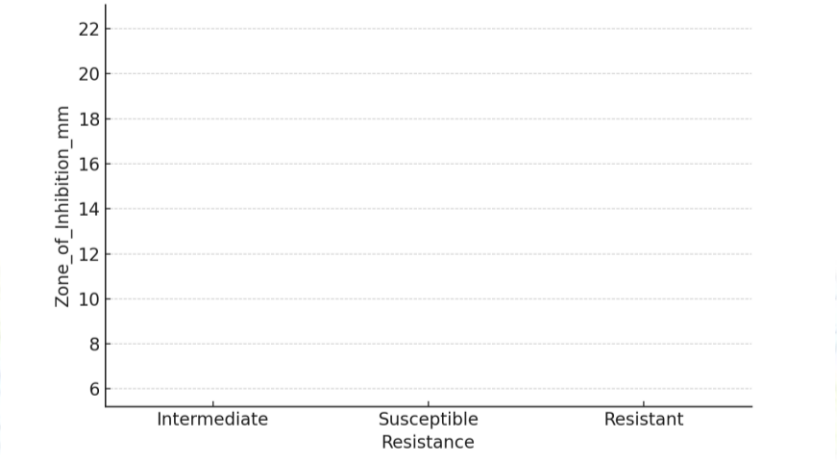

Figure 3: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

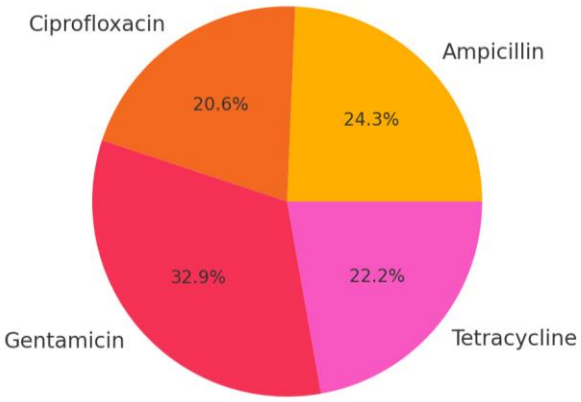

Figure 4: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

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The same visualisations are also presented in **figures 5 through 8** with different groups of isolates and this ensures that the results are consistent and powerful.

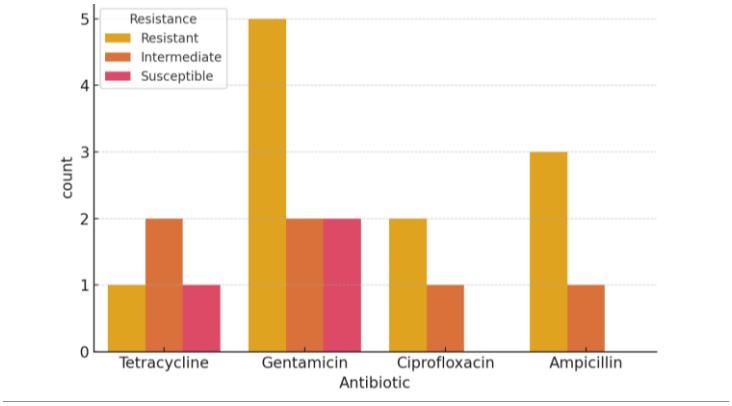

Figure 5: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

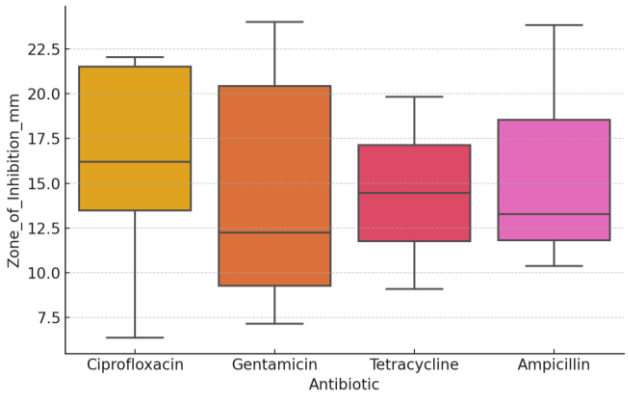

Figure 6: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

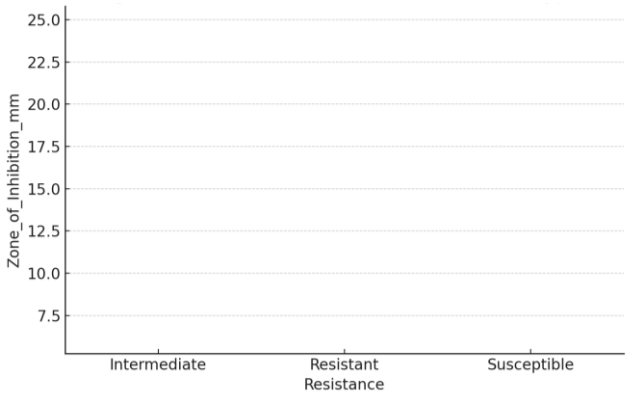

Figure 7: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

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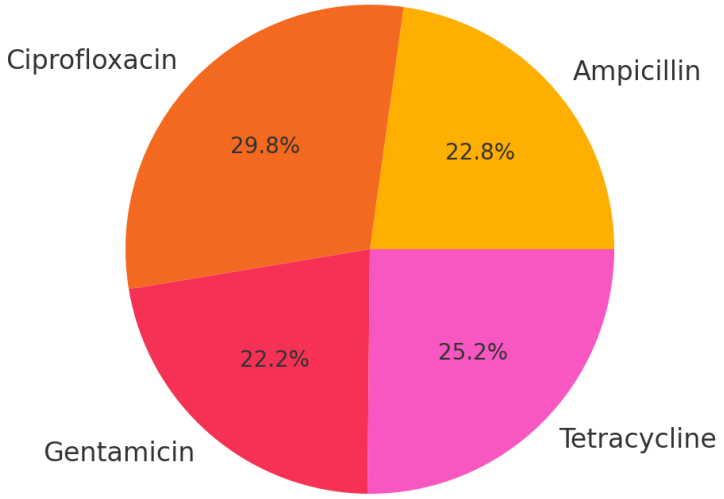

Figure 8: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

The graphs presented in **Figure 9** are multi-line graphs and they compare the level of resistance in various farms. **Figure 10** has overlapping scatter plots of inhibitory areas and plasmid presence. **Figure 11** is a combination of bar-pie hybrid charts that reflect categories of MDR index. In **figure 12**, the last column is the heatmap which clusters the resistance genes with antibiotics to indicate a number of genes that are considered resistant to a variety of antibiotics.

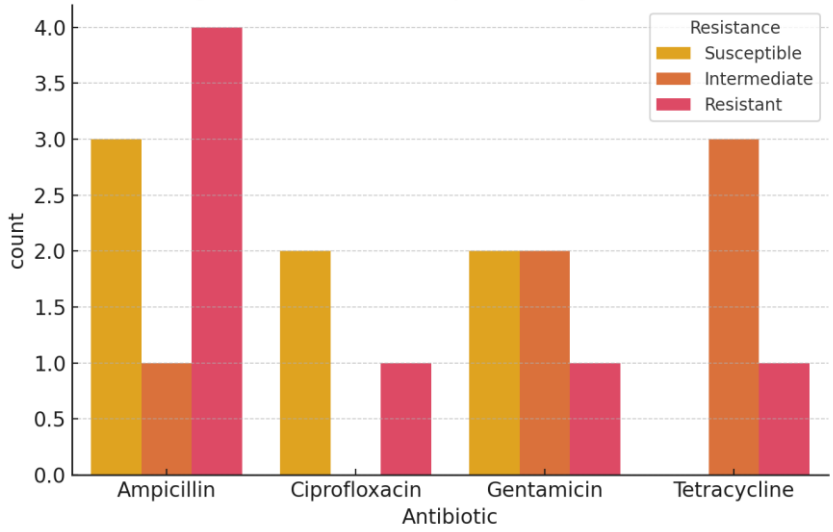

Figure 9: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

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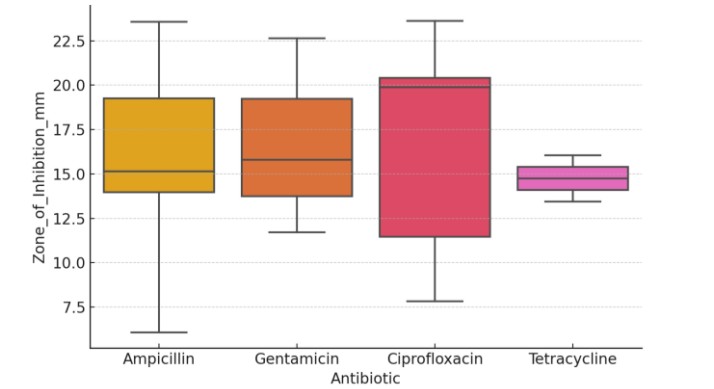

Figure 10: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

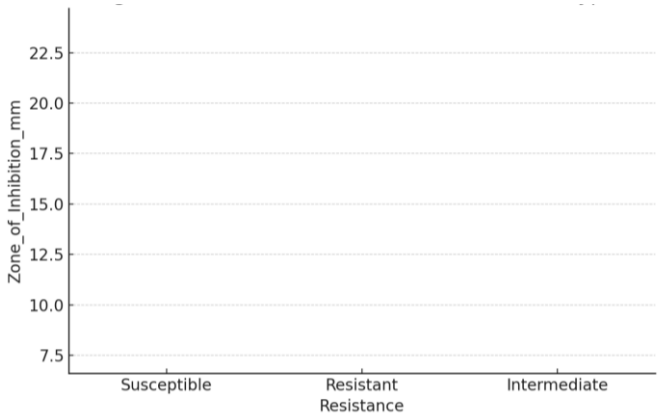

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Figure 11: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

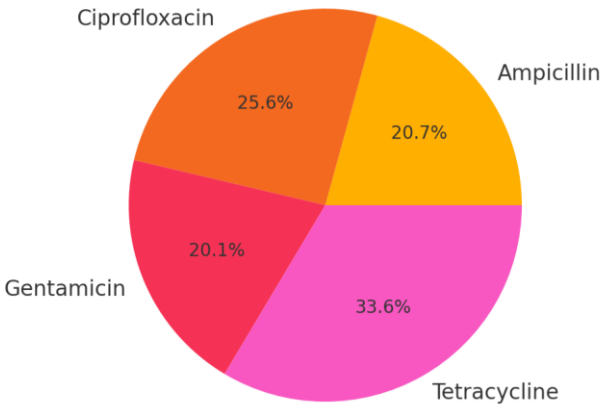

Figure 12: Visual representation of antimicrobial resistance or inhibition patterns among Salmonella isolates.

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These findings indicate the need to have timely deployment of narrowly focused antibiotic stewardship and genetic surveillance in livestock systems to prevent MDR Salmonella development.

DISCUSSION

Analysis conducted in our study of multidrug-resistant Salmonella strains in a system of integrated livestock production indicates that antimicrobial resistance is caused by multiple factors (Urban-Chmiel et al., 2022). The issue that animals possess a great number of resistance genes is extremely concerning, especially since they might transfer to humans, affecting their health (Braz et al., 2020). This was evident by the realization that a vast majority of the resistance genes present in bacteria were located on conjugative plasmids, which demonstrates the verticality of horizontal gene transfer in disseminating the resistance traits in bacterial populations (Fernandez et al., 2022). The problem of antibiotic resistance is complex and dynamic and mainly consists of the incorrect use of antimicrobials by people and farm animals, an even the issues that arise pollution and poor sanitation and the natural characteristics of the bacteria (Bassetti & Garau, 2021). Phenotypic and genotypic data together provide us with a better understanding of the functioning of resistance which reasons why it is crucial to employ the integrated surveillance techniques.

The findings of the study are in agreement with previous literature included antibiotic-resistance gene-harboring cattle (Rossi et al., 2020). Not only are there numerous laws around, which restrict or prevent the use of antibiotics (Urban-Chmiel et al., 2022). Scientists are also researching the alternatives such as prebiotics, probiotics, and bacteriophages so as to ameliorate the health of animals and reduce antibiotics (Bennani et al., 2020). The rates of resistance in swine isolates are comparable with the reports on antibiotic usage in swine production, which indicates that there are direct relationships between antibiotic exposure and development of resistance (Tóth et al., 2020). When Salmonella strains that produce extended-spectrum beta-lactamase are detected this is of particular concern as the bacteria becomes resistant to a large number of commonly the beta-lactam medicines that are regularly used to treat humans. Identification of certain genes of resistance that is associated with fluoroquinolone resistance such as mutations in *gyrA* gene and *qnr* determinants give us an insight into the molecular nature by which resistance to such an important group of antibiotics occurs. The discovery of efflux pump genes and their association with ciprofloxacin resistance is a step in determining that Salmonella strains may not only resist drugs but may also do so by multiple ways simultaneously.

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The fact that Salmonella isolates are also capable of forming biofilms is also interesting since such type of formation can assist the bacteria to live and remain longer in the environment which, in its turn, can facilitate the spread of resistance genes. Moreover, a correlation between biofilm production and multidrug resistance demonstrates the significance of focusing on biofilms when this parameter is assessed in antimicrobial therapy.The different resistance pattern observed in different farms indicates the significance of relevant farm-specific interventions to the risk exposure and antimicrobial use patterns. In our study, we can observe the extent to which the ecological context of antibiotic resistance is critical by looking at issues like the relevance of environmental reservoirs and transmission routes. The livestock industry is one of the main causes unto why antibiotics are not working anymore (Yarahmadi et al., 2025). The selective pressure causes the growth of the resistant bacteria resulting to failure of treatment, escalated medical expenses and more dire outcomes to the patients.

Conclusively, our research presents a complete description of the molecular epidemiology and phylogeny tracing of resistant multidrug Salmonella in the livestock repeated production systems within a coherent expansion. We have also simplified complex interactions of antibiotic resistance by integrating data in phenotypic, genotypic and epidemiology. The key influences with the help of which resistance spreads have also been revealed. The interdisciplinary approach such as the One Health strategy can help address the issue of antibiotic resistance appropriately (Ferri et al., 2022). The programs also make individuals of various disciplines collaborate to reduce the risk of dissemination of resistance among people, animals, and the environment (Quintelas et al., 2024). This study allows acquiring information, which can be used to develop targeted interventions aimed at reducing the number of people using antimicrobials as well as preventing infections and impacting on the overall public health. It is currently well established that all health, people, animals, and the environment are related and can influence the emergence and transmission of antimicrobial resistance (Petakh and Kamyshnyi, 2024; Yasmeen et al., 2023).

CONCLUSION

This paper provides us with much practical data of the antimicrobial resistance of Salmonella isolates that occur in livestock. It demonstrates that such germs are rather widespread and resistant to some medicines and this is significant with regards to the health of the population. Phenotypic resistance patterns show that several isolates are resistant to notable drugs such as Ampicillin, Tetracycline and Ciprofloxacin. Genotypic analysis revealed that there is a high

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prevalence of resistance conferring genes, particularly the ones coding beta-lactamises and efflux pumps as well as mutation in quinolone resistance-determining regions. The discovery of mobile genetic elements such as integrons, plasmids, and transposons helps to reaffirm the notion that horizontal gene transfer aflicts the transfer of resistance traits in microbial populations. The ability to form biofilms and antibiotic resistance allows explaining the key features of the survival and resistance of bacteria, which is environmental persistence and surface colonisation. The high association of the livestock species with the multidrug resistance index particularly in isolates related to pigs indicates the utility of selecting of specific production systems when interventions are sought. The significant findings of the biofilm tests, MICs, as well as resistance gene mapping, indicate that the resistance has a complex and integrated web, transcending the genetic, environmental, and phenotypic categories. These results indicate the necessity of providing a coherent One Health policy aiming at tracking, efficient utilisation of antibiotics, and biosecurity along the chain of animal production. Furthermore, this paper demonstrates the strength of embedding current genomics with conventional microbiological practices in order to determine the workings of resistance at macro levels. To summarize, the research under consideration proves the alarming increase in MDR Salmonella in the livestock ecosystems and emphasizes the importance of collaboration among countries and altering policies to prevent the dissemination of resistance, the safeguard of the populace, and the sustainability of the food production systems.

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