Phenotypic and Genotypic Antibiotic Resistance of *Salmonella* isolated from humans and associated backyard chickens in South Khorasan Province, Iran

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Abstract

Introduction: As significant zoonotic agents, *Salmonella* species represent a substantial challenge to global health. The lack of regulation in antimicrobial use within small-scale poultry production often encourages the prevalence of resistant strains, enhancing the cross-transmission of these pathogens between humans and avian hosts. This study assessed the phenotypic and genotypic antimicrobial resistance (AMR) of *Salmonella* in backyard chickens and their associated human contacts in the villages of South Khorasan Province, Iran, to evaluate the impact of unregulated antibiotic use in small-scale poultry farming.

Methods: Sixteen *Salmonella* isolates from 197 human and 785 chicken samples were tested against 14 antimicrobials using disc diffusion. Six resistance genes were subsequently analyzed via PCR.

Results: Ampicillin and tetracycline showed the highest phenotypic resistance rates (85.71% and 78.57%, respectively, in chickens; and 100% in humans). The most prevalent resistance genes were *tetA* (93.75%), *sulI* (87.5%), and *tetB* (50%), whereas the lowest detection rates were observed for *gyrA* (0%), *floRA* (12.5%), and *sulII* (12.5%) Notably, isolates from the identical families exhibited same resistance profiles and genes.

Conclusion: The observed AMR patterns in both humans and chickens indicate some similarities in their resistance profiles. While the limited number of isolates, particularly from human sources, necessitates caution in generalization, these findings suggest that a "One Health" approach is essential for monitoring and managing the dissemination of resistant *Salmonella* strains in small-scale farming.

Keywords:

Chickens; Drug Resistance; Humans; One Health; PCR; *Salmonella*.

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1. Introduction

Salmonellosis represents a major zoonotic threat, characterized by a wide spectrum of clinical manifestations in humans and animals. These range from acute gastroenteritis to more severe conditions, such as bacteremia and extra-intestinal infections affecting multiple organ systems. [1, 2]. Its endemicity, high transmissibility, integration into the food production chain and zoonotic potential collectively, make it a major public health concern [3].Contaminated poultry eggs and meat products are the major sources of human Salmonella infection [4].

Intestinal infection caused by nontyphoid *Salmonella* serotypes is usually self-limiting, although effective antimicrobial therapy is essential if spread beyond the intestine occurs [2]. Antimicrobial agents have long been considered the primary strategy for treatment and control of *Salmonella* infections [5]; However, Inappropriate antibiotic usage has significantly driven the emergence of resistant *Salmonella* strains [6]. This resistance is largely mediated through various mechanisms such as biofilm formation, alterations in antibiotic targets, β-lactamase production, efflux pumps and horizontal gene transfer [6].

On the other hand, the trend of owning backyard chickens has been rising in the last 10 years and in 2020, due to Covid-19 stay at home orders, an additional surge in popularity was seen and Concurrently, cases of salmonellosis from live poultry exposure have also risen [7].This style of breeding, especially in rural areas, is associated with a lack of biosecurity principles and it increases the risk of zoonotic diseases, including *Salmonella* [8].

Due to their public health importance, the identification and serotyping of zoonotic *Salmonella* serovars, determination of their antimicrobial resistance (AMR) profiles and characterization of resistance-associated genes are essential for effective infection control and mitigation of disease outcomes [9]. While various studies have explored *Salmonella* AMR in different sectors across

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Iran, research that concurrently addresses both human and animal isolates within the same domestic environment is relatively scarce. To our knowledge, this may represent one of the first AMR investigations in backyard chickens and humans in contact with them in this region and it can provide baseline data. Consequently, this study aimed to evaluate, antimicrobial resistance genes (ARGs) and AMR patterns of *Salmonella* isolated from these sources in the villages of this region.

2. Materials and methods

In our study AMR was assessed phenotypically and genotypically in 10 *Salmonella* Typhimurium and 4 *Salmonella* Infantis isolates collected from backyard chickens and 2 *S.* Typhimurium isolates recovered from children. These 16 isolates were previously identified from a larger screening of 197 human stool specimens and 785 chicken fecal samples (processed as 147 pools) from households with a close human-backyard chicken interface. These samples were collected from at least six households in each of the ten villages (each with >50 households) in South Khorasan Province, based on the criteria of owning at least five backyard chickens and the presence of a mother and a child aged 5–12 years. Human-backyard chicken contact was defined as members of the same household sharing a domestic environment and directly handling the poultry. The two human *S.* Typhimurium isolates were recovered from households where backyard chickens were also positive for the same serovar. All isolates were confirmed via microbiological techniques, serotyping, and conventional polymerase chain reaction (PCR) prior to the current phenotypic and genotypic resistance profiling. The present study specifically focuses on providing an in-depth phenotypic and genotypic AMR profiling of these 16 characterized strains.

2.1. Detection of antimicrobial resistance genes

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We identified the ARGs by PCR. All primer sequences were confirmed for specificity and accuracy via the NCBI database and Primer-BLAST tool (Table 1). PCR amplification for *tetA*, *tetB*, *sulI* and *sulII* was performed in 25 µL volumes using an annealing temperature of 72°C, following the protocol by [10]. Similarly, *gyrA* and *florA* were detected according to [11, 12], with annealing temperatures set at 60°C and 55°C, respectively.

Visualisation of the amplified products was performed using ultraviolet light, following electrophoresis on a 1.2% agarose gel (Sinaclon, Iran) previously stained with ethidium bromide (Sinaclon, Iran) [9].

Table 1. Primers used for the detection of antibacterial resistance genes in *Salmonella* serovars

Antimicrobial agent	Target gene	sequence(5′ to3′)	Product size(bp)	References
Tetracycline	*tetA*	F: GCGCCTTTCCTTTGGGTTCT R: CCACCCGTTCCACGTTGTTA	831	[13]
Tetracycline	*tetB*	F: CCCAGTGCTGTTGTTGTCAT R: CCACCACCAGCCAATAAAAT	723	[13]
Sulfonamide	*SulI*	F: TCACCGAGGACTCCTTCTTC R: CAGTCCGCCTCAGCAATATC	331	[13]
Sulfonamide	*SulII*	F: CCTGTTTCGTCCGACACAGA R: GAAGCGCAGCCGCAATTCAT	435	[13]
Florfenicol	*florA*	F:AACCCGCCCTCTGGATCAAGTCAA R: CAAATCACGGGCCACGCTGTATC	548	[9]
Enrofloxacin	*gyrA*	F: AGTCCTATCTCGACTACGCGAT R: AGTCGACGGTTTCCTTTTCCAG	324	[11]

2.2. Antibiotic Susceptibility Testing

To determine resistance profiles, *Salmonella* strains were subjected to disk diffusion assays on Mueller-Hinton agar, as recommended by the CLSI guidelines (M100, 2025 edition). All antimicrobial disks were provided by Padtan Teb Co (Iran). We tested a range of concentrations, specifically: ampicillin (10 µg), cefoxitin (30 µg), cefepime (30 µg), ceftiofur (30 µg), gentamicin (10 µg), amikacin (30 µg), tetracycline (30 µg), oxytetracycline (30 µg), doxycycline

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(30 µg), florfenicol (30 µg), lincomycin-spectinomycin (100 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), and trimethoprim-sulfamethoxazole (52 + 240 µg). Results were interpreted by measuring the zones of inhibition and aligning them with the manufacturer's and CLSI's susceptibility criteria [10].

2.3. Statistical analysis

The distribution of antimicrobial resistance genes was compared between *Salmonella*-positive samples, including *S*. Typhimurium isolates recovered from backyard chickens and humans,as well as *S*. Typhimurium and *S*. Infantis isolates recovered from backyard chickens. The presence or absence of each resistance gene was analyzed using Fisher's exact test due to the limited number of isolates. Furthermore, phenotypic antimicrobial susceptibility profiles were compared between *Salmonella*-positive samples based on susceptibility categories (susceptible, intermediate, and resistant). Differences in antimicrobial susceptibility patterns were evaluated using the Fisher's exact test because of the small sample size. All statistical analyses were performed using SPSS version 31.0 (IBM Corp, Armonk, NY, USA). A P-value < 0.05 was considered statistically significant.

3. Results

All 16 *Salmonella* isolates were screened for six ARGs associated with four antimicrobial classes (Figure 1).Regarding the strains recovered from backyard chickens and human samples, the *tetA* and *sulI* resistance genes were concurrently detected in 100% of the *S*. Typhimurium isolates. In contrast, the distribution of the *tetB* resistance gene was more restricted and varied by host and serovar; while it was identified in 58% of the *S*. Typhimurium and 25% of the *S*. Infantis isolates from backyard chickens, it was conspicuously absent in the human samples. In *S*. Infantis isolates, *tetA* was detected in 75% and *sulII* in 50% of the strains while the *sulII* gene was not

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136 detected in any *S.* Typhimurium isolates. Notably, both *florA* and *gyrA* resistance genes showed
137 low or no presence among the isolates; the *florA* resistance gene was only sporadically detected
138 (16.66% in backyard chickens) and *gyrA* resistance gene was not identified in any of the tested
139 serovars (0%). Overall, among the screened antimicrobial resistance genes, the highest
140 prevalence was observed for *tetA* (93.75%), followed by *sulI* (87.5%) and *tetB* (50%). In
141 contrast, the lowest detection rates were recorded for *gyrA* (0%), *florA* (12.5%) and *sulII*
142 (12.5%), espectively (Table 2).

143 The distribution of antimicrobial resistance genes was compared between *S.* Typhimurium
144 isolates recovered from backyard chickens and humans, as well as between *S.* Typhimurium and
145 *S.* Infantis isolates from backyard chickens. Although differences in the frequency of some
146 resistance genes were observed among groups, none of these differences reached statistical
147 significance (Fisher's exact test, $P > 0.05$).

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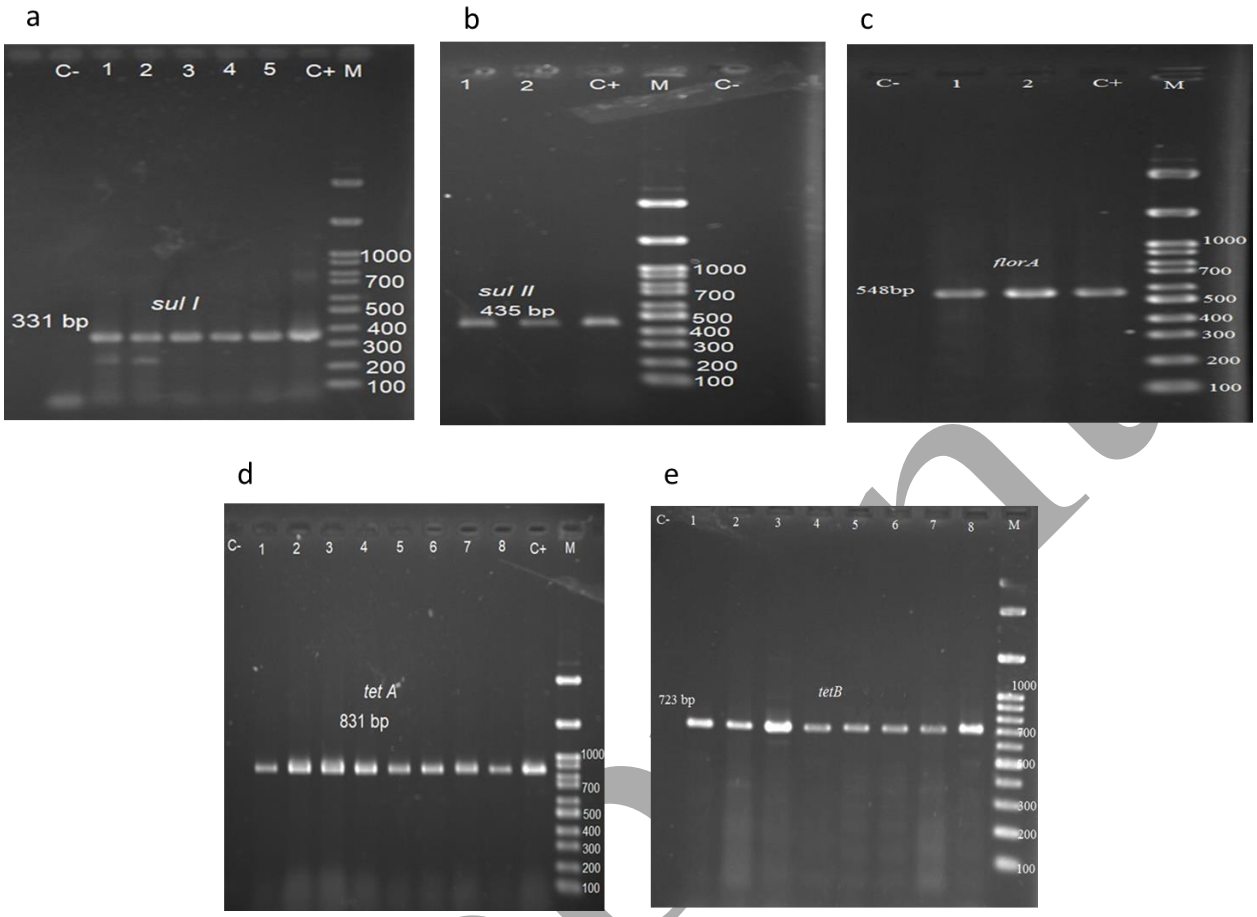


Figure 1. PCR amplification results for the identification of target resistance genes: (a) *sulI* (331 bp), (b) *sulII* (435 bp), (c) *florA* (548 bp), (d) *tetA* (831 bp), and (e) *tetB* (723 bp). C-: Negative control (sterile distilled water); C+: Positive control (Standard *Salmonella* strain, Microbial Collection, Faculty of Veterinary Medicine, University of Tehran); M: 100 bp ladder.

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Table 2. Distribution of the antimicrobial resistance genes in *Salmonella* serovars

Salmonella serovars	Host	Antimicrobial resistance genes						Total isolates (n)
		tetA	*tetB*	*sulI*	*sulII*	*florA*	*gyrA*	
S. Typhimurium	Backyard chickens	100% (10)	58% (7)	100% (10)	0%	16.66%(2)	0%	10
S. Typhimurium	humans	100%(2)	0%	100%(2)	0%	0%	0%	2
S. infantis	Backyard chickens	75% (3)	25% (1)	50% (2)	50% (2)	0%	0%	4
Total (%)		93.75%	50%	87.5%	12.5%	12.5%	0%	

The antimicrobial susceptibility patterns of *S.* Typhimurium and *S.* Infantis isolates against 14 antibiotics, used in veterinary and human medicine were examined (Table 3).

All isolates recovered from both humans and backyard chickens exhibited complete susceptibility to second and third-generation cephalosporins, including cefoxitin, cefepime, and ceftiofur. This total sensitivity also extended to ciprofloxacin, trimethoprim-sulfamethoxazole, and aminoglycosides (gentamicin and amikacin) across all tested samples.

In terms of antibiotic resistance among the 14 backyard chicken isolates, the highest resistance patterns were observed against ampicillin (85.71%, 12/14) followed by tetracycline (78.57%, 11/14). About ampicillin all *S.* Typhimurium isolates (100%, 10/10) and half of the S. Infantis strains (50%, 2/4) were resistant. Regarding tetracycline *S.* Typhimurium and *S.* Infantis showed 80% (8/10) and 75% (3/4) resistance, respectively. Oxytetracycline and linco-spectin showed 57.14% (8/14) resistance in backyard chicken isolates; this accounted for 60% (6/10) of *S.* Typhimurium and 50% (2/4) of *S.* Infantis.

Additionally high susceptibility rates were recorded among backyard chicken isolates for florfenicol and enrofloxacin. The total susceptibility to florfenicol was 92.85% (13/14), including

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90% (9/10) of *S.* Typhimurium and 100% (4/4) of *S.* Infantis isolates. The general susceptibility for enrofloxacin was 85.7% (12/14), including 80% (8/10) of *S.* Typhimurium and 100% (4/4) of *S.* Infantis isolates. Similarly, doxycycline showed a favorable susceptibility profile in poultry, with 60% (6/10) of *S.* Typhimurium and 75% (3/4) of *S.* Infantis isolates susceptible.

Human-derived *S.* Typhimurium isolates (n=2) exhibited 100% resistance to both ampicillin and tetracyclin.

Notably, AMR test in human and backyard chicken isolates from the same households showed identical antibiogram profiles and matching resistance genes.

Phenotypic antimicrobial susceptibility patterns were also compared between *S.* Typhimurium isolates from different hosts and between *S.* Typhimurium and *S.* Infantis isolates from backyard chickens. No statistically significant differences were observed in antimicrobial susceptibility categories (S/I/R) between the compared groups ($P > 0.05$).

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Table 3. Antimicrobial resistance /susceptibility phenotypes of isolated *Salmonella* serovars

Strains	*S.* Typhimurium			*S.* Typhimurim			*S.* Infantis		
Host	Backyard chickens (n=10)			Human(n=2)			Backyard chicken (n=4)		
Antimicrobials	S	I	R	S	I	R	S	I	R
ampicillin	0	0	10	0	0	2	1	1	2
Cefoxitin	10	0	0	2	0	0	4	0	0
cefepime	10	0	0	2	0	0	4	0	0
Ceftiofur	10	0	0	2	0	0	4	0	0
Gentamicin	10	0	0	2	0	0	4	0	0
Amikacin	10	0	0	2	0	0	4	0	0
Tetracycline	1	1	8	0	0	2	1	0	3
Oxytetracycline	3	1	6	2	0	0	1	1	2
Doxycycline	6	3	1	0	1	1	2	2	0
Florfenicol	9	0	1	2	0	0	4	0	0
Lincospectin	2	2	6	2	0	0	2	0	2
Enrofloxacin	8	2	0	2	0	0	4	0	0
Ciprofloxacin	10	0	0	2	0	0	4	0	0
Trimethoprim-Sulfamethoxazole	10	0	0	2	0	0	4	0	0

S = Susceptible; I = Intermediate resistance; R = Resistance

4. Discussion

Salmonellosis remains the second most commonly reported gastrointestinal infection in humans after *Campylobacter*; Therefore the growing AMR in *Salmonella* makes the worldwide sickness burden even worse [14, 15]. Projections indicate that antibiotic-resistant pathogens could cause about 10 million deaths yearly by 2050 [16]. At the same time, the growing interest in natural and local poultry farming has led to an increase in backyard farming. However, the recent outbreak of *Salmonella* linked to this small-scale operation has emerged as a significant public health concern [17].

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Given the general health significance of *Salmonella* and the growing concern about AMR, the present study analyzed 16 *Salmonella* isolates collected from humans and backyard chickens, including phenotypic antimicrobial susceptibility testing and molecular detection of ARGs. The integration of PCR-based resistance gene detection with disk diffusion susceptibility testing offers a preliminary view of resistance dynamics.

Regarding the phenotypic antimicrobial susceptibility patterns, high resistance rates to ampicillin and tetracycline were observed in the present study. These findings are in strong agreement with other domestic reports; for instance, Atash faraz et al. and Hosseini et al. similarly documented substantial resistance to these agents [18, 19]. This phenomenon likely stems from the widespread and indiscriminate prescription of antibiotics, which exerts significant selective pressure on *Salmonella* populations.

However, within the country, these patterns are not uniform. When compared with other Iranian reports, notable disparities emerge. For example, Sales et al. reported entire resistance to amikacin and intermediate resistance degrees to gentamicin (26.92%), ciprofloxacin (15.38%), and ampicillin (11.54%) in *S.* Typhimurium isolates from pediatric cases, while our human isolates confirmed entire susceptibility to amikacin, gentamicin and ciprofloxacin [20]. Similarly, Moghaddami et al. reported resistance to ampicillin (8%) in children, whereas in our study, all human isolates exhibited complete resistance to ampicillin (100%), although both studies shared a common finding of complete susceptibility to trimethoprim-sulfamethoxazole [21]. These discrepancies highlight the fluctuating nature of AMR patterns across different clinical settings and time periods within the country.

On a global scale, the resistance profiles of our isolates show a mixed pattern of concordance and divergence. Consistent with our findings, Castro Vargas et al. identified ampicillin as one of

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the antibiotics with the highest resistance levels within the poultry production chain [16]. Meanwhile, international surveillance data from 148 countries (1991–2023) have proven a growing incidence of resistance to fluoroquinolones and tetracycline class antibiotics in chickens [22]. Michela et al. also reported high resistance of *S.* Infantis isolates to fluoroquinolones, ampicillin and tetracycline [23]. Other reports show different trends. For instance, Koro et al. reported negligible resistance to ampicillin and tetracycline, which stands in stark contrast to the high rates we observed. However, their results aligned with ours regarding the complete susceptibility of *Salmonella* isolates to gentamicin, ciprofloxacin, cefotaxime, and trimethoprim-sulfamethoxazole [14].

These varying degrees of concordance and divergence with international data highlight the complexity of antimicrobial resistance patterns in *Salmonella*. While some alignment suggests a global trend, the divergence may be attributed to several factors, including variations in circulating strains, geographical differences in antimicrobial selective pressures and the specific host species studied.

Regarding the genetic basis of this resistance, tetracycline resistance showed a high degree of concordance between phenotypic profiles and genotypic determinants. This correlation was evidenced by the fact that all phenotypically resistant *Salmonella* isolates harbored at least one of the *tet* genes, which underscores the role of efflux pump mechanisms in mediating tetracycline resistance [24]. These findings align with Perry et al., who reported a significant correlation between phenotypic resistance and the presence of tetracycline resistance genes in poultry layer farms [25].

Furthermore, the lower resistance observed toward Doxycycline compared to Tetracycline suggests that, newer generation tetracyclines might still retain partial efficacy.

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In the present study *sulI* gene was dominant in relation to sulfonamides. Interestingly, despite the high carriage rate of *SulI* gene, all isolates remained phenotypically susceptible to the Trimethoprim-Sulfamethoxazole (SXT) combination. These findings partially diverge from the results of Petrin et al., who observed phenotypic sulfamethoxazole resistance in 24% of their isolates, primarily associated with *sulI* or *sulII* genes. However, Petrin et al. detected nine strains having the *sulII* gene that were phenotypically susceptible; that is much like our study [24]. Conversely, Hosseini et al. found *sulII* gene to be the predominant gene and reported a high level of phenotypic resistance to the SXT combination [19]. This stark contrast suggests that the presence of the gene alone does not necessarily confer phenotypic resistance. It may instead depend on gene expression levels or complicated regulatory mechanisms. Therefore, these resistance genes may remain silent or be insufficiently expressed under standard laboratory conditions, requiring further molecular investigations to clarify the factors influencing their expression.

Moreover, the absence of *gyrA* resistance genes was noted; in addition to this, a high susceptibility of isolates to fluoroquinolones in particular enrofloxacin and ciprofloxacin, was observed, both of which differ from worldwide trends [23]. These findings emphasize the need for continued monitoring to ensure the sustained effectiveness of enrofloxacin in both local and global setting.

Furthermore, a high concordance was observed between the phenotypic and genotypic results for florfenicol, both of which showed low resistance rates. This correlation suggests that florfenicol remains a relatively effective therapeutic option for managing these infections.

When compared to other regional studies, such as Ghoddousi et al., notable variations were observed. While Ghoddousi et al. reported high phenotypic resistance to tetracycline (100%) and

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281 florfenicol (72%) in *S.* Infantis; they found that all florfenicol-resistant isolates carried the *floR*
282 gene, whereas none of the tetracycline-resistant isolates possessed *tetA* [9]. In contrast, in the
283 present study, the *floR* gene was not detected in any *S.* Infantis isolate, and all were
284 phenotypically susceptible to florfenicol. Moreover, all tetracycline -resistant *S.* Infantis isolates
285 carried the *tetA* gene. These variations suggest that distinct resistance mechanisms may prevail in
286 different *Salmonella* populations, further highlighting the impact of host source and regional
287 factors on antimicrobial resistance profiles.

288 Notably, in two households where *Salmonella* isolates were obtained from children and
289 backyard chickens, the human and poultry isolates exhibited identical antibiograms and
290 resistance genes. This concordance suggests possible epidemiological linkage between human
291 and backyard chicken isolates at the human-animal interface.

292 **5. Conclusion**

293 These findings highlight a broader public health challenge, as the emergence of multidrug-
294 resistant (MDR) *Salmonella* strains – often originating from avian sources – continues to
295 exacerbate the worldwide risk of AMR, with profound implications for the poultry industry and
296 human health. [14]. This situation is particularly concerning in backyard farming, which often
297 lacks stringent biosecurity measures and professional veterinary oversight, potentially leading to
298 the unregulated use of antibiotics and the subsequent selection of resistant strains. Furthermore,
299 the close proximity between humans and backyard animals creates a unique ecological niche
300 where resistant bacteria can persist and circulate within the shared environment. Such dynamics
301 increase the risk of these MDR strains entering the broader food chain or contaminating local
302 water and soil. Therefore, our findings underscore the necessity of integrated surveillance and

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the implementation of antimicrobial stewardship programs in small-scale farming to mitigate the spread of AMR and safeguard public health.

Given the critical threat of MDR strains, investigation of additional resistance genes, including *β-lactam* and expanded epidemiological investigations are essential to elucidate the evolution and dissemination of ARGs at the human-animal interface. Comprehensive studies of this nature are required to map the resistomes of both poultry and human isolates, providing a foundation for more precise disease control and antibiotic management strategies.

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Author's Contributions

Conceptualization, study design and final approval: All authors; Lead Research, implementation and first Draft: Zeynab Alizadeh Sorond; Lead Supervision, intellectual guidance and critical revision: Kaveh Parvandar Asadollahi; Equal Supervision: Kiarash Ghzvini, Gholamreza Nikbakht Brujeni.

Ethical Considerations

All ethical standards have been upheld. The collection of samples used in this study was approved by the Ethics Committee of Mashhad University of Medical Sciences (Approval No: 1398.842.IR.MUMS.MEDICAL.REC). As this research focused exclusively on pre-existing archived isolates, no direct intervention with human or animal subjects was involved.

Conflict of interest

The authors declared no conflict of interest.

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Data availability

The datasets generated and analysed during the current study are not publicly available due to the privacy of the participants, but are available from the corresponding author on reasonable request.

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