

Antibiotic Susceptibility Profile of *Avibacterium paragallinarum* Isolated from Commercial and Backyard Poultry in Iran

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Abstract

Introduction: The increasing prevalence of multidrug-resistant (MDR) strains of *Avibacterium paragallinarum*, the causative agent of infectious coryza (IC), is a significant concern in poultry farming. Knowledge of the antimicrobial susceptibility profiles of circulating isolates can contribute to the effective control of IC.

Methods: In this study, 22 *A. paragallinarum* isolates from poultry flocks across different regions of Iran were evaluated for susceptibility to 23 antibiotics using Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC). Additionally, resistance genes [*tet(A)*, *tet(B)*, *tet(C)*, *tet(G)*, *sul2*, and *strA*] were detected via PCR.

Results: The highest resistance rates were observed against erythromycin (90.9%), followed by enrofloxacin, and ofloxacin (81.8%). Except for two isolates (AP-1 and AP-16), all remaining isolates were MDR. Four isolates (AP-2, AP-3, AP-9, and AP-11) exhibited resistance to between 14 and 17 antibiotics concurrently. Among resistance genes, *tet(B)* (100%) and *sul2* (86%) were most frequent, followed by *tet(G)* (59%) and *strA* (18%); *tet(A)* and *tet(C)* were not detected. MIC analysis showed that 77.3% of isolates had MIC ≥ 16 $\mu\text{g/mL}$ for tetracycline, and 86.4% had MIC ≥ 32 $\mu\text{g/mL}$ for streptomycin. A consistent correlation was observed between antibiotic susceptibility test results and the presence of resistance genes. Ceftriaxone was the most effective agent tested, followed by florfenicol. None of the isolates were susceptible to erythromycin or ofloxacin.

Conclusion: These findings provide insights into the antibiotic susceptibility profiles of *A. paragallinarum* and could be served as a foundation for future national control and diagnostic strategies.

Keywords: Antibiotic resistance, *Avibacterium paragallinarum*, Infectious coryza, Multidrug resistance, Resistance genes.

1. Introduction

Infectious coryza (IC) is an acute and highly contagious respiratory disease of poultry caused by *Avibacterium paragallinarum*. The typical clinical signs include facial swelling, sinusitis, conjunctivitis, and purulent nasal discharge. The disease leads to growth retardation in broilers and a marked reduction in egg production among laying hens. Outbreaks of *A. paragallinarum* infection have been reported worldwide, affecting both small- and large-scale poultry operations. Beyond its impact on flock performance, IC represents a significant economic burden by lowering productivity and increasing treatment costs, while also contributing to the dissemination of antimicrobial resistance (AMR) within poultry production systems and the environment [1, 2].

In Iran, *A. paragallinarum* infection has been documented previously in poultry flocks, and the disease is believed to be widespread nationwide. Studies conducted at the Razi Vaccine and Serum Research Institute have confirmed its occurrence across multiple provinces (Alborz, East Azerbaijan, Kermanshah, Mazandaran, Razavi Khorasan and Tehran ...), affecting both commercial layer and backyard flocks [1, 3].

Given the scale of Iran's poultry industry and the lack of comprehensive epidemiological data on bacterial diseases, systematic research on IC is essential not only for improving flock health and productivity but also for supporting antimicrobial resistance control. At the flock level, control and prevention of IC rely primarily on biosecurity measures and vaccination, while targeted antimicrobial therapy may also play a role in disease management. However, the excessive or improper use of antibiotics has intensified the risk of antimicrobial resistance in *A. paragallinarum* [4]. Recent reports have identified multidrug-resistant (MDR) strains of this pathogen, largely linked to the unregulated and frequent administration of antibiotics in poultry farms [5-8]. Such findings are particularly concerning, as they highlight the potential for horizontal transfer of resistance genes from commensal bacteria to pathogenic species involved in IC [9].

In the present study, the phenotypic and genotypic characteristics of antimicrobial resistance in *A. paragallinarum* isolates from Iran were comprehensively investigated for the first time. In addition, the relationship between antimicrobial susceptibility patterns and the presence of selected resistance genes was evaluated.

2. Materials and Methods

2.1 Bacterial culture

A total of 22 *A. paragallinarum* isolates were examined in this study. Isolates were obtained from poultry flocks across six provinces of Iran: Alborz, East Azerbaijan, Kermanshah, Mazandaran, Razavi Khorasan and Tehran. Birds exhibiting severe clinical signs of IC (e.g., marked facial swelling, dyspnea, or inability to eat or drink) were humanely euthanized by cervical dislocation prior to sample collection. Following euthanasia, sterile swabs were used to collect samples from the infraorbital sinus and choanal cleft. For bacteriological isolation, samples were streaked onto defibrinated horse blood agar supplemented with 0.0025% nicotinamide adenine dinucleotide (NAD) or co-cultured with *Staphylococcus epidermidis* as a source of NAD.

Presumptive identification of *A. paragallinarum* was based on colony morphology, Gram staining, and biochemical characterization, including catalase and oxidase reactions. When required, carbohydrate fermentation profiles and species-specific polymerase chain reaction (PCR) assays were employed for confirmatory identification, following previously established protocols [10].

2.2 Antimicrobial susceptibility testing

Because *A. paragallinarum* is a fastidious organism and no specific antimicrobial susceptibility testing (AST) standards are available for this species in either the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST), susceptibility testing was performed according to general CLSI (2023) guidelines. Columbia blood agar (CBA) containing 7% defibrinated horse blood and 0.0025% NAD was used for bacterial cultivation.

Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method using 23 antibiotic discs (PadtanTeb, Tehran, Iran) commonly used in veterinary and human medicine: ampicillin, amoxicillin, cephalixin, ceftriaxone, ceftiofur, difloxacin, doxycycline, danofloxacin, erythromycin, florfenicol, flumequine, fosfomycin, gentamicin, linco-spectin, neomycin, enrofloxacin, ofloxacin, penicillin, streptomycin, Trimethoprim-Sulfamethoxazole, oxytetracycline, and tiamulin.

Fifty microliters of a fresh culture suspension in brain heart infusion (BHI) broth supplemented with 0.0025% NAD, adjusted to a 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL), were evenly spread onto each CBA plate. The reference strain *Escherichia coli* ATCC 25922 was included as a quality control organism. Plates were incubated at 37 °C for 18-24 hours under 5-8% CO₂, and inhibition zone diameters were measured with a caliper in millimeters.

Resistance and susceptibility were interpreted using criteria reported in previous studies, applying *Haemophilus* spp. breakpoints for antibiotics not listed by CLSI or EUCAST. For linco-spectin and flumequine, no specific interpretive data were available, and ciprofloxacin criteria were used for danofloxacin due to its similar chemical structure. Isolates exhibiting resistance to three or more antimicrobial classes were classified as MDR.

2.3 Genomic tracking of antimicrobial resistance

2.3.1 DNA extraction

DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's guidelines. The extracted DNA was stored at -20°C.

2.3.2 PCR amplification

PCR amplification was performed in a 25 µL reaction mixture containing 0.4 µM of each forward and reverse primer (sequences in Table 1), 400 µM dNTP mix (SinaClon, Tehran, Iran), 2 mM MgCl₂, and 1.25 U Taq DNA polymerase (SinaClon, Tehran, Iran). The thermal cycling program included an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 52-69 °C for 1 minute (specific temperatures for each primer set are detailed in Table 1), and extension at 72 °C for 2 minutes, with a final extension at 72 °C for 10 minutes. Amplified products were analyzed on a 1% agarose gel and visualized using a UV transilluminator.

Table 1. Oligonucleotide sequences of primers* used for the detection of genes associated with resistance to tetracycline, streptomycin, and sulfamethoxazole in *A. paragallinarum*.

Gene	Primers	5'→3'	PCR product size (bp)	Annealing Temperature (°C)
<i>tet(A)</i>	tetA-L tetA-R	GTAATTCTGAGCACTGTCGC CTGCCTGGACAACATTGCTT	956	65
<i>tet(B)</i>	tetB-L tetB-R	CTCAGTATTCCAAGCCTTTG ACTCCCCTGAGCTTGAGGGG	430	52
<i>tet(C)</i>	tetC-F tetC-R	CCTCTTGCGGGATATCGTCC GGTTGAAGGCTCTCAAGGGC	505	62
<i>tet(G)</i>	tetG-F tetG-R	GCAGCGAAAGCGTATTTGCG TCCGAAAGCTGTCCAAGCAT	680	62
<i>sul2</i>	sul2-F sul2-R	GCGCTCAAGGCAGATGGCATT GCGTTTGATACCGGCACCCGT	284	69
<i>strA</i>	strA-F strA-R	CCAATCGCAGATAGAAGGC CTTGGTGATAACGGCAATTC	548	55

*: The primers used in this study were selected from previously published studies.

2.4 Determination of minimum inhibitory concentration (MIC)

MIC values for tetracycline and streptomycin (Sigma-Aldrich, St. Louis, MO, USA) were determined following CLSI guidelines for *Haemophilus influenzae* and *Haemophilus parainfluenzae*.

Briefly, a fresh 16-hour culture of each isolate in BHI broth supplemented with 0.0025% NAD was adjusted to a 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL) and distributed into eight 1 mL tubes. A 512 mg/L solution of each antibiotic was added to the first tube, and serial two-fold dilutions were prepared across the remaining tubes, yielding concentrations ranging from 256 to 2 mg/L. *E. coli* ATCC 25922 was included as a quality control strain. Two tubes without antibiotics served as positive growth controls, while a tube containing antibiotic but no bacteria served as a negative control. Tubes were incubated at 37 °C for 18-24 hours in a shaking incubator. The MIC was recorded as the lowest antibiotic concentration that completely inhibited visible bacterial growth. MIC determinations for tetracycline and streptomycin were performed in duplicate to ensure reproducibility.

3. Results

3.1. Antimicrobial susceptibility of *A. paragallinarum*

The antibiotic susceptibility profiles of 22 *A. paragallinarum* isolates recovered from commercial and backyard poultry flocks are summarized in Table 2. The corresponding interpretation criteria (zone diameters in mm) and detailed information on the studied isolates are presented in Table 3 and Figure 1, respectively.

The highest AST resistance rates were observed against erythromycin (90.9%), enrofloxacin and ofloxacin (81.8%), danofloxacin, difloxacin, oxytetracycline, and tiamulin (72.7%), and flumequine (68.2%). In contrast, all isolates were fully susceptible to ceftriaxone (100%). Notably, intermediate susceptibility was observed for some antibiotics, including cephalixin (40.9%) and gentamicin (36.4%), indicating variable responses that may influence therapeutic choices.

MDR analysis (Table 2, and Figure 1) demonstrated that isolates AP-9, AP-2, and AP-3 were resistance to 17, 16, and 15 antibiotics, respectively. Overall, 20 of the 22 isolates (90.9%) exhibited resistance to three or more antimicrobial classes, indicating a widespread MDR pattern among *A. paragallinarum* isolates.

Table 2. MDR profiles, antimicrobial resistance genes (ARGs), and MICs of streptomycin and tetracycline in 22 *A. paragallinarum* isolates.

¹ STR, streptomycin.

² TET, tetracycline.

³ A dash (—) indicates susceptibility of the isolate to the tested antibiotics.

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Isolate ID	Date of Isolation	Location	Flocks	MIC (µg/mL)		MDR group	Resistance genes					
				STR ¹	TET ²		<i>tet(A)</i>	<i>tet(B)</i>	<i>tet(C)</i>	<i>tet(G)</i>	<i>sul2</i>	<i>strA</i>
AP-1	2018	Razavi Khorasan-Mashhad	Commercial	128	32	— ³	×	✓	×	×	✓	×
AP-2	2018	Alborz-Karaj	Backyard	64	256	AM10, AMX25, CL10, DF10, D30, DFX5, E15, FM30, N30, NFX5, OFX5, P10, S10, SXT, T30, TM30	×	✓	×	×	✓	×
AP-3	2019	Tehran-Tehran	Backyard	64	32	AMX25, CL10, DF10, D30, DFX5, E15, FM30, FOS50, GM10, NFX5, OFX5, S10, SXT, T30, TM30	×	✓	×	×	×	✓
AP-4	2019	Tehran-Tehran	Backyard	128	256	AM10, AMX25, CL10, D30, E15, GM10, N30, P10, S10, SXT, T30, TM30	×	✓	×	×	✓	✓
AP-5	2019	Tehran-Tehran	Commercial	128	64	AMX25, CL10, D30, E15, N30, S10, SXT, TM30	×	✓	×	✓	✓	×
AP-6	2019	Tehran-Varamin	Commercial	256	4	AMX25, CL10, CEF30, DF10, DFX5, E15, FM30, N30, NFX5, OFX5, S10, SXT, TM30	×	✓	×	✓	✓	×
AP-7	2019	Tehran-Varamin	Commercial	256	128	AMX25, CL10, DF10, D30, DFX5, E15, FM30, NFX5, OFX5, S10, T30, TM30	×	✓	×	✓	✓	×
AP-8	2023	Alborz-Nazarabad	Commercial	32	512	AMX25, DF10, DFX5, E15, FM30, NFX5, OFX5, SXT	×	✓	×	✓	×	✓
AP-9	2023	Alborz-Shahriar	Commercial	64	128	AM10, AMX25, CL10, CN30, DF10, D30, DFX5, E15, FM30, LS, N30, NFX5, OFX5, S10, SXT, T30, TM30	×	✓	×	✓	✓	×
AP-10	2023	Tehran-Tehran	Backyard	256	32	CL10, DF10, DFX5, E15, FM30, NFX5, OFX5, S10, SXT, T30, TM30	×	✓	×	✓	✓	×
AP-11	2023	Tehran-Tehran	Backyard	4	8	CL10, DF10, D30, DFX5, E15, FM30, GM10, LS, NFX5, OFX5, S10, SXT, T30, TM30	×	✓	×	×	×	✓

AP-13	2023	Tehran-Tehran	Backyard	128	16	CL10, CEF30, DF10, E15, FM30, NFX5, OFX5, P10, S10, T30, TM30	✕	✓	✕	✓	✓	✕
AP-14	2023	Tehran-Tehran	Backyard	4	2	CN30, E15, LS, NFX5, OFX5, P10, TM30	✕	✓	✕	✕	✓	✕
AP-15	2023	Tehran-Tehran	Backyard	128	16	CEF30, D30, DFX5, E15, GM10, N30, NFX5, OFX5, S10, SXT, T30, TM30	✕	✓	✕	✓	✓	✕
AP-16	2024	Alborz-Karaj	Commercial	4	1	—	✕	✓	✕	✕	✓	✕
AP-17	2024	Kermanshah-Kermanshah	Backyard	64	8	DF10, DFX5, E15, FM30, FOS50, GM10, N30, NFX5, OFX5, T30	✕	✓	✕	✓	✓	✕
AP-18	2024	Tehran-Tehran	Backyard	64	128	DF10, D30, DFX5, E15, FM30, NFX5, OFX5, T30, TM30	✕	✓	✕	✓	✓	✕
AP-19	2024	East Azerbaijan-Marand	Backyard	64	16	CL10, DF10, D30, DFX5, E15, FM30, LS, NFX5, OFX5, T30, TM30	✕	✓	✕	✓	✓	✕
AP-20	2024	East Azerbaijan-Marand	Backyard	256	64	DF10, D30, DFX5, E15, LS, NFX5, OFX5, S10, T30, TM30	✕	✓	✕	✕	✓	✕
AP-22	2024	Tehran-Tehran	Commercial	128	64	CL10, DF10, DFX5, E15, FM30, GM10, NFX5, OFX5, S10, T30	✕	✓	✕	✓	✓	✕
AP-23	2024	East Azerbaijan-Marand	Backyard	256	64	DF10, DFX5, E15, FM30, FOS50, GM10, LS, NFX5, OFX5, P10, SXT, T30, TM30	✕	✓	✕	✓	✓	✕
AP-24	2024	Mazandaran-Amol	Backyard	64	32	DF10, DFX5, E15, FM30, NFX5, OFX5, T30	✕	✓	✕	✕	✓	✕

Table 3. Disk diffusion susceptibility results for 22 *A. paragallinarum* isolates.

Antibiotic	Interpretation criteria (zone diameter, mm)			Results ¹		
	S ² (≥)	I (range)	R (≤)	S (%)	I (%)	R (%)
Amoxicillin (AMX25) ³	≥ 18	14-17	≤ 13	45.45	18.18	36.4
Ampicillin (AM10) ⁴	≥ 15	12-14	≤ 11	68.18	18.18	13.6
Cephalexin (CN30) ³	≥ 21	18-20	≤ 17	50.00	40.91	9.1
Ceftiofur (CEF30) ³	≥ 23	20-22	≤ 19	59.09	27.27	13.6
Ceftriaxone (CRO30) ³	≥ 26	23-25	≤ 22	100.00	0.00	0.0
Colistin (CL10) ²	≥ 15	12-14	≤ 11	18.18	22.73	54.5
Danofloxacin (DFX5) ³	≥ 24	21-23	≤ 20	18.18	9.09	72.7
Difloxacin (DF10) ³	≥ 24	21-23	≤ 20	27.27	0.00	72.7
Doxycycline (D30) ³	≥ 19	16-18	≤ 15	27.27	22.73	50.0
Enrofloxacin (NFX5) ³	≥ 24	21-23	≤ 20	18.18	0.00	81.8
Erythromycin (E15) ⁴	≥ 23	20-22	≤ 19	9.09	0.00	90.9
Florfenicol (FF30) ³	≥ 25	22-24	≤ 21	95.45	4.55	0.0
Flumequine (FM30) ²	≥ 20	17-19	≤ 16	27.27	4.55	68.2
Fosfomycin (FOS50) ^{3, 4}	≥ 24	21-23	≤ 20	86.36	0.00	13.6
Gentamicin (GM10) ^{3, 4}	≥ 20	17-19	≤ 16	31.82	36.36	31.8
Linco-Spectin (LS) ²	≥ 21	18-20	≤ 17	59.09	13.64	27.3
Neomycin (N30) ³	≥ 19	16-18	≤ 15	50.00	18.18	31.8
Ofloxacin (OFX5) ³	≥ 25	22-24	≤ 21	13.64	4.55	81.8
Oxytetracycline (T30) ³	≥ 19	16-18	≤ 15	18.18	9.09	72.7
Penicillin (P10) ³	≥ 14	11-13	≤ 10	63.64	13.64	22.7
Streptomycin (S10) ^{3, 4}	≥ 15	12-14	≤ 11	22.73	18.18	59.1
Tiamulin (TM30) ²	≥ 22	19-21	≤ 18	0.00	27.27	72.7
Trimethoprim–Sulfamethoxazole (SXT) ³	≥ 16	13-15	≤ 12	27.27	22.73	50.0

¹ Results are presented as the percentage of isolates classified as susceptible, intermediate, or resistant.

² Abbreviations: S, susceptible; I, intermediate; R, resistant. Interpretation criteria were based on CLSI M100 (2023) and VET01S-ED7 (2024). Because specific breakpoints for *Avibacterium paragallinarum* are not available, interpretive breakpoints established for *Haemophilus* and *Pasteurella* spp. were applied, as appropriate

³ Criteria adapted from the PadtanTeb brochure (based on CLSI 2023 guidelines for *H. influenzae* and *H. parainfluenzae*).

⁴ Criteria adapted from Luna-Galaz et al. (2016).

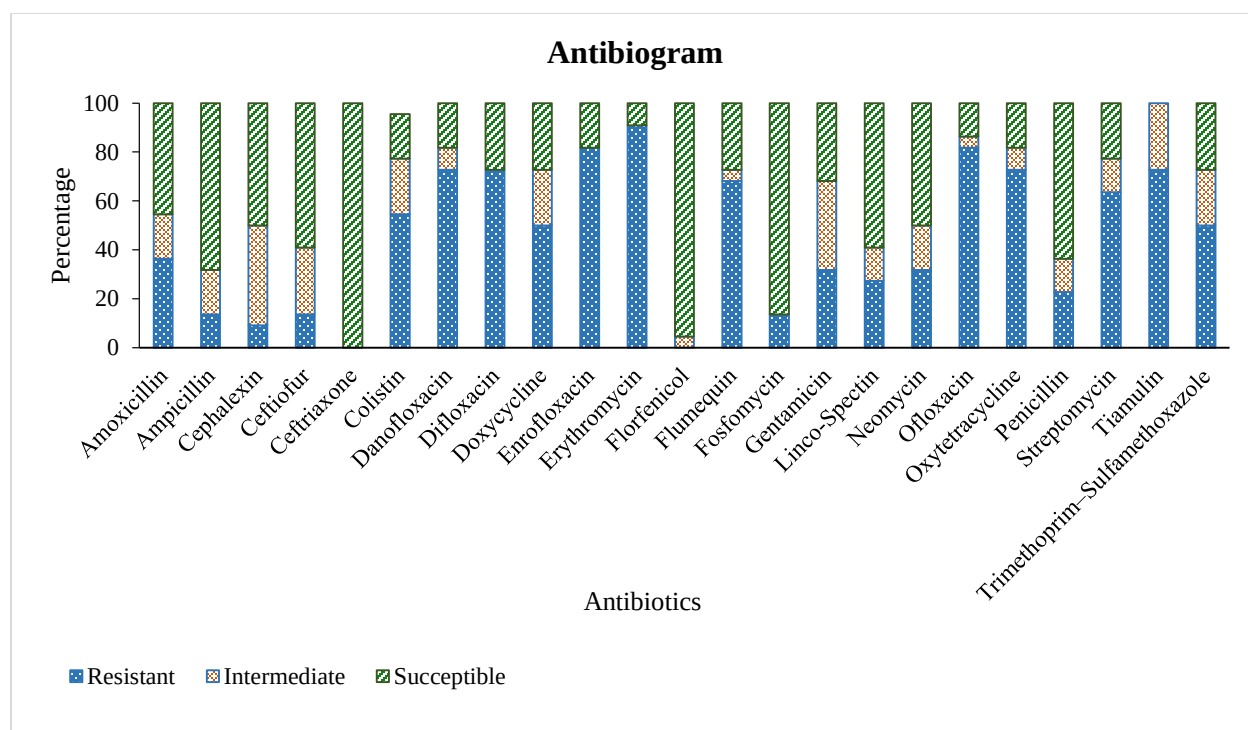


Figure 1. Antibiotic resistance patterns of 22 *A. paragallinarum* isolates determined by disk diffusion.

3.2. MIC

MIC testing for tetracycline and streptomycin confirmed the resistance patterns observed in disk diffusion (Table 2). For tetracycline, more than 86.36% of the isolates exhibited MICs $\geq 8 \mu\text{g/mL}$, with 77.3% showing MICs $\geq 16 \mu\text{g/mL}$; only isolates AP-6, AP-14, and AP-16 remained fully susceptible. Streptomycin resistance was similarly widespread, with 81.8% of isolates demonstrating MICs $\geq 64 \mu\text{g/mL}$, whereas only AP-11, AP-14, and AP-16 were susceptible. Overall, MIC values clustered predominantly between 64 and 128 $\mu\text{g/mL}$, underscoring the high prevalence of resistance.

3.3. Genomic tracking of antimicrobial resistance

The majority of the 22 *A. paragallinarum* isolates harbored at least one resistance-associated gene, particularly for tetracycline. A consistent correlation between phenotypic and genotypic resistance to tetracycline was observed, aligning with the AST results. However, the *tet(B)* gene was identified in four isolates (AP-1, AP-6, AP-14, AP-16) that did not exhibit phenotypic resistance

to tetracycline. Among tetracycline resistance genes, *tet(B)* and *tet(G)* were the most prevalent detected in 100% and 59% of isolates, respectively, while *tet(A)* and *tet(C)* were absent.

The *sul2* gene was absent in only three isolates (AP-3, AP-8, and AP-11), resulting in a prevalence of 86%. In contrast, *strA* was present in only 18% of isolates, despite over 59% showing phenotypic streptomycin resistance. This discrepancy highlights a notable gap between genotypic determinants of resistance and phenotypic resistance. A comprehensive summary of resistance gene detection for each isolate is presented in Table 2.

4. Discussion

IC significantly affects egg production and growth performance in poultry, resulting in substantial economic losses for producers. Vaccination and antimicrobial therapy are commonly used for disease control [1]; however, increasing reports of AMR in *A. paragallinarum* emphasize the need for regional surveillance. Data regarding the antimicrobial susceptibility and resistance patterns of *A. paragallinarum* in Iran is limited. Therefore, understanding the prevalence and dissemination of AMR is essential for managing the growing resistance crisis and requires comprehensive surveillance of bacterial resistance patterns.

Phenotypic antimicrobial susceptibility testing demonstrated that 90.9% of the isolates in the present study exhibited MDR, consistent with previous reports [5, 7, 8, 11]. A major contributor to MDR is the extensive use of antimicrobials in poultry production systems [5, 9]. These agents are frequently administered not only for treatment of bacterial infections, but also for prevention of secondary bacterial infections during viral outbreaks and as prophylactic additives in feed. Therapeutic antimicrobial use has been shown to facilitate the dissemination of plasmids carrying multiple resistance genes [9]. The increasing global prevalence of MDR among human and veterinary pathogens is another major concern due to the limited development of new antimicrobial agents and the continuous emergence of resistance mechanisms. In addition, resistance patterns vary considerably among geographic regions and antimicrobial classes [4]. In this study, evaluating 22 *A. paragallinarum* isolates against 23 antibiotics revealed phenotypic resistance to erythromycin, enrofloxacin, and ofloxacin, and susceptibility to ceftriaxone and florfenicol, largely consistent with earlier studies [2]. Notably, in contrast to previous reports of high

ampicillin resistance, 68.2% of current isolates were susceptible, potentially due to the larger sample size in this study.

Evidence from different geographic regions and poultry production systems shows considerable variation in the antimicrobial susceptibility profile of *A. paragallinarum*. A review of phenotypic resistance in 143 isolates from Asia (India, Thailand, Indonesia, Taiwan), Africa (Uganda), and the Americas (Mexico, Ecuador, Peru, Panama) reported resistance rates of 47.6% for trimethoprim–Sulfamethoxazole, 77.4% for neomycin, 72.7% for streptomycin, 77.8% for erythromycin, and 38.9% for ampicillin. These resistance rates were comparable to those observed in the present study for trimethoprim–Sulfamethoxazole; however, notable differences were identified for neomycin, streptomycin, erythromycin, and ampicillin, further supporting regional variability in antimicrobial resistance patterns. Additionally, Latin American studies report lower resistance to co-trimoxazole, highlighting the geographic diversity of antimicrobial susceptibility profiles in *A. paragallinarum* [4].

Cao et al. (2024) tested 21 isolates using the disk diffusion method and reported very high resistance rates to ampicillin (95.2%), streptomycin (95.2%), sulfamethoxazole (90.2%), and tetracycline (85.7%) [5]. These findings differ substantially from those observed in the present study, especially for ampicillin, for which only 13.6% resistance was detected. While tetracyclines have traditionally been recommended for the treatment of IC, the increasing prevalence of resistance suggests that their therapeutic efficacy should be reevaluated [4, 5]. Heuvelink et al. (2018), who analyzed 44 isolates from 25 outbreaks in the Netherlands, found complete susceptibility to ampicillin, consistent with the relatively high susceptibility observed in the present study (66.7%). However, they also reported considerable resistance to tetracycline, sulfamethoxazole, and erythromycin [6].

In the present study, the highest susceptibility rates were observed for ceftriaxone (100%), florfenicol (95.4%), and fosfomycin (86.4%). In 2024, seven *A. paragallinarum* strains isolated from broilers co-infected with *Ornithobacterium rhinotracheale* in the Netherlands also showed complete susceptibility to florfenicol, consistent with our findings [11]. Proposed mechanisms for shared resistance or susceptibility patterns include horizontal gene transfer among bacterial species [9]. Nsengimana et al. (2022) reported a fatal case in a wild piscivorous bird co-infected with

Klebsiella pneumoniae and *A. paragallinarum*, where resistant strains persisted despite enrofloxacin treatment at 10 mg/kg [12]. Notably, Gayatri (2010) found all six Indian isolates tested were 100% susceptible to enrofloxacin, chloramphenicol, ampicillin, and kanamycin [13].

Due to the lack of standardized consensus regarding primer selection for detection of ARGs associated with different resistance mechanisms, primers from highly reliable published sources were used in this study. We identified the *strA* gene in four isolates, while one isolate (AP-4) simultaneously carried both *sul2* and *strA*. The *sul* genes (*sul1*, *sul2*, and *sul3*) confer sulfonamide resistance by encoding an alternative dihydropteroate synthase that bypasses inhibition of folate synthesis, while the *strA* gene encodes an aminoglycoside phosphotransferase that inactivates streptomycin through enzymatic modification [14, 15]. These resistance determinants are highly transferable via plasmids. Our findings demonstrate the genotypic presence of MDR in *A. paragallinarum*, consistent with previous reports in the family *Pasteurellaceae* [5, 16]. Similar findings have been reported in Ugandan isolates carrying *strA*, *blaTEM*, *tet(C/A)*, and *sul2* [16]. Likewise, characterization of plasmids pYMH5 and pA14 from Taiwanese *A. paragallinarum* isolates identified *sul2*, *strA*, *mbeCy*, and *aphA1* [17].

The discrepancy between phenotypic tetracycline resistance (72%) and genotypic detection of tetracycline resistance genes (100%) may be attributable to the presence of resistance genes in isolates exhibiting intermediate or susceptible phenotypes, variable expression of resistance determinants, or methodological limitations associated with bacterial cultivation and susceptibility testing [18, 19]. Similarly, the difference between phenotypic streptomycin resistance (59.1%) and genotypic detection of *strA* (18%) suggests the possible involvement of additional aminoglycoside resistance determinants that were not investigated in the present study.

Previous studies have demonstrated variable distributions of tetracycline resistance genes among bacterial species. Momtaz et al. (2012) found *tet(B)* (52%) as the predominant tetracycline resistance genes among bacterial chicken *E. coli* isolates, whereas *tet(A)* was not detected [20]. Similarly, Sheikhsaran et al. (2018) reported frequencies of *tet(D)* (4.4%), *tet(A)* (14.4%), *tet(B)* (18.4%), and *tet(C)* (2%) among clinical Enterobacteriaceae isolates [21].

The *tet(A)* and *tet(B)* genes encode efflux pumps that represent common tetracycline resistance mechanisms in Gram-negative bacteria and are widely distributed among bacterial genera, including *A. paragallinarum* [22]. The high prevalence of *tet(B)* observed in the present study is consistent with previous reports describing tetracycline resistance determinants in *A. paragallinarum* and other members of the family *Pasteurellaceae* [16, 17].

Given the long-term use of aminoglycosides and tetracyclines for treatment of IC [7], the strong agreement between elevated MIC values and the genotypic detection of *tet(B)* and *tet(G)* suggests tetracyclines may have limited therapeutic value against IC in the studied region. Nevertheless, tetracyclines may remain useful in regions where local isolates continue to demonstrate phenotypic susceptibility [8, 23].

High levels of tetracycline resistance have been reported in *A. paragallinarum*, with Guo et al. (2022) reporting that 85% of Chinese isolates collected during 2019–2022 exhibited tetracycline MIC values ≥ 64 $\mu\text{g/mL}$ [5, 7]. In comparison, 77.3% of the isolates in this study showed MIC values ≥ 16 $\mu\text{g/mL}$. A complete phenotypic-genotypic concordance was observed for streptomycin resistance, suggesting reduced clinical utility of this antimicrobial agent for IC treatment. MIC determination is considered the reference method for antimicrobial susceptibility testing; however, its clinical interpretation should account for differences between *in vitro* susceptibility and *in vivo* therapeutic efficacy [19].

A major challenge in the prevention and treatment of IC is the absence of standardized MIC testing protocols and clinical breakpoints for *A. paragallinarum*. Experimental variables, including inoculum density, culture medium composition, and incubation conditions, can substantially influence MIC results and subsequent antimicrobial selection [19, 24]. Furthermore, the limited number of available studies investigating MIC values for *A. paragallinarum* have employed heterogeneous methodologies [6, 8, 25]. Consequently, veterinarians should prioritize therapeutic decisions based on local antimicrobial resistance patterns, clinical experience, and regional surveillance data.

Information on antimicrobial resistance mechanisms in *A. paragallinarum* remains limited. A study indicated that outer membrane vesicles (OMVs) transiently mediate transfer of ARGs under

antimicrobial exposure, including chloramphenicol, erythromycin, tetracycline, and streptomycin; however, this transfer does not appear to represent a stable genetic trait, and repeated subculturing may reduce OMV production [9]. This phenomenon may contribute to variations in antimicrobial susceptibility observed among repeatedly cultured laboratory isolates.

5. Conclusion

In summary, resistance to various antibiotics, including aminoglycosides, sulfonamides, and tetracyclines, was frequently observed among the isolates. The findings of the present study indicate a very high prevalence of tetracycline resistance, suggesting that its use in treating IC may be ineffective. Ceftriaxone, a third-generation cephalosporin, was identified as the most effective antimicrobial drug in this study. These findings highlight the necessity for region-specific antimicrobial guidelines and ongoing surveillance to maintain the efficacy of the remaining treatment options.

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Authors' Contribution

Conceptualization, Methodology, and Supervision: AN, MB, SGH, and ACH.

Formal analysis and investigation: All authors.

Drafting of the manuscript: AN, MB, and LMH.

Writing - review and editing: All authors.

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Conflict of Interest

The authors declare that they have no known conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Preprint

References

- [1] Bashashati M, Moradi Haghighi L, Nouri A, Hassanzadeh M, Abdoshah M, Sabouri F. Genetic diversity of *Avibacterium paragallinarum*: uncovering novel genotypes in Iranian poultry farms. *Avian Pathol.* 2026;55(2):161-70. [<https://10.1080/03079457.2025.2584352>].
- [2] Peighambari SM, Nouri A, Barzegari R, Ghorbani A. Isolation and Molecular Identification of *Avibacterium paragallinarum* Isolated from Commercial Layer and Backyard Chickens in Iran. *Iran J Vet Med.* 2022;16(3). [<https://10.22059/IJVM.2022.335367.1005216>].
- [3] Poorshamohammad C, Hassanzadeh M, Nouri A, Bozorgmehrifard MH, Abdoshah M, Sabouri F, et al. Molecular Identification of *Avibacterium paragallinarum* in Backyard and Commercial Layer Chickens Isolated in Iran. *Vet Res Forum.* forthcoming;17 (7):489-96. [<https://10.30466/vrf.2025.2064747.4815>].
- [4] Nhung NT, Chansiripornchai N, Carrique-Mas JJ. Antimicrobial resistance in bacterial poultry pathogens: a review. *Front Vet Sci.* 2017;4:126. [<https://10.3389/fvets.2017.00126>].
- [5] Cao X, Huang X, Lin Y, Sun J, Liu P, Dong X, et al. Prevalence and genomic-based antimicrobial resistance analysis of *Avibacterium paragallinarum* isolates in Guangdong Province, China. *Poult Sci.* 2024;103(6):103751. [<https://10.1016/j.psj.2024.103751>].
- [6] Heuvelink A, Wiegel J, Kehrenberg C, Dijkman R, Soriano-Vargas E, Feberwee A. Antimicrobial susceptibility of *Avibacterium paragallinarum* isolates from outbreaks of infectious coryza in Dutch commercial poultry flocks, 2008–2017. *Vet Microbiol.* 2018;217:135-43. [<https://10.1016/j.vetmic.2018.03.008>].
- [7] Guo M, Chen X, Zhang H, Liu D, Wu Y, Zhang X. Isolation, serovar identification, and antimicrobial susceptibility of *Avibacterium paragallinarum* from chickens in China from 2019 to 2020. *Vet sci.* 2022;9(1):27. [<https://10.3390/vetsci9010027>].
- [8] Luna-Castrejón LP, Buter R, Pantoja-Núñez GI, Acuña-Yanes M, Ceballos-Valenzuela K, Talavera-Rojas M, et al. Identification, HPG2 sequence analysis, and antimicrobial susceptibility of *Avibacterium paragallinarum* isolates obtained from outbreaks of infectious coryza in commercial layers in Sonora State, Mexico. *Avian Dis.* 2021;65(1):95-101. [<https://10.1637/aviandiseases-D-20-00103>].
- [9] Xu J, Mei C, Zhi Y, Liang Z-X, Zhang X, Wang H-J. Comparative genomics analysis and outer membrane vesicle-mediated horizontal antibiotic-resistance gene transfer in *Avibacterium paragallinarum*. *Microbiol Spectr.* 2022;10(5):e01379-22. [<https://10.1128/spectrum.01379-22>].
- [10] Chen X, Mifflin J, Zhang P, Blackall P. Development and application of DNA probes and PCR tests for *Haemophilus paragallinarum*. *Avian Dis.* 1996;40 (2):398-407. [<https://doi.org/10.2307/1592238>].
- [11] Stępień-Pyśniak D, Dec M, Hauschild T, Kurska O, Marek A, Wilczyński J, et al. Case reports involving coinfection with *Avibacterium paragallinarum* and *Ornithobacterium rhinotracheale* in broiler chickens and *Avibacterium endocarditis* in broiler breeding hens in Poland. *Avian Pathol.* 2024;53(4):291-302. [<https://10.1637/11265-082015-ResNote.1>].
- [12] Nsengimana O, Habarugira G, Ojok L, Ruhagazi D, Kayitare A, Shyaka A, et al. Infectious coryza in a grey crowned crane (*Balearica regulorum*) recovered from captivity. *Vet Med Int.* 2022;8(2):822-6. [<https://10.1002/vms3.766>].
- [13] Gayatri R, Ashish R, Yadav MM. Antimicrobial sensitivity pattern of *Haemophilus paragallinarum* isolated from suspected cases of infectious coryza in poultry. *Vet World.* 2010;3(4):177-81.

- [14] Venkatesan M, Fruci M, Verellen LA, Skarina T, Mesa N, Flick R, et al. Molecular mechanism of plasmid-borne resistance to sulfonamide antibiotics. *Nat Commun.* 2023;14(1):4031. [<https://10.1038/s41467-023-39778-7>].
- [15] Sundin G, Bender C. Dissemination of the *strA-strB* streptomycin-resistance genes among commensal and pathogenic bacteria from humans, animals, and plants. *Mol Ecol Notes.* 1996;5(1):133-43. [<https://10.1111/j.1365-294x.1996.tb00299.x>].
- [16] Byarugaba D, Minga U, Gwakisa P, Katunguka-Rwakishaya E, Bisgaard M, Christensen H, et al. Demonstration of antibiotic resistance genes *strA*, *blaTEM*, *tetA*, *tetC* and *sul2* in *Avibacterium paragallinarum*. *Afr J Microbiol Res.* 2011;5(22):3624-7. [<https://10.5897/AJMR11.186>].
- [17] Hsu Y-M, Shieh HK, Chen W-H, Sun T-Y, Shiang J-H. Antimicrobial susceptibility, plasmid profiles and haemocin activities of *Avibacterium paragallinarum* strains. *Vet Microbiol.* 2007;124(3-4):209-18. [<https://10.1016/j.vetmic.2007.04.024>].
- [18] Yee R, Dien Bard J, Simner PJ. The genotype-to-phenotype dilemma: how should laboratories approach discordant susceptibility results? *J Clin Microbiol.* 2021;59(6):10.1128/jcm.00138-20. [<https://10.1128/JCM.00138-20>].
- [19] Kowalska-Krochmal B, Dudek-Wich. The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. *Pathogens.* 2021;10(2):165. [<https://10.3390/pathogens10020165>].
- [20] Momtaz H, Rahimi E, Moshkelani S. Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Vet Med (Praha).* 2012;57(4):193-7. [<https://10.17221/5916-VETMED>].
- [21] [Sheykhsaran E, Bannazadeh Baghi H, Soroush Barbaghi MH, Alizadeh N, Memar MY, Etemadi S, et al. The rate of resistance to tetracyclines and distribution of *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *tetJ* and *tetY* genes in *Enterobacteriaceae* isolated from Azerbaijan, Iran during 2017. *Physiol Pharmacol.* 2018;22\(3\):205-12.](#)
- [22] Grossman TH. Tetracycline antibiotics and resistance. *Cold Spring Harb Perspect Med.* 2016;6(4):a025387. [<https://10.1101/cshperspect.a025387>].
- [23] Luna-Galaz G, Morales-Erasto V, Peñuelas-Rivas C, Blackall P, Soriano-Vargas E. Antimicrobial sensitivity of *Avibacterium paragallinarum* isolates from four Latin American countries. *Avian Dis.* 2016;60(3):673-6. [<https://10.1637/11398-022616-ResNote.1>].
- [24] Butaye P, Devriese LA, Haesebrouck F. Effects of different test conditions on MICs of food animal growth-promoting antibacterial agents for enterococci. *J Clin Microbiol.* 1998;36(7):1907-11. [<https://10.1128/JCM.36.7.1907-1911.1998>].
- [25] Guo M, Liu D, Chen X, Wu Y, Zhang X. Pathogenicity and innate response to *Avibacterium paragallinarum* in chickens. *Poult Sci.* 2022;101(1):101523. [<https://10.1016/j.psj.2021.101523>].