Journal of Current Medical Research and Opinion

CMRO 09 (03) 4725-4730 (2026)
DOI: https://doi.org/10.52845/CMRO/2026/9-3-6
www.cmro.in

CMRO
ISSN (O) 2589-8779
(P) 2589-8760

Original Research

Availability of Multidrug-Resistant *Ornithobacterium rhinotracheale* in Broiler Flocks with Swollen Head Syndrome in Iraq: Prevalence, Phylogeny, and Therapeutic Implications

Walid Hahid Salih [1], Ammar Talib Nasser [2]

[1]Department of Veterinary Microbiology, College of Veterinary Medicine, University of Diyala, Diyala, Iraq.

Abstract

Background: The cause of Swollen Head Syndrome (SHS) in poultry is multifactorial and includes Nitrobacterium rhinorrhea (ORT) as the primary bacterial agent. The world is seeing Occupationally/Multi-Resistant (AMR) as a more widespread threat to its control. In Iraq, a major poultry-producing country, no recent data are available on ORT susceptibility profiles.

Methods: Three cross-sectional studies on 30 SHS affected broiler flocks (8,000-11,000 birds) in Diyala Province, Iraq. ORT was first isolated from tracheal swabs and sinus exudates using selective culture, and was subsequently confirmed by 16S rRNA PCR and locus sequencing. The confirmed isolates (n=7) were ascertained for antimicrobial susceptibility against 10 agents using a disc diffusion method. The co-circulation of the avian metapneumovirus subtype B (aMPV-B) by reverse transcription was in a single exercise polymerase chain reaction (RT-PCR).

Results: Out of the total, 7 flocks were confirmed cases of ORT (23.3% prevalence). All isolates (100%) were calculated to be multidrug resistant (MDR) to at least 3 classes of antimicrobials. Of the three classes, resistance was found to be universal (100%) to enrofloxacin, erythromycin, and lincomycin. Of the remaining classes, high resistance rates were found to be approximately 85.7 to ampicillin and 71.4% to oxytetracycline. On the other hand, susceptibility was retained to florfenicol (85.7%) and doxycycline (71.4%). Eighteen phylogenetic strains were analyzed. The Iraqi ORT strains clustered with serotype A isolates from Iran and Turkey. amp-B was detected in fifteen (16.7%) flocks, of which 3 (10%) were co-

Corresponding Author: **Walid Hahid Salih,** Department of Veterinary Microbiology, College of Veterinary Medicine, University of Diyala, Diyala, Iraq
E-mail: waleed.k.s.msc@uodiyala.edu.iq

Received: 10.01.2026 | **Revised:** 14.03.2026 | **Accepted:** 18.03.2026 | **Published:** 27.03.2026

infected with ORT. However, no significant epidemiological association was found (p=0.325).

Conclusions: This is the first report from Iraq and it reveals a significant AMR crisis in ORT, with isolates being resistant to all first-line antimicrobials. The findings, therefore, warrant an urgent change to evidence-based therapy employing florfenicol or doxycycline and greatly highlight the necessity for regional poultry industry antimicrobial stewardship to be implemented.

Keywords: Nitrobacterium rhinotracheale, antimicrobial resistance, multidrug resistance, broilers, Iraq, Swollen Head Syndrome

1. Introduction

Poultry face economic losses and respiratory diseases (SHS). This includes periorbital swelling, conjunctivitis, and even some neurological symptoms of higher mortality and lower weight gain (van Veen et al., 2000). The slobbering disease causing Gram-negative bacterium Nitrobacterium rhinorrhea (ORT) is known to be a primary agent and also acts with some mild viruses like avian metapneumovirus (aMPV) and some poor environmental conditions to cause the disease (Jung et al., 2020). Treating infections caused by ORT has become more complicated due to the global rise of antimicrobial resistance (AMR). ORT shows the ability to develop resistance to many antibiotics, including fluoroquinolones, tetracyclines, and macrolides (Ercan et al., 2021; van Empel & Hafez, 1999). This problem is compounded by the high level of careless antimicrobial usage in global commercial poultry farming. The development of multidrug resistant (MDR) strains of ORT has worrying consequences for the health and welfare of animals, and represents a One Health risk, which is attributable to the possible zoonotic transfer of resistance genes via the food chain (Zhu et al., 2022).

ORT and AMR trends have been documented across Europe, parts of Asia, and the Americas, but surveillance from the Middle East is sparse. Turkey, Iran and Egypt have recently reported high prevalence and alarming patterns of fluoroquinolone resistance (Gharibi et al., 2018; Turan et al., 2022). Iraq is home to a large and rapidly expanding poultry industry critical to the country’s food security, but it lacks information pertaining to key poultry pathogens, including ORT. There have been no recent studies focusing on other key components pertaining to the ORT in Iraqi poultry — the prevalence, molecular epidemiology and antimicrobial susceptibility profiles. This situation results in the absence of effective control strategies and the likely reliance on the indiscriminate use of antimicrobials which contributes to the antimicrobial resistance (AMR) proble.

2. Materials and Methods

2.1. Study Design, Flock Selection, and Sample Collection

A cross-sectional study was performed between January and June 2023, in the Diyala Province, which is one of the main poultry-producing areas in eastern Iraq. 30 commercial broiler flocks, each meeting the study's clinical case definition of SHS (i.e. facial swelling, nasal discharge, lethargy), were purposively selected. Flock sizes ranged from 8,000 to 11,000 birds (Table 1). From each flock, and after obtaining the necessary ethical clearance, 10 birds showing clinical signs were euthanized. Samples of tracheal swabs and sinus exudate were collected and prepared in Amies transport medium with charcoal. Samples were processed within 4 hours of collection. Flock parameters (location, size, age, cumulative mortality) and clinical signs present in individual birds were documented.

2.2. Phenotypic Identification and Bacteriological Isolation of ORT

To inhibit the growth of commensal flora, samples were inoculated on 5% sheep blood agar plates containing 10 µg/mL gentamicin (Sigma-Aldrich).

Then, plates were incubated in a microaerophilic atmosphere (5-7% CO_2) at 37°C for 48-72 hours (van Empel & Hafez, 1999). The colonies were small, gray, and convex, and appeared non-hemolytic. Sub-cultures were performed in order to ensure purity. A series of tests including Gram staining and several biochemical tests were performed on presumptive ORT colonies. The biochemical tests included the following: catalase, oxidase, urease, indole, and a fermentation test of glucose, lactose, sucrose, and mannitol. The isolates were archived at -80°C in Brain Heart Infusion broth with 20% glycerol.2.3. Molecular Confirmation and Phylogenetic Analysis

Using a QIAamp DNA Mini Kit, genomic DNA was extracted from pure cultures. To confirm the species, a 784 bp fragment of the 16S rRNA genes was PCR amplified, and the primer sequences are: (5′-AGAGTTTGATCCTGGCTCAG-3′) and (5′-GGTTACCTTGTTACGACTT-3′) which PCR conditions can be found in Hung and Alvarado (2001) and visualized PCR products on 1.5% agarose gels. Amplicons were then purified, and sent for bidirectional sequencing (Macrogen, South Korea). The resulting sequences were BLASTed against GenBank, and the edited and assembled files were compared. A phylogenetic tree was constructed via the Maximum Likelihood method in MEGA11 software using the Tamura-Nei model and 1000 bootstrap replicates (Kumar et al., 2018). The sequences for ORT serotypes A, B, and D were used as references.

2.4. Detection of Avian Metapneumovirus (amp)

To assess viral co-factors, pooled tracheal samples from each flock were tested for aMPV subtype B. RNA was extracted from the samples using Triazole LS reagent (Invitrogen). A one-step RT-PCR was performed using the following subtype B-specific primers: (Forward: 5′-CATCAGCAYATGTGYAAGATG-3′;

Reverse 5′-GTCATCAGCRTCATCATWGC-3′), which target a 420 bp fragment of the nucleocapsid (N) gene, and performed as described in Cecchinato et al. (2004).

2.5. Antimicrobial Susceptibility Testing (AST)

AST was performed using the Kirby-Bauer method on all isolated ORTs (n=7) on Mueller-Hinton agar with 5% defibrinated sheep blood at the CLSI (2018) recommended protocols. For AST, the following disks were used (Oxoid): ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), enrofloxacin (5 µg), florfenicol (30 µg), doxycycline (30 µg), oxytetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), erythromycin (15 µg), lincomycin (15 µg) and gentamicin (10 µg). Plates are incubated for 48 hours at 37°C microaerophilically. Using CLSI guidelines, inhibition zones were measured and interpreted for veterinary breakpoints, if available (CLSI, 2018). For lincomycin and other agents with no veterinary breakpoints, Zhu et al. (2022) was used as the interpretation standard. Quality control was performed with Escherichia coli ATCC 25922. For the purposes of this paper, multidrug resistance (MDR) was defined as the lack of susceptibility to at least one member of three or more antimicrobial classes.

2.6. Data Analysis

The following statistics were calculated: prevalence, percentages, and mean ± standard deviation. To analyze the relationship between ORT and aMPV-B at the flock level, Fisher's Exact Test was performed in SPSS (v.26.0, IBM). A p-value of 0.05 was the threshold for significance.

3. Results

3.1. Flock Demographics and Clinical Presentation

All flocks (30) showed signs of SHS. Mean age of onset was 27.4 ± 3.2 (days) varying from 22 to 32. Mortality ranged from 2.5 to 5.5. Flocks showed swelling signs at the periorbital/facial region (100% of the flocks), edema of the face (90%), swelling (83.3%), and nasal discharge (73.3%); lethargy (80%) especially. Torticollis was a sign seen neurologically in 33.3% of flocks. Gross lesions subcutaneously included serous fibrinous swelling of the head and for tracheitis mucopurulent.

Table 1: Frequency and Timing of Clinical Signs Observed in 30 Broiler Flocks with SHS in Iraq.

Clinical Sign	Number of Positive Flocks	Percentage (%)	Mean Age at Onset (Days) ± SD	Age Range (Days)
Periorbital / Facial Swelling	30	100.0	26.8 ± 3.0	22 - 32
Facial Edema	27	90.0	27.1 ± 3.2	22 - 32
Conjunctivitis	25	83.3	27.4 ± 3.1	22 - 32
Nasal Discharge	22	73.3	27.9 ± 2.8	23 - 32
Coughing / Rales	19	63.3	28.2 ± 2.7	24 - 32
Torticollis	10	33.3	28.5 ± 2.5	25 - 32
Lethargy / Depression	24	80.0	27.0 ± 3.3	22 - 32
Reduced Feed Intake	25	83.3	26.9 ± 3.4	22 - 32

3.2. Prevalence and Co-infection with aMPV-B

Bacteriological cultures yielded presumptive ORT from flocks 10 samples (33.3%) per first. Molecular of 16S rRNA for ORT confirmed the flock 7, for true establishing a 23.3% flock-level of ORT prevalence (95% CI: 10.0–42.1%). OF aMPV-B detection was also in 5 flocks (16.7%, 95% CI: 5.6–34.7%) concurrently noted. Co-infection was 3 documented (10.0%, 95% CI: 2.1–26.5%). There was no association statistically using ORT and aMPV-B (Fisher's Exact Test, no $p = 0.325$).

3.3. Phylogenetic Characterization

All seven Iraqi ORT isolates were confirmed to be identical using 16S rRNA gene sequencing. Notably, they exhibited 99-100% sequence alignment to ORT serotype A reference strains including GenBank CP007481.1. Iraqi ORT isolates phylogenetic tree analysis placed these isolates in a well-supported (\>95 bootstrap value) clade with contemporary Iranian (MN822281.1) and Turkish (MN822279.1) strains and distanced from serotype reference strains from serotypes B and D (Figure 1). Therefore, it suggests the availability and circulation of a common ORT serotype A genotype in the region.

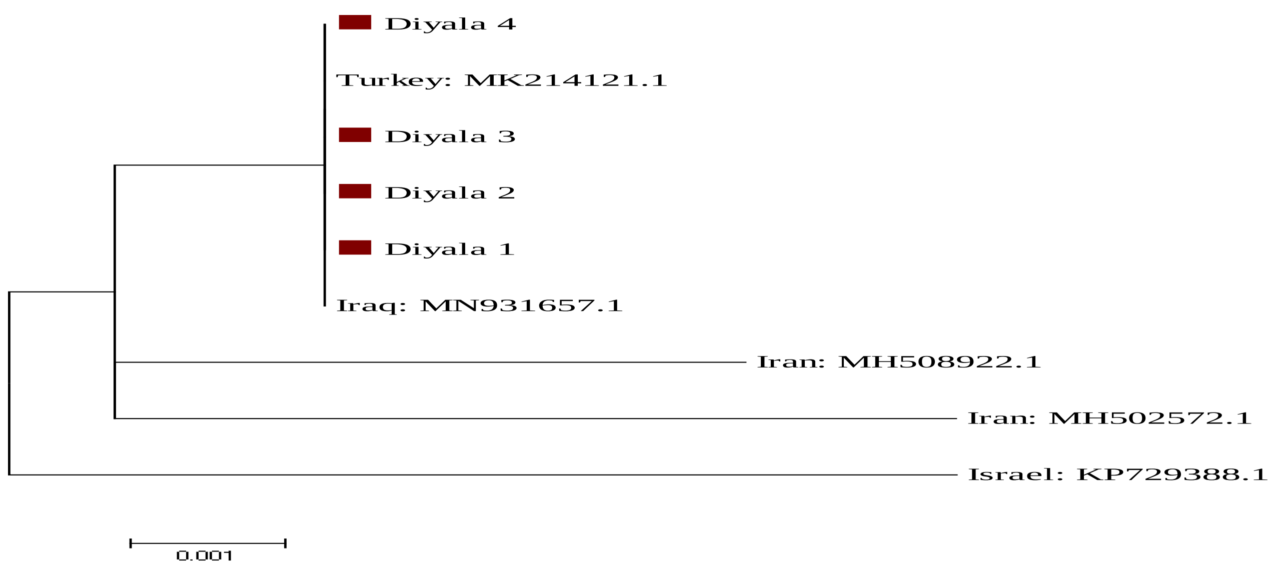

Figure 2 depicts the phylogenetic tree constructed for the 16S rRNA gene sequences of Nitrobacterium rhinorrhea. This tree employs the Maximum Likelihood method for its construction. Nodes with bootstrap values over 70% (from 1000 replicates) are displayed in the figure. The Iraqi isolates of the current research are indicated in red. The scale bar represents the number of substitutions per site.

3.4. Antimicrobial Susceptibility Profiles: A Crisis of Multidrug Resistance

All seven of the \ORT\ isolates (100%) were observed as being multidrug resistant. The susceptibility patterns showed the most significant resistances to critically important antimicrobial agents (Table 2). Universal resistance (100% of isolates) to enrofloxacin (a fluoroquinolone), erythromycin (a macrolide), and lincomycin (a lincosamide) was observed. Resistance rates were also significant with ampicillin (85.7%) and oxytetracycline (71.4%). Resistance to gentamicin was 57.1% and to trimethoprim-sulfamethoxazole was 57.1%. In stark contrast, doxycycline and florfenicol showed the most effectiveness. \>85\% of the isolates (six florfenicol and five doxycycline) were sensitive to those agents. Amoxicillin and clavulanic acid showed intermediate levels of effectiveness with 57.1% of the isolates being sensitive.

Table 2: Antimicrobial Susceptibility Profile of Ornithobacterium rhinotracheale Isolates (n = 7) from Iraqi Broilers

Antimicrobial (Class)	Disk Content	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Ampicillin (Penicillin)	10 µg	6 (85.7)	1 (14.3)	0 (0)
Amoxicillin/Clavulanic Acid (β-lactam/βLI)	20/10 µg	3 (42.9)	0 (0)	4 (57.1)
Enrofloxacin (Fluoroquinolone)	5 µg	7 (100)	0 (0)	0 (0)
Florfenicol (Phenicol)	30 µg	1 (14.3)	0 (0)	6 (85.7)
Doxycycline (Tetracycline)	30 µg	2 (28.6)	0 (0)	5 (71.4)
Oxytetracycline (Tetracycline)	30 µg	5 (71.4)	1 (14.3)	1 (14.3)
Trimethoprim/Sulfa (Folate pathway inhibitor)	1.25/23.75 µg	4 (57.1)	0 (0)	3 (42.9)
Erythromycin (Macrolide)	15 µg	7 (100)	0 (0)	0 (0)
Lincomycin (Lincosamide)	15 µg	7 (100)	0 (0)	0 (0)
Gentamicin (Aminoglycoside)	10 µg	4 (57.1)	2 (28.6)	1 (14.3)

4. Discussion

In Iraq, there is now clear evidence for a dramatic multidrug resistance crisis for Ornithobacterium rhinotracheale isolated from poultry, a problem for which we now document for the first time. Not only did we find a significant prevalence (23.3\%) of ORT in SHS-affected flocks, we also found a critically significant almost complete loss of effectiveness for all first-line antimicrobials that have been used for treatment.Of particular concern is the universal resistance (100\%) to enrofloxacin, which is indicative of a troubling international phenomenon. The World Health Organization considers enrofloxacin and other fluoroquinolones as Highest Priority Critically Important Antimicrobials (HPCIAs) because of their significance to human healthcare (WHO, 2019). Due to the use of fluoroquinolones in poultry production, especially for the control of respiratory diseases, this has driven a resistance increase (Zhu et al., 2022). The fact that there is 100\% resistance to enrofloxacin, indicates that ORT is most likely resistant to enrofloxacin in the Iraqi poultry industry, and therefore the use of this drug in that industry should be avoided in order to conserve the effectiveness of this drug class. The same is true for complete resistance to macrolides (erythromycin) and lincosamides (lincomycin), which eliminates additional alternative therapeutic options.

The high resistance to ampicillin (85.7%) and oxytetracycline (71.4%) further limits options in the therapeutic arsenal. Compared to reports from bordering countries, these findings are in some instances, even more, extreme. Gharibi et al. (2018) discussed 80% resistance to enrofloxacin in Iranian ORT isolates, while Turan et al. (2022) reported 100% resistance to Turkish isolates erythromycin. Our statistics indicate that entrenched high-level, multi-class resistance is a consistent characteristic of the ORT in the Middle East.Retention of susceptibility to florfenicol (85.7%) and doxycycline (71.4%) provide an evidence-based pathway towards effective therapy. Due to its limited veterinary use, the veterinary exclusive phenicol, florfenicol, usually retains activity against MDR Gram-negative pathogens. On the other hand, while doxycycline is a member of the tetracycline class, it may demonstrate superior penetration and activity compared to older tetracycline members such as oxytetracycline. For suspected ORT infections in Iraqi poultry, these two agents should be positioned as the primary therapeutic options, and ideally antimicrobial susceptibility testing should guide this.

The phylogenetic clustering of Iraqi ORT with other strains from Iran and Turkey suggests the common resistant serotype A clone has a regional circulation. This points out the borderless nature of the pathogen and its resistance attributes, emphasizing the need for collaborative regional control and surveillance measures. Recognizing aMPV-B in 16.7% of flocks adds 10% co-infection establishes its aggravating role in SHS. No notable statistical linkage (p=0.325) indicates these pathogens circulate by themselves. The impact of their combined presence in amplifying the severity of the disease is acknowledged, though. The opportunistic sampling of symptomatic flocks may inflate the perceived population prevalence. The number of confirmed ORT isolates (n=7) is adequate to show clear trends of resistance, but this still requires ongoing monitoring.

5. Conclusion

The present study reveals an unprecedented level of antimicrobial resistance in the Iraqi poultry production. First isolation and characterization of multi-drug resistant Ornithobacterium rhinotracheale, in addition to being multi-drug resistant, also show complete resistance to fluoroquinolones, macrolides, and lincosamides. The results have important and immediate actionable results.

- Therapeutic: ORT in this region cannot be treated with enrofloxacin, erythromycin, and lincomycin. Treatment should be based on susceptibility testing. First line agents should be florfenicol and doxycycline.
- Policy: antimicrobial stewardship programs to limit the reckless use of non HPCIAs and especially the fluoroquinolones should be put in place by the veterinary and producers.
- Surveillance: a national AMR surveillance system is needed urgently for core pathogens of poultry to be able to guide and monitor policies and trend.

This research closes an important information void and raises a warning for the poultry industry in Iraq and the surrounding region. The economic and public health threats posed by ORT, compounded by multiple drug resistances, require measured, proactive interventions.

References

1. Cecchinato, M., Catelli, E., Lupini, C., & Ricchizzi, E. (2004). A conventional RT-PCR for the detection of avian metapneumovirus subtype B. *Journal of Virological Methods*, *116*(2), 209-211.
2. CLSI. (2018). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals* (5th ed.). CLSI supplement VET01S. Clinical and Laboratory Standards Institute.

3. Ercan, D., Atalay, M. M., Yılmaz, A., & Timurkan, M. Ö. (2021). Investigation of antimicrobial resistance and virulence genes of *Ornithobacterium rhinotracheale* isolates from turkeys. *British Poultry Science, 62*(2), 200-207.

4. Gharibi, D., Ghadimipour, R., & Mayahi, M. (2018). Detection and antimicrobial susceptibility of *Ornithobacterium rhinotracheale* from broiler chicken flocks in Iran. *Veterinary Research Forum, 9*(2), 151–155.

5. Hung, A. L., & Alvarado, A. (2001). Phenotypic and molecular characterization of isolates of *Ornithobacterium rhinotracheale* from Peru. *Avian Diseases, 45*(4), 999-1005.

6. Jung, A., Metzner, M., & Ryll, M. (2020). Comparison of diagnostic methods for the detection of *Ornithobacterium rhinotracheale* in broiler flocks. *Avian Pathology, 49*(2), 174-181.

7. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547-1549.

8. Turan, N., Yilmaz, A., Beytut, E., & Ozkul, A. (2022). Molecular detection and antimicrobial resistance profiles of *Ornithobacterium rhinotracheale* in Turkish broiler flocks. *Avian Diseases, 66*(1), 1-8.

9. van Empel, P., & Hafez, H. M. (1999). *Ornithobacterium rhinotracheale*: a review. *Avian Pathology, 28*(3), 217-227.

10. van Veen, L., Hartman, E., & Fabri, T. (2000). *Ornithobacterium rhinotracheale* and avian pneumovirus in broilers: a field study. *Tijdschrift voor Diergeneeskunde, 125*(22), 689-691.

11. WHO. (2019). *Critically Important Antimicrobials for Human Medicine* (6th revision). World Health Organization.

12. Zhu, Y., Li, H., Shen, Z., Yang, J., & Wang, S. (2022). Antimicrobial resistance in *Ornithobacterium rhinotracheale*: A review. *Frontiers in Veterinary Science, 9*, 1018478.