

## Antibiotic Sensitivity of *Ornithobacterium rhinotracheale* Isolates Associated with Respiratory Diseases

### Short Communication

Banani<sup>\*1</sup>, M., Pourbakhsh, S.A.<sup>1</sup> and Deihim, A.H.<sup>2</sup>

1. Avian Diseases Research & Diagnosis Dept., Razi Vaccine & Serum Research Institute,  
P.O. Box 11365-1558, Tehran, Iran

2. Graduated student, Azad University, Garmsar, Iran

Received 26 Apr 2004; accepted 19 Nov 2004

### Summary

187 commercial chicken flocks affected with respiratory diseases were examined for *Ornithobacterium rhinotracheale* isolation. The bacterium was isolated from 105 (56.2%) poultry flocks. Drug sensitivity test using standard disk diffusion technique was performed with 19 antibiotics. All the isolates were susceptible to tiamulin and most of them were susceptible to chloramphenicol and linco-spectin. All the isolates were resistant to sulfamethoxazole-trimethoprim, colistin and neomycin and most of them were resistant to gentamicin, lincomycin, erythromycin, tetracycline, and enrofloxacin. One isolate from a native turkey was also tested. This isolate was resistant to sulfamethoxazole-trimethoprim, colistin, neomycin and gentamicin, but was sensitive to other tested antimicrobials. Because of acquired antibiotic resistance, and various result of antibiotic therapy, it must be stressed to prevent the infection.

**Key words:** *Ornithobacterium rhinotracheale*, poultry, drug sensitivity

### Introduction

Respiratory diseases account for high financial losses in the poultry industry due to the negative effects on the performance of the birds, increased mortality, drops in egg production and antibiotic expenses. *Ornithobacterium rhinotracheale* (*O.rhinotracheale*), named by Vandamme *et al* (1994), has recently been isolated in many countries of the world and has been incriminated as a possible additional

\* Author for correspondence. E-mail: m\_banani@rvsri.com

causative agent in the respiratory disease complex (van Empel & Hafez 1999, Joubert et al 1999, EL-Sukon et al 2002, Soriano et al 2002, Turan & AK 2002). *O.rhinotracheale* was isolated from a broiler and a pullet flock affected with respiratory disorders in Iran (Banani et al 2000). Our findings showed that Ornithobacteriosis is a newly known problem in our poultry industry and also *O.rhinotracheale* is a relatively common pathogen in respiratory cases. Antibiotic therapy failure in some cases of colibacillosis, and even some cases of apparently vaccination failure against respiratory viruses in our country might be attributed to ignore of this new emerging infection. Sensitivity of *O.rhinotracheale* to antibiotics is very inconsistent and appears to depend on the source of the strain (van Empel & Hafez 1999). In this study drug sensitivity of *O.rhinotracheale* isolates was examined *in vitro*.

### Materials and Methods

**Sample.** Between early-2001 and late-2002, live and dead birds of 187 broiler, layer and native chicken flocks and also 3 native turkey flocks with respiratory diseases and increased mortalities submitted to Razi Institute, Karaj were examined for routine diagnostic procedures. 105 *O.rhinotracheale* strains were isolated from tracheal swabs on blood agar with 5g/ml gentamicin after 24h of incubation at 37°C under microaerophilic condition (5% CO<sub>2</sub>). The identification procedure was discussed previously (Banani et al 2001).

**Identification of concomitant infections.** Para-clinical tests were carried out to identify the concomitant pathogens. Serum samples were examined for antibodies to Newcastle disease (ND) and avian influenza (AI) subtype H9N2 by the hemagglutination inhibition test, and to infectious bursal disease (IBD) and infectious bronchitis (IB) by the ELISA test. Isolation of viruses and isolation and identification of other bacterial infections were carried out by standard methods (Swayne et al 1998, Krieg et al 1984).



**Drug susceptibility test.** The test was performed as described by Bauer *et al* (1966). The following 19 antibiotic discs were obtained from Padtan teb Co., Iran and were applied by means of a dispenser: tiamulin (30µg), chloramphenicol (30µg), linco-spectin (15/200µg), tylosin (30µg), penicillin (10µg), ampicillin (10µg), bacitracin (10U), furazolidone (100µg), oxytetracycline (30µg), novobiocin (5µg), flumequine (30µg), enrofloxacin (5µg), tetracycline (30µg), erythromycin (15µg), lincomycin (2µg), gentamicin (10µg), neomycin (30µg), colistin (10U), sulfamethoxazole-trimethoprim (1.25/23.75µg).

### Results and Discussion

*O.rhinotracheale* was isolated from 105 out of 187 (56.2%) chicken flocks. The numbers and percentages of positive flocks in samples submitted from Tehran, Semnan, Mazandaran, Ghom, Khorasan, Esfahan and Kermansha provinces were 72 of 122 (59.0%), 12 of 31 (38.7%), 11 of 17 (64.7%), 5 of 8 (62.5%), 2 of 5 (40.0%), 2 of 3 (66.7%) and 1 of 1 (100%) respectively. In terms of clinical indications, gross and microscopic lesions, serology, virus and bacterial isolations, infected flocks by *O.rhinotracheale* were also diagnosed as having clinical disease associated with *Haemophilus paragallinarum*, *Escherichia coli*, *Pasteurella multocida*, NDV, IBV, IBDV, AIV subtype H9N2 and ILTV.

The results of antibiotic sensitivity tests are shown in Table 1. All the isolates were susceptible to tiamulin, and most of them were completely susceptible to chloramphenicol and linco-spectin. All the isolates were resistant to sulfamethoxazole-trimethoprim, colistin, and neomycin, and most of them were completely resistant to gentamicin, lincomycin, erythromycin, tetracycline, and enrofloxacin.

Enrofloxacin is the most frequently used antibiotic in Iran, and in this report only 4.8% of the isolates were completely sensitive to enrofloxacin. In Germany 90% of strains were resistant to the antibiotic, while those isolated in France were almost

always very sensitive to this antibiotic. *O.rhinotracheale* isolates in the Netherlands were only slightly sensitive to enrofloxacin (van Empel & Hafez 1999).

Table 1. Antibiotic sensitivity test on 105 *O.rhinotracheale* isolates from commercial chicken flocks

Antibiotic	No. of sensitive (%)	No. of intermediate (%)	No. of resistant (%)
Tiamulin	105 (100)	0 (0)	0 (0)
Chloramphenicol	102 (97.1)	2 (1.9)	1 (1)
Linco-spectin	88 (83.8)	15 (14.3)	2 (1.9)
Bacitracin	39 (37.1)	59 (56.2)	7 (6.7)
Tylosin	38 (36.2)	51 (48.6)	16 (15.2)
Furazolidone	35 (33.3)	53 (50.5)	17 (16.2)
Ampicillin	18 (17.1)	62 (59.1)	25 (23.8)
Oxytetracycline	10 (9.5)	32 (30.5)	63 (60)
Novobiocin	10 (9.5)	20 (19.1)	75 (71.4)
Penicillin)	8 (7.6)	62 (59.1)	35 (33.3)
Flumequine	6 (5.7)	49 (46.7)	50 (47.6)
Enrofloxacin	5 (4.8)	39 (37.1)	61 (58.1)
Tetracycline	4 (3.8)	41 (39.1)	60 (57.1)
Erythromycin	4 (3.8)	18 (17.1)	83 (79.1)
Lincomycin	2 (1.9)	0 (0)	103 (98.1)
Gentamycin	0 (0)	1 (1)	104 (99)
Colistin	0 (0)	0 (0)	105 (100)
Neomycin	0 (0)	0 (0)	105 (100)
Sulfamethoxazole-trimethoprim	0 (0)	0 (0)	105 (100)

A literature review reveals that *O.rhinotracheale* isolates from different countries show different antibiotic susceptibility patterns. Strains isolated from the Netherlands were almost all resistant to flumequine, only slightly sensitive to trimethoprim-sulphonamide, but sensitive to tetracycline and ampicillin (van Empel & Hafez 1999), while in our study all the isolates were resistant to trimethoprim-sulphonamide and only 3.8% and 17.1% of them were sensitive to tetracycline and ampicillin respectively. 5.7% of our isolates were sensitive to flumequine. Hafez (1996) showed that 90 to 100% of the strains isolated from Germany were resistant



to neomycin, gentamicin and trimethoprim-sulphonamide and were sensitive to tetracycline, chloramphenicol. In France all strains investigated proved to be sensitive to spectinomycin and tylosin but resistant to gentamicin and colistin (Roger & Leorat 1997). All the strains tested in the USA were sensitive to ampicillin, erythromycin, penicillin, spectinomycin and tylosin (Nagaraja *et al* 1998). In Belgium all the strains were resistant to lincomycin and ampicillin. Less than 10% of the strains were sensitive to tylosin and flumequine. A few strains were sensitive to enrofloxacin and doxycycline and all the strains were sensitive to tiamulin (Devriese *et al* 2001). De Herdt *et al* (2001) remarked that none of the drugs currently used in the control of bacterial diseases of poultry are completely active against circulating *O.rhinotracheale* isolates but one exception is tiamulin. Its application is however not often an option because of its incompatibility with ionophores frequently used as coccidiostats. Because of acquired antibiotic resistance, and various results of antibiotic therapy, it must be stress to prevent the disease and vaccination (van Empel & van den Bosch 1998, De Herdt *et al* 2001).

One isolate from a native turkey was resistant to sulfamethoxazole-trimethoprim, colistin, neomycin, and gentamicin, but was completely sensitive to other tested antibiotics. This is the first report of *O.rhinotracheale* infection in a native turkey from Iran.

#### **Acknowledgement**

The authors wish to thank M.Mohammadtaheri, Dr. R.Momayez and Dr. S.Javadi for their technical assistance.

#### **References**

- Banani, M., Khaki, P., Goodarzi, H., Vandyousefi, J. and Pourbakhsh, S.A. (2000). Isolation and identification of *Ornithobacterium rhinotracheale* from a broiler and a pullet flock. *Pajouhesh & Sazandegi* 46:106-109 (in Persian).

- Banani, M., Pourbakhsh, S.A. and Khaki, P. (2001). Characterization of *Ornithobacterium rhinotracheale* isolates from commercial chickens. *Archives of Razi Institute* 52:27-36.
- Banani, M., Pourbakhsh, S.A., Moazeni Jula, G., Momayez, R. and Ezzi, A. (2002). Natural infection with *Ornithobacterium rhinotracheale* in commercial poultry and experimental infection in specific-pathogen-free chickens. *Pajouhesh & Sazandegi* 55:28-37 (in Persian).
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45:493-496.
- De Herdt, P., Cauwerts, K., Vervloesem, J., and Ducatelle, R. (2001). The relevance and efficacy of *Ornithobacterium rhinotracheale* control in chickens. *World Poultry* 10:32-33.
- Devriese, L.A., De Herdt, P., and Haesebrouck, F. (2001). Antibiotic sensitivity and resistance in *Ornithobacterium rhinotracheale* strains from Belgian broiler chickens. *Avian Pathology* 30:197-200.
- EL-Sukhon, S.N., Musa, A. and Al-Attar, M. (2002). Studies on the bacterial etiology of airsacculitis of broilers in northern and middle Jordan with special reference to *Escherichia coli*, *Ornithobacterium rhinotracheale*, and *Bordetella avium*. *Avian Diseases* 46:605-612.
- Hafez, H.M. (1996). Current status on the role of *Ornithobacterium rhinotracheale* (ORT) in respiratory disease complexes in poultry. *Archiv fur Geflugelkunde* 61:208-211.
- Joubert, P., Higgins, R., Laperle, A., Mikaelian, I., Venne, D. and Silim, A. (1999). Isolation of *Ornithobacterium rhinotracheale* from turkeys in Quebec, Canada. *Avian Diseases* 43:622-626.
- Nagaraja, K.V., Back, A., Sorenger, S., Rajashekara, G. and Halvorson, D. (2000). Tissue distribution post-infection and antimicrobial sensitivity of



- Ornithobacterium rhinotracheale*. Proceedings of the 47<sup>th</sup> Western Poultry Diseases Conference. Pp: 57-60.
- Roger, M-F., Leorat, J. (1997). A l'origine de troubles respiratoires chez la dinde: *Ornithobacterium rhinotracheale* est mieux maitrise. *Filiere Avicole Juin*. 1997:62-63.
- Soriano, V.E., Longinos, M.G., Navarrete, P.G. and Fernandez, R.P. (2002). Identification and characterization of *Ornithobacterium rhinotracheale* isolates from Mexico. *Avian Diseases* 46:686-690.
- Turan, N., AK, S. (2002). Investigation of the presence of *Ornithobacterium rhinotracheale* in chickens in Turkey and determination of the seroprevalence of the infection using the enzyme-linked immunosorbent assay. *Avian Diseases* 46:442-446.
- Vandamme, P., Segers, P. Vancaneyt, M., van Hover, K., Mutters, R. *et al.*, (1994). Description of *Ornithobacterium rhinotracheale* gen. nov. sp. nov. isolated from the avian respiratory tract. *International Journal of Systematic Bacteriology* 44:24-37.
- van Empel, P.C.M., Hafez, H.M. (1999). *Ornithobacterium rhinotracheale*: a review. *Avian Pathology* 28:217-227.
- van Empel, P., van den Bosch, H. (1998). Vaccination of chickens against *Ornithobacterium rhinotracheale* infection. *Avian Diseases* 42:572-578.