

## Antimicrobial susceptibility of *Ornithobacterium rhinotracheale* isolated from broiler chickens in Turkey

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### ABSTRACT

Minimum inhibitory concentrations (MICs) of eight antimicrobial agents were tested for 11 *Ornithobacterium rhinotracheale* isolates and for one type strain. Of the eight antibiotics examined, oxytetracycline was the most active antibiotic with MIC ranges of  $\leq 0.125 - 1 \mu\text{g/ml}$  for *O. rhinotracheale* isolates. All isolates were resistant to gentamicin and neomycin. Resistance to doxycycline occurred only in the type strain. All other isolates were found to be sensitive to doxycycline. Tilmicosin (MICs ranging from  $\leq 0.5 - 4 \mu\text{g/ml}$ ), erythromycin (MICs ranging from  $\leq 0.5 - 8 \mu\text{g/ml}$ ), and penicillin G (MICs ranging from  $\leq 4 - 16 \mu\text{g/ml}$ ) gave good inhibitions, but with most strains in a higher concentration.

**Key words:** *Ornithobacterium rhinotracheale*, MIC, Turkey, antibiotic susceptibility

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### Introduction

*Ornithobacterium rhinotracheale* is a newly-named bacterium by VANDAMME et al. (1994). The organism has recently been isolated in many countries of the world and has been incriminated as a possible additional causative agent in the respiratory disease complex (CHARLTON et al., 1993; DUDOUYT et al., 1995; VAN BEEK et al., 1993; JOUBERT et al., 1999; VAN VEEN et al., 2000; SAKAI et al., 2000). More recently, *O. rhinotracheale* was shown to be associated with respiratory problems, growth depression and arthritis in chickens and turkeys (HAFEZ, 1996; CHIN and DROUAL, 1997; VAN BEEK et al., 1993; VAN VEEN et al., 2000).

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The sensitivity of *O. rhinotracheale* to antibiotics is very inconsistent and appears to depend on the source of the strain (VAN EMPEL and HAFEZ, 1999; VAN VEEN et al., 2001). Acquired resistance has been reported for some antibiotics (CHIN and DROUAL, 1997; DEVRIESE et al., 1995).

The main purpose of the present study was to evaluate the *in vitro* bactericidal activity of eight antibiotics against 11 *O. rhinotracheale* strains isolated from chickens in a previous study in Turkey and one type strain.

### **Materials and methods**

**Bacteria.** Eleven *O. rhinotracheale* isolates from the trachea in chickens, and type strain 0-96069, were included in the study. The 0-96069 strain was obtained from Dr. van Empel (Intervet International Boxmeer, The Netherlands).

**Antibiotic standards.** Standard powders of the following antibacterial agents for laboratory use were obtained from respective manufacturers; oxytetracycline (Sanofi pharmaceuticals, TR), erythromycin (E-5505, Sigma Chemical Company, USA), gentamicin (VETAŞ, pharmaceutical, TR), danofloxacin (Pfizer, TR), penicillin G (Lot-39F0450, Sigma Chemical Company), doxycycline (Hipra, Spain), tilmicosin (Lilly com, Austria), neomycin (N-1876, Sigma Chemical Company, U.S.A.) Stock solutions of antibiotics were prepared freshly on the day of each assay and filter sterilised.

**Media.** Peptone water was used for susceptibility testing due to the organism growing slowly and failing to grow in the antibiotic sensitivity test media.

**Susceptibility testing.** MICs of antibiotics and extract were determined by broth macrodilution using a standard method (ANONYMOUS, 1998). The antibacterial agents were serially diluted from 128 mg/ml to 0.008 mg/ml in doubling dilutions in peptone water.

Bacterial suspension of log phase cultures in peptone water was adjusted by turbidity to yield an inoculum of  $10^5$  cfu/ml, and added to wells. The MIC was defined as the lowest concentration of antibiotic which inhibited visible growth after 48 h of incubation at 37 °C under microaerobic conditions (candle jar).

Interpretation of the in vitro susceptibility findings should be done with caution because standards have not been established for *O. rhinotracheale*. Non-Enterobacteriaceae (except *P. aeruginosa*) guidelines in the National Committee for Clinical Laboratory Standards (ANONYMOUS, 1998) documents were utilized in performing all tests and evaluating the susceptibility data. MIC values of the antibiotics not found in the catalogue were accepted to be sensitive for low dilutions.

### Results

The individual and the arithmetic average of the MIC values of the eight antibiotics tested at 48 h are shown in Table 1.

According to the individual and the arithmetic average of the MIC values of antibiotics, it was determined that oxytetracycline was the most effective antibiotic against *O. rhinotracheale* strains.

The *O. rhinotracheale* Turkey strains were inhibited effectively by penicillin G, doxycycline, oxytetracycline and tilmicosin. None of the strains was inhibited by neomycin or gentamicin. For the gentamicin and

Table 1. Individual and arithmetic average MIC values of different antibiotics against 12 *Ornithobacterium rhinotracheale* strains

Antibiotic	Number of strains with MIC ( $\mu\text{g/ml}$ )										MIC ( $\mu\text{g/ml}$ )	
	0.12	0.25	0.5	1	2	4	8	16	32	64	Range	Arithmetic mean
Penicillin G						4	6*	2			$\geq 4-16 \leq$	4.0
Doxycycline		4	7				1*				$\geq 0.25-8 \leq$	1.0
Danofloxacin		2*			2	4	4				$\geq 0.25-8 \leq$	4.4
Erythromycin			2	4*	1	3	2				$\geq 0.5-8 \leq$	3.0
Oxytetracycline	1	2	5	4*							$\geq 0.12-1 \leq$	0.6
Tilmicosin			5*	2	3	2					$\geq 0.5-4 \leq$	1.5
Neomycin								3	4	5*	$\geq 16-64 \leq$	41.3
Gentamicin									6	6*	$\geq 32-64 \leq$	42.7

\* including type strain

neomycin resistant *O. rhinotracheale* strains, the gentamicin MIC values ranged from 32 to 64 mg/ml (6 isolates 32 mg/ml; 6 isolates 64 mg/ml), and the neomycin MIC values ranged from 16 to 64 mg/ml (3 isolates 16

mg/ml; 4 isolates 32 mg/ml; 5 isolates 64 mg/ml).

The Turkey strains had low doxycycline MIC values (of  $<$  or  $=0.25$ - $0.5$  mg/ml) while the type strain was inhibited by the highest concentration (8 mg/ml). The type strain and one of the Turkey strains were sensitive to danofloxacin, but the others were resistant to danofloxacin.

### Discussion

*O. rhinotracheale* is a new poultry pathogen associated with respiratory disease, decreased growth and increased mortality in chickens and turkeys (CHIN and DROUAL, 1997; HAFEZ, 1996; VAN BEEK et al., 1993). For the control of *O. rhinotracheale* infection, besides the general procedures of biosecurity, treatment of the infected flock with antibiotics has been used (CHIN and DROUAL, 1997; VAN EMPEL and HAFEZ, 1999). Little has been published on antibacterial sensitivity of *O. rhinotracheale* (DEVRIESE et al., 1995; HAFEZ, 1996; VARGA et al., 2001; VAN VEEN et al., 2001).

It has been reported that resistance of *O. rhinotracheale* to antibiotics is varying and that according to the region from which it is isolated the resistance is different (VAN EMPEL and HAFEZ, 1999). In France and Belgium, 98% and 71% of the isolates were found to be sensitive to quinolone *in vitro*, respectively (DEVRIESE et al., 1995; DUDOUYT et al., 1995). Acquired resistance was also seen with quinolone, tetracycline, lincosamide and macrolide (DEVRIESE et al., 1995). VARGA et al. (2001) stated that lincomycin, oxytetracycline and enrofloxacin gave good inhibition; however, MIC values for several strains were found to be high. In Germany, most of the isolates have been resistant to quinolone *in vitro* (HAFEZ, 1994; HAFEZ, 1996). In this study, it has been determined that most (90.9%) of the *O. rhinotracheale* isolates found in Turkey were resistant to quinolone group antibiotics. It is thought that this result can be related to acquired resistance. Contrary to reports (DEVRIESE et al., 1995) no resistance formed against doxycycline of the tetracycline group, and Turkey strains were sensitive.

In practice, penicillin, sulfas and/or tetracyclines are the most commonly used antibiotics for treatment, and other antibiotics (spectinomycin, streptomycin, erythromycin, ceftiofur and tilmicosin) have been used with varying degrees of success related to the produced

resistance (CHIN et al., 1996). It has been reported that Hungary strains are sensitive to erythromycin and tilmicosin (VARGA et al., 2001). In this study, erythromycin and tilmicosin of the macrolid group showed antimicrobial activity against all *O. rhinotracheale* strains. However, while some strains had low MIC values, others were seen to have higher MIC values. These results were expected for erythromycin and tilmicosin (HAFEZ, 1994; CHIN and DROUAL, 1997; VARGA et al., 2001; CHIN et al., 1996; ODOR et al., 1997), but the results contrasted with findings in the Netherlands (VAN VEEN et al., 2001), where isolates were resistant to these antibiotics. It has been found that strains isolated in studies are sensitive to penicillin (DEVRIESE et al., 1995; VARGA et al., 2001; ZORMAN-ROJS et al., 2001). In this study, the strains are within susceptibility limits according to NCCLS criteria. However, when compared to other studies (VAN VEEN et al., 2001; DEVRIESE et al., 1995; VARGA et al., 2001) an increase was observed in MIC values of the isolates against penicillin.

It has been reported that most of the *O. rhinotracheale* strains show resistance against gentamicine and neomycine belonging to aminoglycosides (VAN VEEN et al., 2001; DUDOUYT et al., 1995). In this study, all of the isolates were resistance to gentamicin and neomycin.

The results obtained show that the strains of *O. rhinotracheale* isolated in Turkey between 1999 and 2000 may indicate that acquired antibiotic resistance is unusually common in *O. rhinotracheale*. *O. rhinotracheale* infections have been determined in several bird-species in different parts of the world. Due to this, development of strains, resistant to antibiotics used for the treatment of this fast spreading infection, also brings about serious problems.

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**AK, S., N. TURAN: Osjetljivost prema antibioticima bakterije *Ornithobacterium rhinotracheale* izdvojene iz tovnih pilića u Turskoj. Vet. arhiv 71, 121-127, 2001.**

**SAŽETAK**

Određene su minimalne inhibicijske koncentracije (MIK) osam antibiotika za 11 izolata i jedan referentni soj bakterije *Ornithobacterium rhinotracheale* izdvojenih iz tovnih pilića. Najveća osjetljivost dokazana je za oksitetraciklin s minimalnom inhibicijskom koncentracijom u rasponu od 0,125 do 1 µg/ml za izolate *Ornithobacterium rhinotracheale*. Svi izolati bili su otporni prema gentamicinu i neomicinu. Otpornošću prema doksiciklinu odlikovao se samo referentni soj. Tilmikozin (MIK u rasponu od 0,5 do 4 µg/ml), eritromicin (MIK od 0,5 do 8 µg/ml) i penicilin G (MIK 4 do 16 µg/ml) su inhibirali razmnožavanje većine izolata, ali samo u visokoj koncentraciji.

**Ključne riječi:** *Ornithobacterium rhinotracheale*, minimalna inhibicijska koncentracija, Turska, osjetljivost prema antibioticima

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