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In vitro antibiotic sensitivity of *Ornithobacterium rhinotracheale* strains isolated from laying hens in India

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ABSTRACT

Eighteen isolates of *Ornithobacterium rhinotracheale* isolated from laying hens in India were tested for their susceptibility to various antibiotic agents. Antibiogram pattern of all the 18 *O. rhinotracheale* isolates were determined in Mueller Hinton agar enriched with 10 per cent sheep blood, with antibiotic discs. All the isolates were resistant to amikacin, cloxacillin, co-trimoxazole, gentamicin, metronidazole and triple sulpha. Susceptibility of *O. rhinotracheale* isolates to cephalexin, norfloxacin, pefloxacin, streptomycin and furazolidone was variable. The isolates were sensitive to amoxycillin, ampicillin, chloramphenicol, ciprofloxacin, doxycycline, enrofloxacin, erythromycin, oxytetracycline, and penicillin-G.

Key words: *Ornithobacterium rhinotracheale*, antibiotic susceptibility, layers

Introduction

Ornithobacterium rhinotracheale is an emerging poultry pathogen associated with respiratory disease, poor production performance and retarded growth in chickens and turkeys (VANDAMME et al., 1994; VAN EMPEL and HAFEZ, 1999; CHIN et al., 2003).

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Antibiotics are routinely used in poultry flocks, for the control of bacterial pathogens besides biosecurity measures. Antibiotics are widely used to treat and prevent various bacterial diseases in layer flocks. Indiscriminate use of antibiotics may lead to antibiotic resistance in pathogenic bacteria as well as normal microflora. Very few reports were available on the antibacterial sensitivity of *O. rhinotracheale*. Susceptibility of *O. rhinotracheale* isolates to antibiotics is variable depending on the region of isolation. High rates of acquired resistance of the *O. rhinotracheale* strains from poultry and wild birds against certain antibiotics by *in vitro* antibiotic sensitivity test was reported (FITZGERALD et al., 1998; DEVRIESE et al., 1995). It was indicated that acquired antibiotic resistance was exceptionally frequent in *O. rhinotracheale*, and its natural sensitivity to antibiotics could be determined only with strains from wild birds. The resistance mechanisms active in *O. rhinotracheale* were unknown except in the case of the β-lactams, in which β-lactamase has been demonstrated.

Establishing the antibiotic sensitivity of the avian respiratory pathogen *O. rhinotracheale* was difficult because of the organism's complex growth requirements and the unusually frequent occurrence of resistance (DEVRIESE et al., 2001). It might be possible to interpret the inhibition zone diameters of disc or tablet diffusion tests with *O. rhinotracheale* according to the criteria in use with certain fastidious bacteria of human medical importance (DOERN, 1995).

Studies on antibiotic susceptibility and resistance are very few and hence this study on *in vitro* susceptibility test on *O. rhinotracheale* isolates would be helpful in formulating control measures for *O. rhinotracheale* infection in poultry. Therefore it is important to evaluate the efficacy of various antimicrobial drugs currently used in poultry and to determine patterns of antibiotic sensitivity. In this study we described the antimicrobial susceptibility profiles of *O. rhinotracheale* strains isolated from layers in Tamil Nadu state of India.

Materials and methods

Specimens for isolation. Trachea, lungs, air sacs, swabs of infraorbital sinus exudates, and heart blood and liver samples were collected from layers showing symptoms of respiratory diseases. The collected samples were inoculated onto 10 per cent sheep blood agar media and incubated at 37 °C for 48 h in candle jars. Following incubation, growth characteristics and colony morphology of the cultures were studied. The colonies were subjected to standard biochemical test procedures described by BARROW and FELTHAM (1993) and VANDAMME et al. (1994) for confirmation of *O. rhinotracheale*.

Antibiotic susceptibility. The method was followed described by the National Committee for Clinical Laboratory Standards (NCCLS) for fastidious Gram-negative organism (ANONYMOUS, 1998; MALIK et al., 2003). Antibiogram of the isolates

were carried out in Mueller Hinton agar enriched with 10 per cent sheep blood, with commercially available antibiotic discs. *Ornithobacterium rhinotracheale* colonies grown on blood agar plates were suspended in brain heart infusion broth. The suspension was swabbed on the surface of blood Mueller Hinton agar, followed by the application of antibiotic discs *viz*. amikacin (30 mcg), amoxycillin (30 mcg), ampicillin (10 mcg), cephalexin (30 mcg), chloramphenicol (30 mcg), ciprofloxacin (5 mcg), co-trimoxazole (1.25/23.75 mcg), cloxacillin (30 mcg), doxycycline (30 mcg), enrofloxacin (10 mcg), erythromycin (15 mcg), furazolidone (50 mcg), gentamicin (10 mcg), metronidazole (5 mcg), norfloxacin (10 mcg), oxytetracycline (30 mcg), pefloxacin (5 mcg), penicillin-G (10 units), streptomycin (25 mcg), and triple sulpha (300 mcg). After the application of antibiotic discs, the plates were incubated at 37 ^{0}C in candle jars for 48 h. Antibiotic resistance or susceptibility was determined using the criteria for fastidious Gram-negative organisms as established by NCCLS.

Results

*Bacterial strains***.** A total of eighteen *O. rhinotracheale* isolates were yielded from the samples examined. All the isolates with colony characters of very small colonies, non-haemolytic, grey to greyish white, opaque, convex with entire edge, circular with diameter of 1-2 mm and with butyrous odour were observed on sheep blood agar after 48h of incubation at 37 ^{o}C under anaerobic conditions.

Smears prepared from the colony revealed Gram-negative, highly pleomorphic rod shaped bacteria. The smears prepared from the brain heart infusion broth culture revealed more pleomorphic and thin rods than from agar plates. All isolates were found to be non motile.

Biochemical reactions. No growth was observed on MacConkey agar. No reaction was observed on triple sugar iron agar. All the isolates were positive for oxidase, acetyl methyl carbinol production, β-galactosidase (ONPG) and urease activity. The isolates were negative for catalase, citrate utilization, indole, methyl red reaction, nitrate reduction, phenylalanine deamination and gelatin liquefication. All the isolates were positive for arginine dehydrolase and negative for Lysine and Ornithine decarboxylases.

Antibiogram. Antibiogram patterns of all *O. rhinotracheale* isolates were determined. The antibiotic sensitive pattern shown by the 18 isolates to 20 antibiotics and antibacterials are presented in Table 1 and 2. In the present study, hundred percent resistance to amikacin, cloxacillin, co-trimoxazole, gentamicin, metronidazole and triple sulpha was observed. Susceptibility of *O. rhinotracheale* isolates to cephalexin, norfloxacin, pefloxacin, streptomycin and furazolidone was variable. All the isolates were sensitive to amoxycillin, ampicillin, chloramphenicol, ciprofloxacin, doxycycline, enrofloxacin, erythromycin, oxytetracycline, and penicillin-G.

Table 1. *In vitro* antibiotic sensitivity of *Ornithobacterium rhinotracheale* isolates (isolate Nº 1-9)

S. Nº	Antimicrobial drug	Disc content mcg	Interpretation			Diameter of zone of inhibition in mm Isolate No.								
			Resistant	Intermediate	Sensitive									
			mm or less	mm	mm or more	1	2	3	4	5	6	7	8	9
1.	Amikacin	30	14	15-16	17	10	10	R	R	11	10	<10	<10	10
2.	Amoxycillin	30	13	14-17	18	23	22	24	22	22	23	22	23	22
3.	Ampicillin	10	11	12-14	15	21	21	22	21	22	21	20	21	21
4.	Cephalexin	30	11	12-14	15	14	13	13	14	13	13	14	14	14
5.	Chloramphenicol	30	12	13-17	18	26	28	26	27	26	28	28	26	26
6.	Ciprofloxacin	5	15	16-20	21	22	20	22	20	21	22	21	20	20
7.	Co-Trimoxazole	1.25/23.75	10	11-15	16	R	R	10	R	R	R	R	R	R
8.	Cloxacillin	30	11	12-16	17	R	R	R	R	R	R	R	R	R
9.	Doxycycline	30	12	13-15	16	22	21	24	22	23	24	22	22	22
10.	Enrofloxacin	10	12	13-17	18	22	20	20	21	22	23	22	22	22
11.	Erythromycin	15	13	14-22	23	22	21	21	22	22	21	20	21	20
12.	Furazolidone	50	11	12-14	15	16	14	16	16	16	14	14	14	14
13.	Gentamicin	10	12	13-14	15	10	R	<10	<10	<10	<10	<10	R	R
14.	Metronidazole	5	11	12-14	15	R	R	R	R	R	R	R	R	R
15.	Norfloxacin	10	12	13-16	17	14	15	15	14	13	15	15	14	14
16.	Oxytetracycline	30	14	15-18	19	19	18	18	18	19	18	18	18	18
17.	Pefloxacin	5	11	12-14	15	14	14	14	15	14	14	14	14	14
18.	Penicillin-G	10 units	19	20-27	28	26	24	26	26	24	25	26	24	24
19.	Streptomycin	25	11	12-14	15	13	12	12	13	13	13	13	12	12
20.	Triple Sulpha	300	10	11-15	16	10	R	10	10	R	<10	10	10	10

Table 2. *In vitro* antibiotic sensitivity of *Ornithobacterium rhinotracheale* isolates (Isolate No. 10-18)

S. N°	Antimicrobial drug	Disc content mcg	Interpretation: Resistant mm or less	Intermediate mm	Sensitive mm or more	Diameter of zone of inhibition in mm, Isolate N°: 10	11	12	13	14	15	16	17	18
1.	Amikacin	30	14	15-16	17	10	10	R	R	11	10	10	10	10
2.	Amoxycillin	30	13	14-17	18	22	23	22	24	22	22	23	22	22
3.	Ampicillin	10	11	12-14	15	21	22	21	22	22	21	21	21	21
4.	Cephalexin	30	11	12-14	15	13	13	12	14	13	12	14	13	14
5.	Chloramphenicol	30	12	13-17	18	28	28	26	27	26	26	27	26	26
6.	Ciprofloxacin	5	15	16-20	21	22	22	23	24	22	24	22	22	23
7.	Co-Trimoxazole	1.25/23.75	10	11-15	16	R	10	10	10	R	10	10	10	10
8.	Cloxacillin	30	11	12-16	17	R	R	R	R	R	R	R	R	R
9.	Doxycycline	30	12	13-15	16	24	22	22	22	21	21	22	21	22
10.	Enrofloxacin	10	12	13-17	18	21	20	22	20	22	21	22	20	21
11.	Erythromycin	15	13	14-22	23	22	22	21	22	22	22	21	22	22
12.	Furazolidone	50	11	12-14	15	16	16	15	16	14	14	14	16	14
13.	Gentamicin	10	12	13-14	15	10	<10	10	10	10	10	10	10	10
14.	Metronidazole	5	11	12-14	15	R	R	R	R	R	R	R	R	R
15.	Norfloxacin	10	12	13-16	17	14	14	14	15	14	14	14	14	14
16.	Oxytetracycline	30	14	15-18	19	18	18	19	18	19	19	18	19	18
17.	Pefloxacin	5	11	12-14	15	13	14	14	13	15	15	14	15	14
18.	Penicillin-G	10 units	19	20-27	28	26	26	26	26	25	27	26	26	26
19.	Streptomycin	25	11	12-14	15	13	13	12	13	14	13	13	12	12
20.	Triple Sulpha	300	10	11-15	16	10	10	10	10	10	10	10	10	10

Discussion

Of the eighteen *O. rhinotracheale* isolates tested, none of them was inhibited effectively by amicacin, cloxacillin, co-trimoxazole, gentamicin, metronidazole and triple sulfa. This result was expected for sulfadimethoxine and trimethoprim sulfa (MALIK et al, 2003; SORIANO et al., 2003) where complete resistance to sulfa methoxine in majority of isolates and an increasing trend of resistance to gentamicin and trimethoprim sulfa was reported. The results of the present study were in accordance with most of the *O. rhinotracheale* strains isolated from many countries, which showed resistance against gentamicin, trimethoprim sulfa and amikacin (DUDOUYT et al., 1995; ZORMAN-ROJS et al., 2000; AK and TURAN, 2001; VAN VEEN et al., 2001).

Susceptibility of *O. rhinotracheale* isolates to cephalexin, norfloxacin, pefloxacin, streptomycin and furazolidone was variable. The results obtained with ampicillin and amoxycillin in this study were in agreement with earlier studies in which *O. rhinotracheale* was found to be very susceptible to ampicillin, amoxycillin and amoxycillin supplemented with clavulanic acid *in vitro* (MALIK et al., 2003; ZORMAN-ROJS et al., 2000). The antibiotic sensitivity of Dutch isolates of *O. rhinotracheale* to amoxycillin and tetracycline steadily decreased in a successive period of four years (VAN VEEN et al., 2001).

However, these results were in contrast to those of DEVRIESE et al. (2001) in which *O. rhinotracheale* was found to be resistant to ampicillin and, possibly, the test methods, criteria for sensitivity and resistance might have differed. It might be possible to interpret inhibition zone diameters of disc diffusion tests with *O. rhinotracheale* according to the criteria in use with certain fastidious bacteria (DOERN, 1995; DEVRIESE et al., 2001).

The results obtained with doxycycline, enrofloxacin, erythromycin, oxytetracycline and penicillin G in this study were in agreement with earlier studies, in which *O. rhinotracheale* was found to be very susceptible to these antibiotics *in vitro* (DEVRIESE et al., 1995; DUDOUYT et al., 1995; AK and TURAN, 2001; VAN VEEN et al., 2001). It was indicated that acquired antibiotic resistance was unusually common in *O. rhinotracheale*.

The results of antibiotic sensitivity tests conducted for the *O. rhinotracheale* isolates in the present study correlated with the findings of ODOR et al. (1997) with respect to tetracycline and erythromycin, but differed regarding the susceptibility towards penicillin. The findings of antibiotic sensitivity tests conducted for the *O. rhinotracheale* isolates in the present study were in accordance with the reports of ZORMAN-ROJS et al. (2000), except towards the antibiotic enrofloxacin.

Since the poultry in and around Namakkal, are constantly exposed to various stress factors, such as adverse climatic conditions, farmers continuously use various antibiotic drugs. The continuous use of drugs might have resulted in the development of acquired antibiotic resistance in the *O. rhinotracheale* isolates of the present study for antibiotics

such as cloxacillin, co-trimoxazole, gentamicin, metronidazole and triple sulpha as reported by DEVRIESE et al. (1995), AK and TURAN (2001), VAN VEEN et al. (2001), MALIK et al. (2003) and SORIANO et al. (2003).

Though enrofloxacin was extensively used by poultry farmers in Namakkal area, still the drug sensitivity for *O. rhinotracheale* is retained and this differed from the findings of ZORMAN-ROJS et al. (2000).

The indiscriminate use of various antimicrobial drugs might have resulted in the development of acquired antibiotic resistance in *O. rhinotracheale* isolates. Treatment with such antimicrobial drugs is hardly useful in controlling *O. rhinotracheale* infections. A monitoring programme for *O. rhinotracheale* for antibiotic susceptibility is needed to design control measures.

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SAŽETAK

Osamnaest izolata bakterije *Ornithobacterium rhinotracheale* iz kokoši nesilica u Indiji pretraženo je na osjetljivost prema različitim antibioticima. Osjetljivost je određivana difuzijskim postupkom na Mueller-Hintonovu agaru obogaćenom s 10% ovčje krvi. Svi su izolati bili otporni na amikacin, kloksacilin, kotrimoksazol, gentamicin, metronidazol i sulfa pripravke. Osjetljivost je kolebala prema cefaleksinu, norfloksacinu, pefloksacinu, streptomicinu i furazolidonu. Izolati su bili osjetljivi na amoksicilin, ampicilin, kloramfenikol, ciprofloksacin, doksiciklin, enrofloksacin, eritromicin, oksitetraciklin i penicillin G.

Ključne riječi: *Ornithobacterium rhinotracheale*, osjetljivost, antibiotici, nesilice