

Identification of Respiratory Tract Bacteria from Poultry in and Around Jabalpur

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Abstract Among various prevalent poultry disease, respiratory diseases are most common. Mostly bacterial pathogen involved in respiratory infections are *E. coli*, *Pasteurella* spp., *Haemophilus* spp., *Mycoplasma* spp., *Ornithobacterium rhinotracheale*, is the new and difficult respiratory tract bacteria to isolate and grow in the laboratory. It has also been associated with retarded growth, reduction in egg production and can lead to economic losses to the poultry industry. A total of 166 serum samples, 176 nasal swabs and tracheal swab each, 167 lung pieces from same poultry were collected in transport media from 09 different poultry farms/outlets in and around Jabalpur. Isolation was performed in different media viz. nutrient agar, MacConkey agar, Eosin methylene blue agar (EMB) and 10% blood agar with and without carbon dioxide and biochemical test viz. indole, methyl red, VP, citrate, urease, oxidase, catalase and

triple sugar iron agar (TSI) were performed. The respiratory tract bacteria isolated from nasal swabs were *Escherichia coli* 22.15%, *Klebsiella* spp. 13.06%, *Staphylococcus* spp. 15.34% and *Streptococcus* spp. 6.25%, and from tracheal swab predominant bacteria were *E. coli* 24.43%, *Klebsiella* spp. 9.09%, *Staphylococcus* spp. 11.36% and *Streptococcus* spp. 7.38%, respectively. While from lung pieces the percentage bacteria which were isolated were *E. coli* 19.76%, *Klebsiella* spp. 10.17%, *Staphylococcus* spp. 9.58% and *Streptococcus* spp. 2.99%, respectively. From poultry 8.43% of serum samples were screened positive for *O. rhinotracheale* antibodies with ELISA kit.

Keywords *Ornithobacterium rhinotracheale*, Respiratory tract infection, Elisa, Poultry, Jabalpur.

Introduction

Ornithobacterium rhinotracheale is a slow growing, pleomorphic, gram-negative, rod-shaped, relatively new bacterium associated with poultry diseases. Before 1994, the bacterium was named *Pasteurella*-like, *Kingella*-like or Pleomorphic Gram-Negative Rod (PGNR) and also the name TAXON 28 were used. Currently the bacterium is often referred to as *Ornithobacterium rhinotracheale* (ORT) and its in-

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Table 1. Sample collection.

Sl. No.	Poultry farms outlets	Nasal swab	Tracheal swab	Long pieces	Serum sample
1.	A	16	09	09	16
2.	B	21	21	21	18
3.	C	12	12	12	12
4.	D	15	15	15	15
5.	E	35	35	35	35
6.	F	48	48	41	47
7.	G	06	06	04	0
8.	H	13	20	20	13
9.	I	10	10	10	10
	Grand total	176	176	167	166

fection, as ornithobacteriosis, which is a contagious disease of avian species, primarily turkeys and chickens, causing respiratory distress, decreased growth and mortality [1]. Respiratory diseases are very common in poultry. *Ornithobacterium rhinotracheale* are among the minor microorganisms responsible for these diseases [2]. However, many of the infections, caused by *O. rhinotracheale*, are not recognized as such either because the causative agent cannot be isolated or because investigators are not aware of the possibility that *O. rhinotracheale* can cause infection other than the more well-known respiratory ones [3, 4].

Jabalpur, one of the most leading poultry rearing pockets in Madhya Pradesh, is constantly facing the threat emerging respiratory diseases even after vaccination of known viral and bacterial diseases. Regardless of whether birds are raised for meat, eggs, breeding or show purposes, respiratory infections result in decreased performance and ultimately lead to economic loss. Clinical signs and postmortem of *Ornithobacterium rhinotracheale* infections are similar to other bacterial and viral infections. Isolation and identification of the causative agent is essential for differential diagnosis. So, present research work was aimed at isolation, identification and antibiotic susceptibility of gentamicin resistant respiratory tract bacteria of poultry with special reference to *Ornithobacterium rhinotracheale* and determination of its circulating antibodies in poultry in and around Jabalpur, Madhya Pradesh.

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Materials and Methods

Collection of sample

A total of 685 samples consisting of 166 serum samples, 176 nasal swabs, 176 tracheal swab, 167 lung pieces from same poultry showing respiratory disease symptoms were collected in transport media from 09 different poultry farms/outlets in and around Jabalpur, Madhya Pradesh as presented in Table 1.

Isolation and identification of respiratory tract bacteria

Samples were cultured in nutrient agar, MacConkey agar, 10% sheep blood agar and incubated at 37°C with and without 05 to 10% CO₂. Colony characteristics were observed and Grams staining was performed. The following conventional biochemical tests like : indole, methyl red, Voges Proskauer, citrate, oxidase, catalase, triple sugar iron agar, urease and fermentation of various sugars with acid and gas production was performed for identification [6].

Serological identification

Due to several reports about *O. rhinotracheale* that it is a new and tedious bacterium to cultivate in laboratory a reliable serological identification method was performed using commercially available ELISA kits (IDEXX, Germany) to detect its antibodies in chicken population. The test was carried out according to manufacturer's recommendations. Briefly, serum samples were diluted at 1 : 100 ratio and assayed as duplicated samples. Optical density (OD) of the samples was measured by ELISA reader. The interpretation of the ELISA readings was made after calculating the sample to positive ratio (S/P). Samples having 1.0 S/P or higher were considered positive while samples with 0.499 S/P or less were considered negative. S/P between 0.500 and 0.999 were considered suspected.

Table 2. Incidence of respiratory tract bacteria from tracheal swab.

Sl. No.	Poultry farms outlets	Tracheal swab	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.	Unidentified bacteria
1.	A	09	03	-	01	-	02
2.	B	21	04	01	02	01	03
3.	C	12	02	02	01	01	-
4.	D	15	02	01	01	01	-
5.	E	35	10	03	04	05	02
6.	F	48	13	07	07	03	02
7.	G	06	02	-	02	-	-
8.	H	20	04	01	02	02	01
9.	I	10	03	01	-	-	01
	Total samples	176	43	16	20	13	11

Results and Discussion

Incidence of respiratory tract bacteria from nasal swab

In an attempt to isolate respiratory tract bacteria from poultry, each of 176, nasal and tracheal swabs along with 167 lung pieces were collected in transport media from 09 different poultry farms/outlets in and around Jabalpur. The bacteria which are isolated from nasal swab were *E. coli* 22.15%, *Klebsiella* spp. 13.06%, *Staphylococcus* spp 15.34% and *Streptococcus* spp 6.25%, respectively. The percentage of bacteria isolated from tracheal swab were *E. coli*, *Klebsiella* spp. *Staphylococcus* spp. and *Streptococcus* spp. were 24.43%, 9.09%, 11.36% and 7.38%, respectively as depicted in Table 2. While the percentage respiratory tract bacteria isolated from 167 lung pieces is presented in Table 3. Incidence of *E. coli*, *Klebsiella* spp. *Staphylococcus* spp. and *Streptococcus* spp were 19.76%, 10.17%, 9.58% and 2.99%, respectively.

Biochemical tests

The non hemolytic pin pointed growth in 10% sheep blood agar which do not grow in MacConkey's agar were subjected to biochemical test and were found positive to oxidase, urease, VP and fermented carbohydrate such as glucose, lactose and maltose. While they were negative to MR, indole, citrate, catalase, triple sugar iron agar and were unable to ferment sucrose. Similarity in both the isolates indicates that they may be originated from same source.

Seroprevalence of *Ornithobacterium rhinotracheale* by ELISA

For assessment of immune status as well as serological identification of *Ornithobacterium rhinotracheale* (serotypes A-M), an antibody ELISA test kit (IDEXX ORT, Germany was procured to carry out the investigation. In 96% well ELISA plate a total of 166 serum samples in duplicate were used for screening the birds. The ELISA was performed ac-

Table 3. Incidence of respiratory tract bacteria from lung pieces.

Sl. No.	Poultry farms outlets	Lung pieces	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.	Unidentified bacteria
1.	A	09	02	-	-	-	03
2.	B	21	03	04	02	-	03
3.	C	12	02	03	01	-	-
4.	D	15	03	01	01	-	-
5.	F	35	07	02	02	02	02
6.	F	41	10	05	04	01	03
7.	G	04	01	-	02	01	-
8.	H	20	03	01	03	01	-
9.	I	10	02	01	01	-	-
	Total samples	167	33	17	16	05	11

cording to manufacture instruction. Total of 14 (8.43%) serum samples were found positive for *Ornithobacterium rhinotracheale* antibodies.

In the present study, conventionally pin pointed non hemolytic growth in 10% sheep blood agar at 37°C under 10% carbon di oxide for 48h incubation, which further do not grow on MacConkey agar were suspected for *O. rhinotracheale*. Suspected isolates were further subjected to battery of biochemical tests mentioned by Markey et al. [5]. But none of the isolates were confirmed as *O. rhinotracheale*. *O. rhinotracheale* is a tedious, difficult and fragile bacterium to grow in laboratory. Previous works like [2, 6] reported the requirement for the growth of *O. rhinotracheale*. One of the main reasons for no isolation could be the overgrowth by rapid-growing bacteria masking the *O. rhinotracheale* colonies, as reported earlier [3]. In our study isolation of various pathogenic bacteria from respiratory tract infection in poultry indicates that frequent and indiscriminate use of antibiotics in poultry farms which may be the cause of isolation of pathogenic bacteria, like *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp. [7, 8]. Our findings are in agreement with the previous workers who also isolated other bacteria from respiratory tract, alone or in conjunction with ORT [4].

In the present study, a total of 14 (8.43%) serum samples were found positive for *Ornithobacterium rhinotracheale* antibodies indicating that antibodies are in circulation [9] as indicated in previous studies also. As organism is fastidious and have special requirement it is generally not isolated with other respiratory tract bacteria in the laboratory. Frequency of *O. rhinotracheale* infection incidence may vary depending on the season and is usually common in winter months. Suzuki et al. [10] some of the co workers analyzed the use of a commercial ELISA for the detection of antibody against ORT in turkey serum and reported the estimates for the apparent prevalence and true prevalence at flock-level to be 30 and 17%, respectively. The differences in the results of reports might be due to the difference in topographical location, season age of the chicks and stage of the infec-

tion

This is a preliminary work carried out in respect to *Ornithobacterium rhinotracheale*. Much more exhaustive research is the need to know the exact role of ORT bacteria in respiratory tract infection of poultry.

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