Journal of Advanced Veterinary Research

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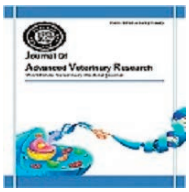

Phenotypic and Molecular Characterization of *Ornithobacterium rhinotracheale* Isolates in Broiler Chickens

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ARTICLE INFO

Original Research

Received:
13 August 2020

Accepted:
11 September 2020

Keywords:

Molecular characterization, *Ornithobacterium rhinotracheale*, Pathogenicity, Phenotypic identification, Serology

ABSTRACT

Ornithobacterium rhinotracheale (ORT), is a bacterium that cause respiratory tract infection, has led to significant problems in the intensive poultry production. The current study aimed to isolate and identify ORT from broiler chickens, to detect antibacterial sensitivity and resistance of ORT isolates, and to test experimental infection of ORT in broiler chickens. One hundred eighty samples including tracheas, lungs and air sacs were subjected to isolation and phenotypic identification. Twelve suspected ORT isolates were used for molecular identification. Agar gel precipitation test was used to determine serotype of ORT isolates. Antibacterial sensitivity and resistance of ORT isolates were tested against 14 antibacterial drugs using standard disk diffusion technique. Pathogenicity of ORT was tested by experimental infection in broiler chickens. Results revealed that the incidence of ORT infection in broiler chickens in Assiut Governorate is 17.77% using isolation and phenotypic methods of identification, while it is 3.33% using molecular identification. Serological identification of ORT isolates indicated that all tested isolates, were belonged to serotype A. All ORT isolates were resistant to gentamycin, amoxycillin and cephradine with 100% incidence, where, 100% isolates were sensitive to colistin and doxycycline, 50% of isolates were sensitive to ampicillin and streptomycin, and 16.67% of isolates were sensitive to neomycin and trimethoprim. Living Newcastle attenuated vaccine, Lasota vaccine, exaggerates the clinical signs and lesions of ORT experimental infection.

Introduction

Ornithobacterium rhinotracheale (ORT) infection, also known as ornithobacteriosis, is a contagious disease of avian species, primarily turkeys and chickens, causing respiratory distress, decreased growth, and mortality (Chin and Charlton, 2008; Chin *et al*., 2008). Among the avian species, a new bacterial respiratory disease in chickens was observed in South Africa and named *Ornithobacterium rhinotracheale* (Vandamme *et al*., 1994). ORT was incriminated as a possible additional causative agent in respiratory disease complex (El-Gohary and Awaad, 1998; Ellakany *et al*., 2019). ORT has been isolated throughout the world (Tabatabai *et al*., 2010; Chernyshev *et al*., 2011; Churria *et al*., 2011). In Egypt this problem was studied (El-Gohary and Awaad 1998; Amal, 2002; Shahata, *et al*., 2006; Hegazy *et al*., 2015; Ellakany *et al*., 2018).

Ornithobacterium rhinotracheale is a Gram-negative, pleomorphic, rod-shaped bacterium of the rRNA superfamily V, in the taxonomic neighborhood of the genera *Cytophaga, Riemerella, Flavobacterium* (Vandamme *et al*., 1994; Canal *et al*., 2005).

The disease spreads horizontally by direct and indirect contact. Vertical transmission was proven, since some researchers isolated ORT at a very low incidence from reproductive organs, hatching eggs, infertile eggs and dead embryos (El-Gohary, 1998; Shahata, *et al*., 2006; Chin *et al*., 2008).

The severity of clinical symptoms, duration of the disease and mortality have been described to be highly variable (Bisgaard *et al*., 2008). Signs include depression, decreased food intake, reduced weight gains, nasal discharge, sneezing, and facial edema appear in chickens at 3-6 weeks of age. Also, sudden death with or without respiratory signs has been reported in chickens with nervous signs (Chin and Charlton, 2008; Chin *et al*., 2008). In postmortem examination, ORT infection associated with tracheitis, pericarditis, sinusitis, exudative pneumonia and yoghurt-like exudates in the air sacs (Banani, 2001). However, as these lesions are not sufficiently specific to diagnose the disease, laboratory tests are needed for definitive diagnosis (Ellakany *et al*., 2018).

Accurate diagnosis must be substantiated by isolation and identification of the causative organisms and/or detection of

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ISSN: 2090-6277/2090-6269/

antibodies using serological examination. Currently many reports discussed the laboratory diagnosis of ORT using molecular identification techniques such as polymerase chain reaction (PCR) and 16S ribosomal gene sequencing (Ozbey *et al*., 2004). On the other hand, still the isolation of the bacterium is necessary for serotyping, determining the antimicrobial susceptibility for an effective therapy and producing autogenous vaccines (Vandamme *et al*., 1994; Hafez and Sting, 1996; Hegazy *et al*., 2015).

In the current study, authors aimed to isolate and identify *Ornithobacterium rhinotracheale* from broiler chickens using phenotypic and molecular methods. Also, this study had other objectives including, detection of antibacterial sensitivity and resistance patterns of ORT, and pathogenicity testing of ORT in broiler chickens.

Materials and methods

Sampling

A total of 180 samples including, 60 lung samples, 60 air sac samples and 60 trachea samples, were collected from freshly dead and sacrificed diseased broiler chickens, aged from 20 to 42 days old. All examined birds showing clinical signs of respiratory disease manifested as cough, sneezing, rales, nasal discharge, conjunctivitis and swollen head. Postmortem examination showed yogurt like air sacculitis and pneumonia. Samples were collected from different broiler flocks at different localities of Assiut Governorate. Samples collected under aseptic condition and were subjected to bacteriological examination.

Isolation

Cultures were made by inoculating samples into brain heart infusion (BHI) broth and then were sub-cultured on 10% sheep blood agar media supplemented with 10 ug/ml of gentamicin sulphate to inhibit the overgrowth of other enterobacteriaceae according to Back *et al*. (l996), or sub-cultured into trypticase soya agar. Samples also sub-cultured into MacConkey agar media. Cultured media were then incubated at 37°C for 48 h under 7.5-10% CO_2 tension by using gas bags (oxoid) in candle jar according to Vandamme *et al*. (1994), and then examined for suspected ORT colonies.

Phenotypic identification

The suspected colonies were examined for their colonial morphology (Shape, size, color and appearance), films were prepared from the suspected pure colonies and stained with Gram's stain, then examined microscopically according to Cruickshank *et al*. (1975). The suspected isolates of ORT were tested biochemically by oxidase test and catalase test beside another identification method API 20 NE. ORT colonies were inoculated into 5 ml of BHI suspension media and inoculated in different test in API 20 NE strip by using micropipette or syringe, then incubated at 37°C for 48 h under 7.5% CO_2 tension. The interpretation of the test was according to the instructions of the kit's producer.

Molecular identification

Total genomic DNA was extracted from 12 randomly selected phenotypically identified ORT isolates, including 6 isolates from lung samples, 4 isolates from air sac samples and 2 isolates from trachea samples, using QIAamp DNA mini extraction kit (Qiagen, Germany) according to the manufacturer's instructions. The DNA concentration was determined by UV spectrophotometer (Beckman DU 640, CA, USA) and adjusted to be 50 ng/µl. Three microliters of each template were used for the PCR. Species identification was confirmed using the ORT species-specific 16S rRNA gene PCR assay (Hafez, 2002). In brief, the primer sequences were: 5-GAGAATTAATTTACGGATTAAG-3 (forward) and 5-TTCGCTTGGTCTCCGAAGAT-3 (reverse) (Biobasic, Canada). The parameters for all reactions were described in the following profile; initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 45 seconds and extension for 45 seconds at 72°C. The final extension took 10 minutes at 72°C. The PCR product (784 bp) was seen by electrophoresis in a 1.5% agarose gel stained with ethidium bromide for visualization performed in a horizontal gel chamber plate. The running buffer was 0.5X TBE (Tris borate EDTA (pH 8.3). The 1 kb plus DNA ladder was used as a reference standard molecular weight marker.

Serological identification

Six ORT isolates, which were previously identified using phenotypic and molecular methods of identification, were subjected to serological identification using Agar Gel Precipitation technique according to Türkyilmaz (2006).

Detection of Antibacterial Sensitivity and Resistance Patterns of ORT using Standard Disk Diffusion method

Sensitivity and resistance of ORT isolates to 14 antibacterial agents including; Ampicillin (10 ug), Amoxycillin (10 ug), Colistin sulfate (10 ug), Cephradine (30 ug), Flrofenicol (30 ug), Neomycin (30 ug), Erythromycin (15 ug), Vancomycin (30 ug), Doxycycline (30 ug), Gentamycin (10 ug), Trimethoprim (5 ug), Tetracycline (30 ug), Streptomycin (10 ug) and Levofloxacin (5 ug) were determined by using standard disk diffusion method described by Back *et al*. (1997).

Single colonies of ORT isolates were picked up from blood agar and cultured into broth culture and incubated at 37°C for 18 h, about 2 ml of this broth culture were transferred to be inoculated into trypticase soya agar by using sterile pasture pipette, then all excess fluids were removed with pipette and the plate was leaved to be dry. The antibiotic discs were applied at adequate spacing to the surface of plate with sterile forceps and the agar plate was incubated for 24 h at 37°C. Judgment of the test was done following incubation; the degree of sensitivity was estimated by measuring the visible and clear zone of inhibition produced by diffusion of the used antibacterial agent from the disc into surrounding medium. Interpretation of the results was performed according to Bauer *et al*. (1966) and Koneman *et al*. (1997).

Pathogenicity testing

Sixty, one-day old chicks were kept separately and fed on antibiotic free ration in cleaned and disinfected isolation units. All chicks were examined clinically. Pooled tracheal swabs were collected from examined chicks and isolation trials of pathogens were done with special reference to ORT to ensure their freedom of infections. ORT isolates (broth cultures were adjusted to 10^9 colony-forming units (CFU)/ml), which were previously identified and confirmed by PCR. The standard plate count method technique (Black, 1996) was used to adjust the number ORT per milliliter in the inoculated brain heart infusion (BHI) broth.

At 10th day old, chicks were randomly divided into 3 equal groups; first group were subjected to coarse spray of Newcastle disease vaccine, Lasota strain containing10^6 EID 50/ bird at 10th day old and then inoculated with ORT isolates via aerosol

at 14th day old, each chick received a 100 ml of BHI broth containing 10^9 CFU of ORT organism/ml. The second group were inoculated with ORT isolates via aerosol at 14th day old with the same dose as first group. The third group were inoculated with sterile BHI broth via aerosol and kept as a non-infected negative control. All infected and control chicks were observed daily for clinical signs. Ten birds from each group were sacrificed at 7th and 14th days post infection and samples were collected and subjected to postmortem and bacteriological examination for re-isolation of the ORT.

Results

Isolation and phenotypic identification of ORT

Examination of broiler chickens in different localities of Assiut Governorate showed typical clinical signs and postmortem lesions of ORT infection. The results revealed that by using isolation on blood and MacConkey agar media and biochemical reactions including oxidase, catalase and API 20 NE strips, ORT incidence was 32 out of 180 collected samples (17.77%). Incidence of ORT in lung samples was 17 out of 60 (28.3%), which was higher than incidence in trachea samples (6 out of 60; 10%) and incidence in air sac samples (9 out of 60; 15%). Colonies of ORT on blood agar, ranged from 1-3 mm after 48 h, and appeared circular, convex butyrous, opaque grayish white with a distinct odor like that of butyric acid, there was poor adherence of colonies to agar surface. ORT was not hemolytic on blood agar, but some isolates can cause incomplete hemolysis especially after 96 h, ORT also grew on BHI broth and trypticase soya agar but not on MacConkey agar. On Gram stained films, ORT appeared as gram negative, pleomorphic, sometimes appeared in clusters or single plump short rods, non-motile and non-sporulated. Biochemical tests revealed that ORT isolates were catalase negative and oxidase test positive. The result of API 20 NE strips of the ORT isolates (Table. 1) were positive for arginine dihydrolase (ADH), β-galactosidase (ONPG), gelatin liquefaction and Voges – Praskauer (VP) and negative for L- lysine decarboxylase (LDC), ornithine decarboxylase (ODC) and H_2S. The result of sugar fermentation by API 20 NE were negative for glucose, mannitol, Inositol sorbitl and positive for sucrose, arabinose, lactose, fructose, galactose and maltose.

Molecular identification of ORT using PCR

Results pointed out that 6 out of 12 tested isolates were positive for the presence of 16S rRNA gene (784bp), including 4 positive samples out of 6 isolates of lung samples, 2 positive samples out of 4 isolates of air sacs samples and no positive samples out of 2 isolates of trachea samples (Fig. 1).

Table 1. showing API 20 NE strip interpretation

Biochemical test	*Ornithobacterium rhinotracheale*
Catalase	-
Oxidase	+
β- galactosidase (ONPG)	+
Arginine dihydrolase (ADH)	+
L- lysine decarboxylase (LDC)	-
Ornithine decarboxylase (ODC)	-
H2S	-
Urease	+
Nitrate reduction	-
Indole	-
Voges – Praskauer (VP)	+
Gelatin liquefaction	+
Sugar fermentation	
Glucose	-
Mannitol	-
Inositol	-
Sorbitol	-
Sucrose	+
Arabinose	+
Lactose	+
Fructose	+
Galactose	+
Maltose	+

Serological identification

By using Agar Gel Precipitation Test with specific antisera against ORT serotypes A, B, C, D, E, F and G, results revealed that all tested 6 isolates belonged to serotype A of ORT.

Detection of Antibacterial Sensitivity and Resistance Patterns of ORT using Standard Disk Diffusion method

The results were recorded in Table 2, which indicated that, all isolates were resistant to gentamycin, amoxycillin and cephradine with 100% incidence, where, 100% isolates were sensitive to colistin and doxycycline, also 50% of isolates were sensitive to ampicillin and streptomycin, 16.67% of isolates were sensitive to neomycin and trimethoprim.

Table 2. Results of antibacterial sensitivity and resistance of ORT isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Ampicillin	0	0	3	50	3	50
Amoxycillin	6	100	0	0	0	0
Colistin	0	0	0	0	6	100
Cephradine	6	100	0	0	0	0
Florfenicol	3	50	3	50	0	0
Neomycin	0	0	5	83.33	1	16.67
Erythromycin	2	33.33	4	66.67	0	0
Vancomycin	4	66.67	2	33.33	0	0
Doxycycline	0	0	0	0	6	100
Gentamycin	6	100	0	0	0	0
Trimethoprim	0	0	5	83.33	1	16.67
Tetracycline	1	16.67	5	83.33	0	0
Streptomycin	2	33.33	1	16.67	3	50
levofloxacin	6	100	0	0	0	0

Pathogenicity testing

Results of the experimental infection of chickens with ORT isolates by spray exposure at 14th day old, revealed that chickens of the first group, which were previously exposed to ND vaccine at 10th day old; seven days post infection, chickens showed signs of huddling together, ruffled feathers, nasal discharge and conjunctivitis, also depression and decrease appetite with no mortality. Lesions of sacrificed birds were sinusitis and slight tracheal congestion. 14th day post infection, clinical signs were exaggerated with sneezing and conjunctivitis, lesions were moderate tracheitis, bilateral pneumonia and moderate fibrinous airsacculitis.

The second group, which was infected with ORT isolates alone showed no clinical sings or postmortem lesions at 7 days post infection, while, at 14th post infection, it was noticed that clinical signs were nasal discharge, decrease water and feed consumption, depression, sneezing and slight conjunctivitis. Postmortem findings were slight sinusitis, mild tracheitis and mild airsacculitis. The control group was negative for clinical and pathological lesions.

Discussion

Respiratory diseases in birds generate sanitary and economic impacts, especially in the developing countries (Ellakany *et al*, 2018) and may be related to the environment and climate. Clinical signs and lesions are of little value in diagnosis, since many other infectious diseases can produce similar clinical signs and post mortem lesions (Hafez and Sting, 1996).

This study was conducted to isolate and identify ORT in broiler chickens, it was isolated in the present work from different organs as lungs, air sacs and tracheas as recorded by other authors (El-Gohary and Awaad 1998; Joubert *et al*., 1999; Amal, 2002; Rahimi, and Banani 2007; Mayahi *et al*., 2016). Moreover, it was reported that air sacs, lungs and tracheas are the most suitable for primary isolation from cases of respiratory infections (El-Gohary and Awaad, 1998).

In this work, 180 samples were collected from different broiler farms in Assiut Governorate that suffered from respiratory signs, which included nasal discharge, coughing, wet eyes sometimes with lacrimation and sinusitis. These findings were recorded by several authors (El-Gohary and Awaad, 1998; Amal, 2002; Canal *et al*., 2005; Rahimi, and Banani 2007; Asadpour *et al*., 2008).

It was reported that the organism is slowly growing and needs longer incubation period (Travers *et al*., 1996; Amal, 2002). The successful isolation of ORT in present study may be attributed to the addition of gentamicin to the medium, which was effective in suppression of contaminating bacteria. Since it has been shown that most of ORT isolates are resistant to gentamicin, (Back *et al*., 1997). Colonies of ORT on blood agar under CO_2 tension were gray to grayish white, opaque, circular, convex butyrous, sometime with reddish glow and always with distinct odor of butyric acid and these agrees with Shahata, *et al*. (2006). ORT colonies were not hemolytic on blood agar, but some isolates can cause incomplete hemolysis especially after 96 h, ORT also grew on BHI broth and trypticase soya agar but not on MacConkey agar, these characters were also described by previous studies (Hafez *et al*., 1993; Roepke *et al*.,1998; Post *et al*., 1999; Amal, 2002; Asadpour *et al*., 2008; Mayahi *et al*., 2016). Concerning cellular morphology of ORT, it appeared as Gram negative, pleomorphic rods bacteria, as described by Van Empel and Hafez (1999); Amal (2002); Bisshop (2003); Shahata *et al*. (2006) and Espinosa *et al*. (2011).

Biochemical tests revealed that ORT isolates were catalase negative and oxidase test positive and these findings agree with El-Gohary and Awaad (1998); Chin and Charlton (2008) and Ellakany *et al*. (2018). The result of API 20 NE strips of the ORT isolates were positive for Arginine dihydrolase, β- galactosidase, Gelatin liquefaction and Voges-Praskauer and negative for L-lysine decarboxylase, Ornithine decarboxylase and H_2S and these agrees with Amal (2002); Canal *et al*. (2005) and Chin and Charlton (2008). The results of sugars fermentation by API 20 NE were negative for glucose, mannitol, inositol and sorbitol, and positive for sucrose, arabinose, lactose, fructose, galactose and maltose, these results were reported by Amal (2002); Rahimi and Banani (2007); Chin *et al*. (2008) and Mayahi *et al*. (2016).

PCR is considered to be a useful laboratory tool for the definitive identification of suspected ORT isolates, due to difficulty in isolation and biochemical characterization as it is usually associated with overgrown by other bacteria (Chansiripornchai *et al*., 2007; Chin *et al*., 2008; Churria *et al*., 2011; Churria *et al*., 2012). In the present study, 12 of the most suspected isolates were tested by PCR, which is a fast, sensitive, and specific method to identify and characterize a bacterial strain (Hung and Alvarado, 2001; Ozbey *et al*., 2004; Hassanzadeh *et al*., 2010), and results revealed six positive ORT iso-

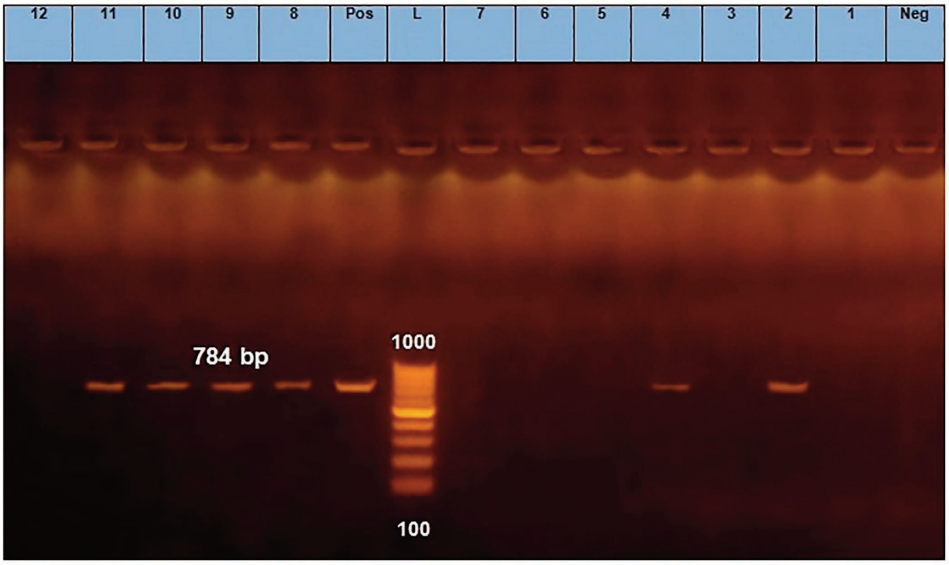

Fig. 1. An agarose gel stained with ethidium bromide, with PCR products of ORT isolates (M: 100 bp DNA ladder, lanes 2,4,8,9,10 and 11: positive for ORT).

lates, these results came in line with many authors (Li and Diao, 2009; Banani *et al*., 2009; Wu *et al*., 2010; Ellakany *et al*., 2018).

The six PCR positive isolates of ORT were subjected to serotyping using agar gel precipitation test with specific antisera against 18 serotype, and results explained that all tested isolates were belonged to serotype A, these result were also noticed by Amal (2002); Chin and Charlton (2008) and Wu *et al*. (2010). It was mentioned that serotype A has been found to be the most prevalent serotype among chicken strains (97%) (Van Empel *et al*., 1997; Amal, 2002; Chin *et al*., 2008).

In the present study, using phenotypic methods of isolation revealed that 32 isolates of ORT from broiler chickens in Assiut Governorates with incidence rate 17.77%, while using PCR revealed that 6 positive samples with incidence ratio 3.33%. These results more or less with in accordance with an incidence rate of 8.6% by Youssef and Ahmed (1996) and El-Gohary and Awaad (1998), 4.3% by El-Gohary *et al*. (1998), 3.2% by El-Gohary and Sultan (1999), 5.8% by Amal (2002), 7.27% by Elbestawy (2010), 10% by Mousavi *et al*. (2012) and 11.66% by Ellakany *et al*. (2018).

Antimicrobial agents are used widely in the veterinary and medical fields. The present work was designed to evaluate invitro susceptibility and resistance of ORT isolates to some commonly and recently used antimicrobial agents. The antibacterial sensitivity of ORT strains could differ according to region, possibly due to inherent genetic differences between breeds and the antibiotic resistance of agents (Odor *et al*., 1997; Malik *et al*., 2003; Türkyilmaz, 2006). In the current study, the results of antimicrobial susceptibility test of ORT isolates showed that all isolates were resistant to gentamycin, levofloxacin, amoxycillin and cephradine, while all isolates were sensitive to colistin and doxycycline and all the isolates were moderately sensitive to erythromycin, neomycin, trimethoprim and tetracycline. Shahata *et al*. (2006) revealed that amoxicillin, enrofloxacin and tetracycline were the most effective drugs against ORT. Asadpour *et al*. (2011) reported that all ORT isolates (100%) were resistant to enrofloxacin, ciprofloxacin, erythromycin, tetracycline, and all of them were susceptible to ceftriaxon, but two isolates (66.70%) were moderately sensitive to amoxicillin and sensitive to florfenicol. Churria *et al*. (2016) reported that all isolates of ORT were non-susceptible to gentamicin, most of them were non-susceptible to enrofloxacin, erythromycin, trimethoprim-sulfamethoxazole, doxycycline and fosfomycin, while they were susceptible to ampicillin and florfenicol. Susceptibility to ampicillin and florfenicol was significantly higher compared to all other antibacterial drugs tested. Mohd-Zain *et al*. (2008) noticed that all the isolates were susceptible to chloramphenicol but were resistant to ampicillin, enrofloxacin and the combination of sulfanomide with trimethoprim. Majority (77.8%) of the isolates of ORT were resistant to amoxicillin. Hegazy *et al*. (2015) reported that three different isolates were sensitive to amoxicillin, ampicillin and doxycycline but were resistant to gentamycine, norofloxacin, ciprofloxacin, cefotaxim, sulphamethoxazole trimethoprim and colistin sulphate.

Van Veen *et al*. (2001) revealed that isolated strains of ORT were sensitive to amoxycillin, tetracycline, enrofloxacin and trimethoprim/sulphonamid, also reported that the percentages of strains sensitive to amoxycillin and tetracycline decreased in successive years from approximately 62 to 14 percent, and four strains were resistant to enrofloxacin or trimethoprim/sulphonamide. ORT strains examined in this research have been shown to be resistant to some of the major antibiotics, perhaps due to inappropriate use of antibiotics for treatment of secondary infections related to the prevalence of respiratory diseases complex in broiler chicken farms. Several antimicrobial agents, including those most recently developed, are becoming inefficient against ORT, reinforcing the hypothesis of continuous resistance transference among them (Banani *et al*., 2001; Devriese *et al*., 2001; Watteyn *et al*., 2016), with an increased resistance for different classes of drugs (Cauwerts *et al*., 2002). Mayahi *et al*. (2016) mentioned that the sensitivity pattern of ORT strains depends on the source of the strain and routinely used antibiotics in the area.

In this study, the pathogenicity of ORT was evaluated by aerosol exposure in 2 weeks old chickens. In chickens of group 1, symptoms appeared to be aggravated by prior administration of ND vaccine, clinical signs appeared as depression, ruffled feather and respiratory signs in form of conjunctivitis, labored respiration, sneezing and nasal discharge, there were decreases in feed and water intake. Regarding the PM lesions, there were severe pneumonia, air sacculitis and tracheitis and that reported also by many authors (Travers *et al*., 1996; Van Empel *et al*., 1996; Amal, 2002). These data matched with Van Empel *et al*. (1996); Amal (2002) and Ellakany *et al*. (2018), who found that vaccination stress caused higher degree of pathogenicity and severe growth retardation than the single infection of ORT.

In group 2, the most characteristic finding of ORT infection alone caused only mild respiratory manifestations with no recorded mortality, Mild to moderate gross lesions in the respiratory system such as mild tracheitis and mild to moderate airsacculitis. This result was noticed by other authors (Van Empel *et al*., 1999; Amal, 2002; Hegazy *et al*., 2015; Ellakany *et al*., 2018; Ellakany *et al*., 2019). Van Empel *et al*. (1996) reported that administration of ND Lasota vaccine at 5 days prior to ORT challenge induced a more serious increase of airsacculitis and pneumonia scores, as compared with both ORT challenge and ND Lasota vaccine administration alone.

No mortalities were recorded in any of the groups, as the environmental conditions were good during the experiment. However, under field conditions, the severity of the ORT infection's clinical signs, duration of the disease and mortality were extremely variable; they were influenced by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter condition, poor hygiene, variable ammonia levels, concurrent disease, and types of secondary infection (Travers *et al*., 1996; Ellakany *et al*., 2018), and the challenged bacteria could easily be re-isolated from the affected organs (Van Empel *et al*., 1999; Amal, 2002; Shahata *et al*., 2006).

Conclusion

It was concluded that the incidence of ORT infection in broiler chickens in Assiut Governorate is 17.77% using isolation and phenotypic methods of identification, while it is 3.33% using molecular method of identification. All ORT isolates, which were serologically tested, using agar gel precipitation test, were belonged to serotype A. Antibacterial sensitivity testing of ORT isolates is essential to determine the drug of choice for treatment. Inappropriate use of antibacterial drugs led to decreased efficacy of them in treatment of bacterial diseases including ORT infection. Living Newcastle attenuated vaccine, Lasota vaccine, exaggerates the clinical signs and lesions of ORT experimental infection.

Acknowledgement

The authors would like to thank the staff members in the Department of Poultry Diseases, Faculty of Veterinary medicine, Assiut University and in Assiut Regional Laboratory, Animal Health Research Institute, Agricultural research Center for offering facilities in processing the materials used in this study.

Conflict of interest

Authors declared no conflict of interests exist.

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