BACTERIOLOGICAL STUDIES ON ORNITHOBACTERIUM RHINOTRACHEALE (ORT) IN CHICKENS

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ABSTRACT

Efforts were directed to study 720 diseased chickens (broilers and broiler breeders) taken from 40 flocks with variable clinical signs including mild to severe respiratory manifestations either alone or associated with growth retardation, arthritis, nervous signs and lameness preceded by increase of mortalities. The mortality rates varied between 0.2% and 16.8%. Ornithobacterium rhinotracheale (ORT) was isolated from 23 out of the 40 flocks (57.5%) and were recovered from various organs including lungs, trachea, air sacs, joints, sinuses, brains and yolk sacs of recently dead and diseased birds, while no strains could be isolated from heart blood, pericardium, liver and eyes. It could be isolated also from hatcheries that suffered from lower hatchability, increased late embryonic death and dead in shell chicks. The serotyping of the isolates by AGPT revealed that they all belonged to serovar A. The sensitivity tests against antibiotics of the 23 isolates of ORT revealed that 100% of the isolates were sensitive to ampicillin, amoxicilline, oxytetracycline, ciprofluxacine and chloramphenicol, while all of them were resistant to gentamycin, streptomycin, colistine and sulphamethoxazale-trimethoprim. Inoculation of S.P.F embroynated chicken eggs by ORT isolated strains revealed that there were no characteristic lesions in embryos except some congestion in the head and neck of the embryos. Also they could be isolated from the yolk sac of newly hatched chicks.

INTRODUCTION

Ornithobacterium rhinotracheale is a recently discovered bacterium, of the rRNA superfamily V, first named in 1994 **(Lopes et al., 2000)**. The first occurrence was reported in wild birds and later shown to be widespread worldwide in commercial poultry **(Vandamme et al., 1994)**. Bacteriological examination revealed the detection of a slowly growing, pleomorphic Gram negative rods (PGNR), which could not be classified into one of the known species **(Charlton et al.,**

1993). This bacterium was initially named Pasteurella-like **(Hafez et al., 1993)** or Kingella-like **(Van Beek et al., 1994)** microorganism. **Bisgaard (1989)** stated that the organism could be classified within a group of bacteria designated taxon 28. By further identification of isolates obtained from the respiratory tract of different species from different countries using genetic taxonomic methods ultimately the name Ornithobacterium was suggested for this new genus within the rRNA superfamily V and the species name rhinotracheale was assigned **(Vandamme et al., 1994)**. It constitutes a distinct species and genus placed in taxonomic neighbourhood of the genera Flavobacterium, Cytophaga, Capnocytophaga and Riemerella **(Hinz et al., 1994)**.

Ornithobacterium rhinotracheale (ORT) infection is considered a serious problem facing the poultry industry. It can affect all avian species (wild and domestic birds) and is associated with respiratory diseases. Air sacculites and pneumonia are the most common features of infection. Other factors, especially respiratory viruses and bacterial infections aggravate these clinical signs. Infection can be transmitted horizontally by aerosol droplets and also vertically in eggs. Currently 18 serovars designated A to R seem to exist **(Van Empel., 1998)**. Most of chicken isolates belong to the serovar A.

The aim of the present work was to isolate ORT from broiler chickens and egg samples from hatcheries, identify the isolates using convential test and API system and study the pathogenicity of ORT strains in S.P.F. embrionated chicken eggs.

MATERIAL AND METHODS

Chickens : 720 broiler chicken samples representing 40 broiler flocks of different ages (1 to 42 day), were collected from different localities including Cairo, Giza, El-Sharkya and El-Behira governorates. The chickens suffered from variable clinical signs including respiratory manifestations, lameness, growth retardation, and nervous manifestations.

Residual unhatched eggs: 543 unhatched eggs with culled chicks were collected from three hatcheries that suffered from problems of decreased hatchability, increased late embryonic death and increased death in shell.

Specimens were collected from moribund and freshly dead birds under aseptic conditions using sterile cotton swabs.

Isolation and identification of ORT bacteria:

Samples from different organs as tracheas, lungs, sinuses, eyes, joints, air sacs, pericardium, bone marrow and liver for primary isolation were cultivated on 10% defibrinated sheep blood agar containing 10 ug/1ml of gentamicin sulphate to inhibit the over growth of other bacteria

according to **Back et al. (1996)**, incubated at 37°C for 24-48 hours in microaerophilic condition using candle jar **(Vandamme et al., 1994 and Travers et al., 1996)**. For isolation of other bacteria ths same samples were also inoculated on MacConky agar, brain heart infusion agar, PPLO broth, MORT medium, tryptic soy agar medium, XLT4 agar, Salmonella Shigella agar, brilliant green agar and Vogel Johnson agar medium, then incubated under microaerophilic condition at 37°C for 24 - 48 hours.

The suspected colonies of ORT were examined for their colonial morphology (shape, size, colour, appearance, elevation, haemolysis and the adhesion to the media). Microscopical films were prepared from the suspected pure colonies and stained with Gram's stain. Other films were prepared from different types of colonies, which grew on other types of media for differential diagnosis.

Pure colonies of ORT were sub-cultured on semi-solid slope agar, peptone water and brain heart infusion broth containing NAD and 1-2% swine or horse serum as enrichment to enhance the growth and multiplication of ORT according to **Opengart (1996)**. ORT strains were tested biochemically according to **Charlton et al. (1993)**. In addition, biochemical identification by using Api system (Api 20E and Api ZYM) was done. The anti-biogram was done according to the technique described by **Back et al. (1997)**.

Preparation of outer membrane protein was made according to **Charles et al. (1994)** using three ORT strains originated from different examined flocks were selected. The outer membrane proteins were separated through sodium dodecyle sulphate (SDS)-polyacrylamide gel-electrophoresis. The destined gel was scanned using computer densitometry scanner. Protein fractions were expressed as percentage area. Molecular weight was also expressed in both marker and samples.

Serotyping of the isolates by agar gel precipitation test (AGPT) Preparation of the antigen for agar gel precipitation test was made according to **Heddleston et al. (1972)** and the test was performed according to the method described by **Van Empel et al. (1997)**.

The antibiogram of ORT isolates was determined according to **Cruickchank et al. (1975)**.

Pathogenicity test in SPF eggs :

Three isolates were selected and cultivated on 10% sheep blood agar for 24 hour at 37°C under microaerophilic condition. The bacteria were harvested from the cultures by washing with 0.15 m saline and concentrated by centrifugation to 10^9 colony forming units per ml. Thirty SPF embryonated chicken eggs of 11-day-old were examined by candling, and inoculated via chorio-

allantoic membrane with 0.2 ml. of peptone broth containing 10^9 CFU and gentamicin sulphate, using tuberculin syring according to **Odor et al. (1997)**. The inoculated eggs were candled daily and the embryos that died within the first 24 hours were discarded as non-specific deaths caused by contamination or injury. The mortalities were then recorded, and were removed from the incubator. Cultures were then made from yolk sac and allantoic fluid of the embryos on sheep blood agar, nutrient agar, Vogel-Johnson agar and MacConky agar. The embryos and the chorio-allantoic membrane were examined for any characteristic lesions. Control groups included five eggs inoculated with saline only and other 5 eggs left without inoculation.

The five embryos were left till hatching date, and then the hatched chicks were examined bacteriologically for ORT.

RESULTS

Isolation of ORT from clinical cases :

From Table (1) it is clear that ORT could be isolated from 23 out of the 40 flocks examined (57.5%); age varied from 1 day to 42-day-old and mortality rate varied from (0.2%- 16.8%).

Incidence of ORT isolation from various organs of examined broiler flocks:

It is evident from Table (2) that the highest rate of isolations of ORT was achieved from the air sacs (63%); followed by lungs (59%); joints (46.6%); tracheas (40%); sinuses (37.5%); brain (30%); yolk sacs (26.6%) and bone marrow (7.1%), while no isolation was achieved from eyes, liver, heart blood and pericardium. On the whole, it was succeeded to recover 376 isolates from 909 organs examined (41.3%).

It is worthy to note that ORT was always isolated in association with other microorganisms. From 19 flocks showing respiratory manifestation, ORT was associated with *E. coli and Mycoplasma gallisepticum*, in 4 flocks with *S. aureus*, in 3 flocks with *P. aeruginosa* and in 2 flocks with *P. aeruginosa* and Salmonella.

Isolation of ORT from hatcheries:

From the results demonstrated in Table (3), it is evident that in hatchery No.1, ORT could be isolated from yolk sacs of one-day-old chicks (16.7%), dead-in-shell chicks (6.8%) and late embryonic deaths (5.7%). *Pseudomonas aeruginosa* was frequently recovered in this hatchery. In hatchery No. 2, ORT could be isolated from late embryonic deaths (13%), dead-in-shell chicks (6.8%) and one-day-old chicks (6.7%) but not from other samples. This hatchery was infected

with Salmonella Montevideo. In hatchery No. 3, ORT could not be isolated from any of the samples examined and only Salmonella species could be isolated. In all cases, ORT could not be isolated from rotten eggs, infertile eggs and early embryonic deaths.

Isolation of ORT on different types of media :

ORT could be isolated successfully on sheep, goat, bovine, buffalo, camel, swine and rabbit blood agar media on primary isolation. Chicken blood agar gave variable degrees of growth of ORT on primary isolation. ORT could also be isolated on chocolate blood agar with better growth of colonies and decreased contamination with other bacteria. ORT could be isolated well on brain heart infusion agar supplemented with NAD and serum, without addition of thallium acetate and crystalline penicillin, which inhibits the growth of ORT. Also ORT could be isolated on tryptic soy agar plates after 24-48 hour incubation. This medium contains enrichment material, which enhances the growth of ORT colonies. No growth was observed in PPLO broth. On the other hand, ORT could not be isolated on other media such as Vogel Johnson agar, XLT4, Salmonella Shigella agar (S.S.), MacConkey agar and Brilliant green agar (Table 4).

Enhancement of the growth of ORT

a - **Sheep blood agar without addition of antibiotics** : It was observed that there was an increase in the number of other bacteria as *E.coli*, Proteus and Staphylococcus spp. on blood agar, which contained antibiotics. Such bacteria overgrew ORT colonies, which are commonly very small in size.

b - **Sheep blood agar containing gentamicin sulphate** : The colonies of ORT on this medium were observed easily due to suppression of the other bacterial contamination.

c - **Sheep blood agar after addition of gentamicin and 1% NAD solution to the media** : It was observed that ORT growth was enhanced by addition of NAD to the medium and addition of gentmycine sulphate to the medium proved to be necessary for selective isolation of ORT. However, blood agar plates without antibiotics should be always used also to prevent missing of the 5%-10% antibiotic susceptible isolates.

Growth at different types of incubation temperature.

The optimum growth was obtained, when the plates were incubated at (37-38°C.) However, ORT could grow also at various temperatures, namely 25, 30, 35, 40, 42°C and bad growth was

observed at 45°C.

Identifecation of the isolated organism.

Examination of smears stained by Gram's stain prepared from suspected colonies revealed the presence of Gram negative, pleomorphic, rod shaped bacterium which was non-capsulated and non-sporulated organism. It appeared as short and plump rods that measured 0.2-0.9 X 1-3 microns.

Colonies on blood agar appeared as pin-point colonies, less than1mm in diameter after 24 hours and by 48 hours, colonies were pin-headed, approximately 1-3 mm in diameter, circular, opaque gray to grayish white, sometimes with reddish glow colouration. The colonies were convex, with smooth surface, rounded, glistening, shinny raised on media surface, with entire edge, non-pigmented, non-adhesive and non-haemolytic. The colonies had a characteristic butyric acid odour.

When the primary cultures were subcultured, the colony size became more uniform. Subcultures of ORT colonies on different types of media could be done aerobically without need to CO2 tension for optimal growth and were obtained after 24 hours incubation. The colonies reached a reasonable size and had characteristic butyric acid odour. ORT grew well in peptone water, brain heart infusion broth and tryptose soy broth with uniform turbidity. ORT isolates grew on semi-solid slope agar and remained viable for 3 months when preserved at 4°C.

Biochemical identification using standard biochemical tests :

The biochemical test results of the isolates compared with the results of 18 reference strains of *O. rhinotracheale* reported by **Hinz et al. (1994)** were given in Table (5). All strains of *O. rhinotracheale* were positive in the oxidase test, negative catalase and methyle red tests and some strains gave positive VP test. No growth was observed on Simmon's citrate agar medium and no motility in motility medium, indole was not detected in motility indole ornithine (MIO) medium. Some strains grew on TSI slants with acid production in the butt and slant portions of the tube. Urea production was variable as determined in both Christensen urea agar slants and broth.

All isolates did not hydrolyze gelatine after 24-48 hours and gave a positive reaction (acid production without gas) in both oxidation and fermentation test tube media. The acid production from carbohydrates was tested in phenol red broth base containing one percent carbohydrate and 2 per cent inactivated chicken serum. Although heavy inocula were used, positive reactions did not appear before 48 to 72 hours incubation. Glucose, galactose, lactose, maltose, and fructose were fermented by majority of isolates. Fermentation reactions of manitol, xylose, sorbitol,

salicin, inositol, glycine and trehalose were variable

Biochemical identification of ORT isolated from broiler chickens using Api 20E

Table (6) demonstrates the reactions of ORT in API 20 E system). The biochemical test results of 23 isolates were compared with three reference strains isolated in South Africa and published by **Travers et al. (1996)** and the reference NO. 4 was published by Van **Empel et al. (1996).** All isolates were (100%) positive for b-galactosidase (ONPG) and arginine dehycholase (ADH), (56.5%) of the isolates reacted positively in the urease test., (73.9%) in Vogous- Proskaver test, while they proved to be negative in lysine decarboxylase, ornithine decarboxylase, citrate utilization, H2s production, tryptophane deaminase, indole and methyle red. Three isolates were positive for gelatine liquifaction after 48-hour incubation. The results of fermentation of glucose, galactose, manitol, inositol, sorbitol, rhaminose, sucrose, melliose, amygtalin and arabinose were variable.

Determination of enzymatic activities of ORT isolates by Api ZYM system :

As shown in table (7), the enzymatic reactions of ORT isolates in comparison to reference strains described by Vandamme et al. (1994) showed uniform results.

Serological Identification of the isolated organism :

Serotyping of all positive isolates by using agar gel precipitation test with specific monovalent antisera against ORT (serovar A to R) revealed that all isolates belonged to *O. rhintracheale* serovar A.

Determination of antibiogram of the isolated ORT strains :

The results as shown in table (8) indicated that all tested isolates (100%) were sensitive to ampicilline, amoxicilline, oxytetracycline, ciprofluxacine and chloramphenicol; (70%) of the strains were sensitive to doxycycline; (35%) to enrofluxacine; (22%) to erythromycin, and (13%) of strains were sensitive to flumiquine. All strains were resistant to gentamycin, streptomycin, sulphamethazol-trymethoprim and colistine sulphate.

Pathogenicity of some ORT isolates to 11-day chicken embryos :

The inoculation of ORT into 11- day -old embryos in SPF eggs caused death of 7 out of 30

cases (33.3%). The death of one embryo on the first day post inoculation is most probably accidental. The 9 other deaths spread, however, on the 4th.-10th. day post inoculation, with highest rate on 4th PI. ORT was recovered from all dead embryos and hatched chicks as illustrated in (Table 9). Congestion in head and neck of dead embryos and chorio allantoic membrane was observed in dead embryos, and distended yolk sac in dead-in-shell. Ornithobacterium could be isolated from yolk sacs of hatched chicks. In two control inoculated and non-inoculated groups, no mortality was observed and the ORT could not be isolated from the control hatched chicks.

Electrophoretic pattern of some ORT field isolates using SDS —PAGE:

The outer membrane proteins (OMP) of three representative strains isolated from lung, joint and yolk sac were prepared and electrophoriezed in SDS-PAGE. Obtained results are shown in Figure (1). The majority of protein bands were located in between 16.233 - 111.19 KDa.

The three strains shared a common molecular weight at 45.525 KD; 34.696KD; 26.383 KD and 16.233KD. The strain no. 2 and strain no.3 were nearly similar to each other with some minor variation in expression of protein bands. The differences were noted in lack of protein expression in bands no. 2,3 and 7 of the strain no. 2, and bands no.2, 3,4,5,6,7,8 in the strain no. 1 in comparison to strain no. three.

DISCUSSION

In the present study, four broad categories of symptoms were identified, the first being primarily upper respiratory signs, which were associated with mortalities and the results revealed that 65.5% of respiratory cases were positive for ORT isolation. This finding is nearly similar to investigation recorded by **Hafez (2002) and Travers (1996)**. The second category included respiratory manifestations associated with growth retardation as mentioned by several authors **(Joubert et al., 1996; Van Beak et al., 1994 and Sprenger et al., 2000)**. The third group showed lameness and arthritis with swelling of infraorbital sinuses. These signs were reported also by **Van Empel (1994); Clark (1996) and Travers et al. (1996)**. The last category showed nervous signs (ataxia, head tremors) followed by respiratory manifestations. Similar observations were reported by **Van Empie and Hafez (1999) and Sakai et al. (2000)**. In all groups there were poor average-daily gain and feed conversion rate as reported by **Van Empel and Hafez (1999)**.

The cases demonstrated in this study suggest that the strain of ORT isolated in Egypt is pathogenic and causes serious problems particularly in older age. Different investigations showed that the ORT was present in the chicken population in Egypt and plays a significant role in the

diseases **(El-Gohary, 1998; El-Gohary and Awaad, 1998)**. Therefore in cases associated with respiratory diseases, lameness problems and head tremors; ORT should be considered as a possible causative agent in association with other primary or secondary pathogens and thus, isolation techniques should be adapted accordingly **(Hafez, 1996 and Sakai et al., 2000)**.

ORT could be isolated, in the present work, from different organs as lungs, air sacs, tracheas, sinuses, joints, brains, pericardium, yolk sacs. This was recorded by other authors, e.g. **El-Gohary and Awaad (1998) and Joubert et al. (1999)**. Moreover, it was reported that the air sacs, lungs and tracheas are the most suitable organs for primary isolations from cases of respiratory infections **(El-Gohary and Awaad, 1998)**, while joints and brains are preferred in cases of arthritis and meningitis **(Joubert et al., 1999)**. Culture of heart blood and liver under field condition has revealed negative results. This finding agrees with **Hafez, (2002)**. Also ORT could be isolated from non-absorbed yolk sac of 4-day-old chicks from 2 separate outbreaks of high moralities and increased number of culls in concomitant infection with Salmonella Montevedeo and associated with *Pseudomonas aeruginosa*.

It is worthy to mention that, ORT was found associated with hatchery problems, and could be isolated in low incidence from dead in shell, late embryonic death and one day old chicks in concomitant infection with Salmonella Montevideo and *Pseudomonas aeruginosa* infection. This finding goes in agreement with that of **El-Gohary (1998); Nagaraja et al. (1998) and Van Empel (1997)**. On the other hand, ORT could not be isolated from infertile eggs or early embryonic death. This was explained by the finding that ORT did not survive on egg shell for more than 24 hours **(Varga et al., 2001)**.

It is to be noted that the organism is slowly growing and needs longer incubation period **(Travers et al., 1996)**. The successful isolation of ORT in the present study is attributed to the consideration of all these points. Moreover, the addition of gentamicin to the medium 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)** recommended the use of 10 ug gentamicin per 1 ml blood agar medium to isolate ORT from contaminated samples with fast growing bacteria as E. coli and Proteus. This is also the opinion of **Van Empel (1996) and DeRosa et al. (1996)**.

The sensitivity of the isolated strains against 13 antimicrobial agents was studied by using disc diffusion technique. The results revealed that the isolates were 100% sensitive to ampicilline, amoxicilline, oxytetracycline ciprofluxacine and chloramphenical. These results resemble those obtained by **El Gohary and Awaad (1998) and Joubert et al. (1999)**. Poor sensitivity of the isolates to enrofluxacine and flumiquine was reported also by **Hafez (1996); Van Beek et al. (1994) and Hinz et al. (1994)**. On the other hand, all strains were resistant to gentamicin,

streptomycin: sulphonamid trimethroprim and colistine.This was in agreement with **Devriese et al. (1995); Dudoyt et al. (1995);; Odor et al. (1997); El Gohary (1998) and Abd-El Ghany (2000).**

The confirmation of strains could be carried out using serological examination with known positive antisera in agar gel precipitation test (AGPT) and ELISA according to **Van Empel (1998) and Hafez and Sting (1999)**. Currently 18 serovars designated (A to R) seem to exist according to **Van Empel (1998)**. Serotyping of the 23 strains of ORT recoverd in the present study by using AGPT revealed that all tested isolates were belonging to serovar A. This finding was supported with the previous results recorded by **Hafez (1996); El-Gohary and Awaad (1998); El-Gohary et al. (1998); Van Empel (1998), AbdEl-Ghany (2000) and Sanaa (2002)**. They mentioned that ORT serovar A is the most prevalent, important and common serotype identified in chicken.

Attempts were also done to investigate the pathogenicity of ORT isolates in embryonated chicken eggs and the possibility of egg transmission to hatched chicks. Inoculation of ORT on CAM of 11-day SPF embryo chicken eggs revealed that, ORT could be propagated in chicken embryos after 24 hours post inoculation and large numbers of bacteria can be isolated from the yolk in pure form. On the other hand, no pathgnomonic lesions could be detected in the inoculated embryos except some congestion in head and neck of the dead embryos and the embryonic mortalities were (33.3%). Also ORT was isolated from the yolk sacs of the newly hatched chicks suggesting the possibility of egg transmission. This finding agrees with **Nagaraja et al. (1998), El Gohary (1998) and Hafez (2002)**.

Characterization of the three ORT field isolates by the outer membrane protein extract using SDS-PAGE technique revealed that there were substantial differences between individual field strains. The majority of the protein bands were located between 16.23- 111.19 KD. The strains had a shared common molecular weight at 45.52 : 34.69 : 26.38 : and 16.23 KD. These results are nearly similar to the results reported by **Hung and Alvarado (2001)** The difference in the protein makeup of the isolated strains may constitute a major problem in trials of vaccine production against ORT infection and preparation of a sub unit vaccine depending on the shared genes between different isolates. This should be taken in consideration on preparation of such vaccine to overcome this obstacle **(Sanaa, 2002)**.

Table (1) : Results of ORT isolation from clinically infected flocks.

No. of examined flocks	No of tested chickens	Mean age (days)	Range of mortality	No. of positive flocks	Percentage of positives
40	720	1 - 42	0.2%-16.8%	23	57.5%

Table (2) : Incidence of ORT isolation from various organs of broiler chicken.

Organs	Total No of examined organs	Total No of ORT isolates	Rate of ORT isolation
Air sacs	240	152	63%
Lungs	160	95	59%
Tracheas	95	38	40%
Peircardium	46	0	0%
Sinuses	24	9	37.5%
Livers	48	0	0%
Eyes	32	0	0%
Joints	60	28	46.6%
Bone marrow	14	1	7.1%
Brains	40	12	30%
Yolk sacs	150	40	26.6%
Total	**909**	**375**	**41.3%**

Table (3) : Isolation of ORT from hatcheries with problems.

Hatcheries	Total No.	No. of ORT +	Isolation %	Other bacterial infection
Hatchery (1)				
I.E	18	0	0	
E.D	29	0	0	
L.D	70	4	5.7	*Pseudomonas aeruginosa* infection
Dead-in-shell	88	6	6.8	
Rotten egg	24	0	0	
One-day chicks	30	5	16.7	
Hatchery (2)				
I.E	56	0	0	
E.D	29	0	0	Salmonella species infection
L.D.	31	4	12.9	
Dead-in-shell	29	2	6.8	
Rotten egg	18	0	0	
One-day chicks	30	1	3.3	
Hatchery (3)				
I.E	27	0	0	
E.D	12	0	0	Salmonella species infection
L.D.	18	0	0	
Dead-in-shell	6	0	0	
Rotten egg	9	0	0	
One-day chicks	30	0	0	

IE = infertile eggs, LD = late embryonic deaths , ED = Early embryonic deaths

Table (4) : Different media used for Isolation of ORT.

Types of media	ORT positive isolates	Reference strains*
-Sheep blood Agar	+	+
-Goat blood Agar	+	N
-Bovine blood Agar	+	+
-Buffalo blood Agar	+	N
-Camel blood Agar	+	N
-Swine blood Agar	+	N
-Rabbit blood Agar	+	N
-Chicken blood Agar	V	N
-MacCkonkey	-	-
-Brain heart infusion Agar	+	+
-PPLO broth	-	-
-MORT	+	N
-Vogel Johnson	-	N
-XL T4	-	N
-Brilliant green	-	N
-S.S. Agar	-	N
-Tryptic soy Agar	+	+

Positive =(+) Negative =(-) Variable =(V) Not tested=(N) *Reference strains growth results were reported by ***Vandamme et al. (1994)*** and Lombardi et al. (1999)

Table (5) : Biochemical identification using standard biochemical tests:

Reactions	ORT isolates(n=23)	Reference strains (18)
Gram stain	Gram negative rods (23)	Gram negative rods
Catalase	Negative (23)	Negative (17)
Oxidase	Positive (23)	Positive (17)
Indole	Negative (23)	Negative (18)
Urease	Positive (13)	Positive (18)
Voges - Proskauer	Positive (17)	Positive (17)
Methyl red	Negative (23)	Negative (18)
Growth on TSI	Positive (4), Negative H_{2s} production	Not tested
Citrate utilization	Negative (23)	Not tested
Ornithine decarboxylase	Negative (23)	Negative (18)
Oxidative/fermentation of -glucose -fructose -lactose -maltose -galactose -mannose	 Positive (18) (variable) Positive (19) (variable) (variable) (variable)	 Positive (15) Positive (17) Positive (18) (variable) (variable) Not tested
Motility test	Non motile	Non motile

The results of 18 reference strains puplished by ***Hinz, (1994)***.

Table (6) : Biochemical identification of ORT isolated from broiler chickens using Api 20E.

	No of suspected isolates																							R 1, 2, 3	R 4
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LDC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ODC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-/+	-/+	-/+	-	-	-	-	-	+
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
URE	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	
GEL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+
GLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MAN	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	
INO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SOR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
RHA	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	
SAC	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	
MEL	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	
AMY	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	
ARA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O/F test	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+	

Three reference strains isolated in South Africa and published by *Travers et al.,(1996)* and the reference NO. 4 was referred to it by *Van Empel et al.,(1996).*
ONPG: Beta galactosidase. ADH : Arginine dehydrolase.LDC : Lysine decarboxylase .ODC : Ornithine decarboxylase.
CIT : Citrate utilization.OX : Cytochrome oxidase. H2S : H_2S production (hydrogen-2-sulfide production).URE : Urease.
TDA : Treptophane deaminase.IND : Indole production.VP : Acetoin production RHA : Rhamnose (Fermentation / Oxidation)
GEL : Gelatinase.GLU : Glucose (Fermentation / oxidation).MAN : Mannitol (Fermentation / Oxidation).
INO : Inositol (Fermentation / Oxidation) SOR : Sorbitol (Fermentation I Oxidation). R=Reference strains

H2S : H_2S production (hydrogen-2-sulfide production).
URE : Urease.
TDA : Treptophane deaminase.
IND : Indole production.
VP : Acetoin production
GEL : Gelatinase.
GLU : Glucose (Fermentation / oxidation).
MAN : Mannitol (Fermentation / Oxidation).
INO : Inositol (Fermentation / Oxidation).
SOR : Sorbitol (Fermentation I Oxidation).
ARA : Arabinose
SAC : Sucrose
MEL : Melibiose
AMY : Amygtalin

Table (7) : Determination of enzymatic activities of ORT isolates by Api ZYM system.

Reactions	ORT isolates	Reference*
N-Acetyl-β-glucosaminidase	Positive	Positive
Phosphohydrolase	Positive	Positive
Acid phosphatase	Positive	Positive
Chymotrypsine	Positive	Positive
Alkaline phosphatase	Positive	Positive
Esterase	Positive	Positive
Esterase lipase	Positive	Positive
Leucine aminopeptidase	Positive	Positive
Valine aminipeptidase	Positive	Positive
Cystine aminopeptidase	Positive	Positive
Trypsine	Positive	Positive
β-galactosidase	Positive	Positive
α-glucosidase	Positive	Positive
β-glucosidase	Negative	Negative
β-Glucoronidase	Negative	Negative
α-Fucosidase	Negative	Negative
α-mannosidase	Negative	Negative
Lipase	Negative	Negative

*Reference strains described by *Vandamme et al. (1994)*

Table (8) : Results of in- vitro sensitivity of Ornithobacterium.

Name of antibiotic	Abbreviation	No. positive	No. tested	Sensitivity %
Ampicilline	Amp	23	23	100%
Amoxicilline	Aml	23	23	100%
Oxytetracycline	OT	23	23	100%
Ciprofluxacine	CIP	23	23	100%
Chloramphenicol	C	23	23	100%
Doxycycline	DO	16	23	70%
Enrofluxacine	Enr	8	23	35%
Erythromycin	E	5	23	22%
Flumiquine	FL	3	23	13%
Gentanycin	CN	O	23	0%
Streptomycin	S	0	23	0%
Sulphamethazol	SXT	0	23	0%
Colistine sulphate	CT	0	23	0%

Table (9) : Pathogenicity of ORT isolates on ECE of 11 day old .

Age of Embryo	*Days post inoculation*	Mortalities		*Lesion in embryos*	ORT reisolation
12d	1st day	1/30	3.3%	Congestion of blood vessels of CAM	ORT+ve Alantoic fluid and Yolk sac
13d	2nd day	0	0%	Congestion of blood vessels of yolk sac and CAM	OR+ve high count in yolk
14d	3rd day	0	0%	Congestion of blood V. of yolk sac and CAM	ORT+ve in yolk
15d	4 rd day	3/30	10 %	Congestion of CAM, Yolk sac, muscular haemorahage, watery yolk	ORT + ve in Yolk sac
16d	5 day	2/30	6.6%	Watery yolk and changed in color	ORT in yolk
17 d	6 day	0	0%	-	Not done
18d	7 day	1/30	3.3%	Late embryonic death	+ve ORT
19d	8 day	1/30	3.3%	Late embryoinc death	ORT + ve
20d	9 day	0	0%	Not done	Not done
21d	10 day	2/30	6.6	Dead in shell	ORT+ ve
1 day old chicks	*1 day*	0	0%	*3/20 ill vitality and distended belly*	ORT + ve in yolk sac
Total No	-	10/30	33.3%	----	-----
Control –inoculated and noninoculated groups	***1day -9day PI****	0/10	0%	---	----

**PI : post inoculation*

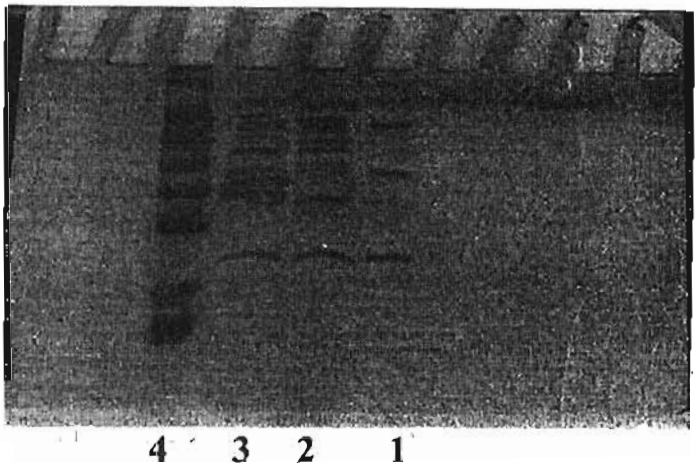

Fig. (1) : Coomassie blue - Stained SDS- PAGE analysis of OMP of ORT. Lanes1,2,3: Outer membrane protein bands of strains no. 1,2,3, Lane 4: marker.

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الملخص العربى

دراسات بكتيرية على الأورنيثوباكتريم رينوتراكيال (أو - أر - تى) فى الدجاج

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تركزت الجهود فى هذا العمل لدراسة ٧٢٠ من دجاج التسمين المريض من واقع ٤٠ قطيع عليه أعراض إكلينيكية مختلفة، شملت الأعراض التنفسية البسيطة والحادة، سواء بمفردها أو مصحوبة بأعراض أخرى كتأخر النمو والتهاب المفاصل والأعراض العصبية والعرج وارتفاع النفوق التى تراوحت نسبته من ٢ر٠٪ حتى ٨ر١٦٪، وقد تم عزل بكتيريا أو - آر - تى من ٢٣ قطيع (٥ر٥٧٪) كانت مأخوذة من عينات أنضاء مختلفة (٣ر٤١٪) شملت الرئتين والأكياس الهوائية والقصبة الهوائية والمفاصل والجيوب الأنفية والمخ وكيس المح من طيور مريضة أو حديثة النفوق. بينما لم يتم عزل أى عترة أو - آر - تى من الدم أو غشاء التامور أو الكبد أو العين. كما أمكن عزلها من معامل التفريخ التى بها شكوى من إنخفاض الفقس وارتفاع معدلات الموت الجنينى المتأخر داخل البيض، وأوضح التصنيف السيرولوجى إنها من النوع A بواسطة إختبار الترسيب المناعى، أظهرت تجارب الحساسية للمضادات الحيوية أن جميع المعزولات حساسة بنسبة ١٠٠٪ للأمبيسيللين والأموكسيسيلين والأوكسيتتراسيكلين والسيروفلوكساسين والكلورامفنيكول ولكن جميعها مقاومة للجنتاميسين والستربتومايسين والكوليستين والسلفاميثاكسازول / ترايميثوبريم. وأثبتت الدراسة عند حقن أو - آر - تى فى بيض مخصب خالى من المسببات المريضة عند عمر ١١ يوم إنه يمكن عزلها من كيس المح بعد ٤ ساعة من الحقن فى صورة نقية وأنها تسبب نفوق للجنين بعد ٤ أيام من الحقن ولاتوجد أعراض تشريحية مميزة غير إحتقان برأس ورقبة الجنين، وأوضحت نتائج التحليل الكهربائى للغشاء البروتينى الخارجى لثلاث عترات معزولة من كيس المح والرئة والمفصل بوجود فروق بينها وأن هناك تشابه بصورة واضحة بين العترة رقم ٢ و ٣.