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Study on some bacterial pathogens causing septicemia and death in ducks with special reference to *Riemerella anatipestifer*

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Abstract

Riemerella anatipestifer and *Escherichia coli* infections are important waterfowl pathogens, causing major economic losses to the duck-producing industry as they are the causative agent of duck septicemia with mortality among ducks. The aim of the present study was isolation and identification of some bacterial pathogens causing septicemia and deathes in ducks. A total of 100 ducks (Muscovy, Pekin and Cross breed) suffered from signs of septicemia, respiratory and nervous manifestations were collected from different farms and small holders at El-Beheira governorate. The obtained results revealed that the total percentage of *Escherichia coli*, *Staphylococci* and *R. anatipestifer* were37%, 23% and 4% respectively. Molecular assays like 16S rRNA based PCR and *R. anatipestifer* specific PCR, were used for confirmation of the *R. anatipestifer* isolates as well as directly from clinical samples. Moreover, *R. anatipestifer* isolates were examined for the presence of some virulence genes that associated with its pathogenicity as (*ompA* and *dnaB* genes), the results showed that both genes were detected in all isolates. Serological identification of *E.coli* isolates (37) was made and showed that they belonged to the following serotypes: O126,O86,O157,O44,O55,and O164 in an incidence of 7,4,7,6,6,4 and3 respectively. The antibiotic sensitivity testing of bacterial isolates was carried out to choose the best effective antibiotics and showed that all isolates shared in their sensitivity to Imipenem and Ofloxacin. PCR assay proved to be highly sensitive, rapid and specific assay for detection of *R. anatipestifer* from pure cultures as well as direct detection from clinical samples.

Key words: *Escherichia coli- Staphylococci -Riemerella anatipestifer – virulence factors- duck- molecular biology- sensitivity test.*

INTRODUCTION

Among the respiratory diseases of ducks, *R. anatipestifer* and *E. coli* infections are of primary economic importance to the duck industry. Since both *E. coli* and *R. anatipestifer* infections occur at about the same age and are not distinguishable by clinical signs or lesions. Colibacillosis continues to be a major cause of mortality in many duck farms, in spite of preventive sanitation and management practices. **(Soliman et al. 2018).**

Multiple bacterial pathogens including *Escherichia coli, Staphylococci* and *P. multocida* become the major threats of duck health globally **(Singh et al. 2013)**. Avian pathogenic *E.coli* (APEC) may cause colisepticemia, yolk sac infection, cellulitis, coligranuloma and omphalitis in ducks and other bird species **(Salehi and Ghanbarpour. 2010).**

E. coli causes a wide variety of problems in ducks at different ages, but the most dangerous illness occurs at 2-6 weeks of age and mortality rates reach up to 43% **(El-Demerdash et al 2015).** It considered as one of the most important and frequently encountered bacterial avian pathogen causing a wide variety of disease syndrome in birds causing up to 30% of poultry mortality **Kaul et al .1992**, Barnes and **Gross.1997** and **Geornaras et al. 2001**.

E.coli species are serologically divided in serogroups and serotypes on the bases of their antigenic composition (somatic or O antigen for serogroups and flagella or H antigen for serotypes) **Roshdy et al. 2012.**

Staphylococci, including *Staphylococcus aureus* are known to cause various diseases from acute septicemia to chronic osteomyelitis in poultry **Amen et al. (2019)**. *Staphylococcus aureus* is responsible for a broad spectrum of clinical signs in poultry including suppurative dermatitis, suppurative arthritis, and septicemic lesions **(Pattison et al. 2008 and Elfeil 2012).**

Duck septicemia caused by *Riemerella anatipestifer* is commonly referred as new duck disease affecting ducks at 1-7 weeks of age and characterized by ocular and nasal discharges, tremors of the head & neck, and in-coordination. In typical cases, affected ducklings may lie on their back with paddling movement of legs **(Soman et al. 2014).**

R. anatipestifer is a gram-negative, non motile, non spore- forming, rod-shaped bacterium **(Segers et al. 1993)**. Several virulence factors have been identified that associate with disease severity, including outer membrane protein A (OmpA) **(Hu et al. 2011)** andvirulence associated protein D (VapD) **(Chang et al. 1998)**. Outer membrane proteins play a very important role in virulence of *R. anatipestifer* and induce a strong antibody response **(Weiser and Gotschlich 1991).**

Diagnosis of *R. anatipestifer* is difficult due to absence of selective and/or indicative media for isolation **(Rubbenstroth et al. 2013).**Several nucleotide amplification methods for rapid diagnosis of *R. anatipestifer* have been developed, such as polymerase chain reaction (PCR) assays targeting the 16S rRNA gene, the ompA gene **(Tsai et al. 2005)** and the dnaB gene **(Hu et al. 2011)** have also been used to characterize the isolates.

A PCR assay that is a valuable tool for the rapid and species-specific identification of *R. anatipestifer* from tissue or bacterial culture, were performed for the further identification and confirmation **(Kardos et al. 2007).**

Selection of the suitable antibiotic for treatment of bacterial infection is significant to treat and control the infection, reduce or prevent mortality of the infected ducks and ensure maximum cost effectiveness by diminishing the unnecessary use of antibiotics to which they are resistant. **(Zhong et al., 2009).**

Therefore, this study aimed for detection of some bacterial pathogens causing septicemia and death in ducks from different farms and small holders at El-Beheira governorate and for detection of the most effective antibiotic against these pathogens using antibiotic disk diffusion test. We aimed also to detect

some virulence factors in *R. anatipestifer* species that may associated with its pathogenicity as (*ompA* and *dnaB* genes).

MATERIALS AND METHODS

Collection of samples:

A total of 100 ducks (Muscovy, Pekin, Cross dreed) showed respiratory signs, watery yellowish green diarrhea, nervous manifestation and cyanosis were collected (each in a separate sterile bag) and examined bacteriologically, 19 clinical samples from these ducks were randomly selected and subjected to direct detection of R. *anatipestifer* by PCR. Samples (liver, heart, spleen, kidney and lung) were collected (from freshly dead and emergency slaughtered ducklings of (1-7 weeks) of age from different farms and small holders at El- Beheira governorate – Egypt. The distribution of these samples from different duck breeds are illustrated in Table (1)

Table (1): Number and types of examined samples

Type of breed	Diseased ducks	Freshly dead ducks	Total
Muscovy	32	26	58
Pekin	14	8	22
Cross breed	13	7	20
Total	59	41	100

Isolation and identification of *E. coli* :

The samples (polymix from each duck) were inoculated in tubes of buffer peptone water and incubated at 37 ^{o} C for 18±2hrs under aerobic condition. A loopful from each tube was separately streaked onto Mac Conkey's agar and eosin methylene blue agar (EMB) and incubated aerobically at 37 ^{o}C for 24 hrs. Suspected colonies were subjected to cultural morphological and biochemical identification according to **Murray et al. (2003).**

Serotyping of *E. coli* strains by slide agglutination test using polyvalent antisera and its corresponding monovalent one: **(Sifin diagnostics gmbh, Berlin, Germany)** according to **(Edwards and Ewing, 1972)**.

Isolation and identification of *Staphylococci:*

The samples were inoculated into nutrient broth and incubated at 37 °C for 24 h. A loopful of the inoculated broth was subcultured onto nutrient agar, Baird Parker agar and Mannitol salt agar medium and incubated aerobically at 37^{o} C for 24-48 hours. Suspected colonies were identified according to morphological features, pigment production, gram staining, catalase test, coagulase test according to **Quinin et al. (2004).**

Isolation and Identification of *R. anatipestifer:*

The collected samples were prepared and inoculated into Brain Heart Infusion (BHI) broth (Hi-media) then incubated at 37°C for 24 hours. The cultures were inoculated onto blood agar and MacConkey agar medium (Oxoid) and incubated at

37°C for 24-48 hours. Suspected colonies were stained by Gram's stain then examined microscopically and subjected to further morphological and biochemical identification **(Quinn et al. 1994).**

Molecular characterization of *R. anatipestifer*:

R. anatipestifer specific PCR and 16S rRNA based PCR were used for confirmation of the isolates and detection of *R. anatipestifer* directly from clinical samples (**Pala et al. 2013) and** (**Tsai et al. 2005)**

Table (2): primer used in PCR reactions for the detection of *Riemerella anatipestifer*

Primer	Primer Design	Amplicon Size bp
16S rRNA-F R	CAGCTTAACTGTAGAACTGC TCGAGATTTGCATCACTTCG	665
specific -F R	TTACCGACTGATTGCCTTCTA AGAGGAAGACCGAGGACATC	546

Detection of virulence genes:

Oligonucleotide Primers: Primers used were supplied from Metabion (Germany) are listed in Table (3).

Table (3): Primers sequences, target genes, amplicon size and cycling conditions

Target agent	Target gene	Primers sequences	Amplified segment(bp)	Prim.denat.	Ampli.(35 cycles)			Final ext.	Reference
					Sec.denat.	Annel.temp	Exten.		
	ompA	CTTGGTATC CAAGGGGA TTATGTTT TTTAACTGA GATGGGTTA ACACCTC	707	94ºC 5 min	94ºC 30 sec	55ºC 40 sec	72 ºC 45 sec	72 ºC 10 min	**Sun et al. 2012**
	dnaB	**DnaB P1** AAACTCAG GCAAAGGT GGCAC **DnaB p2** TGTATGGTA GTTTTGATG CTTTCAA	459	94ºC 5 min	94ºC 30 sec	56ºC 40 sec	72 ºC 45 sec	72 ºC 10 min	**Hu et al. 2011**

Antibiotic sensitivity of bacterial isolates:

Antibiotic sensitivity pattern of the recovered isolates was determined by standard disc diffusion method according to **(CLIS, 2012)** using 11 antibiotics as Azereonam (ATM_{30}), Cefadroxil (CDX_{30}), Cephradine (CRD_{30}), Amikacin (AK_{30}), Amoxicillin (AML_{25}),Amoxycillin+ Clavulinic acid (AMC_{30}), Colistin suphate (CT_{25}), Oxytetracyclin (OT_{30}), Ampiclox (AX_{30}) Imipenem (IPM_{10}), Ofloxacin (OFX_5).

Results:

1-Postmortem examination:

Affected ducklings under investigation were showed respiratory, nervous signs and diarrhea with clinical postmortem findings as air sacculitis, pericarditis, perihepatitis, peritonitis and erosions on the gizzards (septicemia)

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A B C D

E F

Photo A: The ducklings were showed severe nervous manifestation as trembling of head and neck, paddling of legs and ataxia **Photo B,C and D**: Postmortem examination showed liver and spleen were enlarged and mottled, pericarditis; airsacculitis and perihepatitis, **PhotoE**:grey, convex and non-hemolytic dew drop colonies on blood agar, **PhotoF**: Gram-negative cocco-bacilli, short rods organismswith Gram staining.

2- All the results are illustrated in tables 4-11 and photos 1-5

Table (4): prevalence of *E. coli* among the examined duck samples:

Type of breed	No of examined samples	Freshly dead ducks Positive samples NO %	Diseased ducks Positive samples NO %	Total NO %
Muscovy	58	11 19%	13 22,4%	24 41,3%
Pekin	22	3 13,6%	5 22,7%	8 36,3%
Cross breed	20	2 10%	3 10%	5 25%
Overall	100	16 16%	21 21%	37 37%

% calculated according to the No. of samples examined

Table (5): prevalence of *Staphylococcus spp.* among the examined duck samples :

Type of breed	No of examined samples	*S. aureus*		Other *staphylococcus*		Total	
		Dead and diseased		Dead and diseased		NO	%
		NO	%	No	%		
Muscovy	58	10	17.2	5	8.6	15	25.8
Pekin	22	4	18.2	1	4.5	5	22.7
Cross breed	20	2	10%	1	5	3	15
Overall	100	12	12%	11	11%	23	23

% calculated according to the No. of samples examined

Table (6): prevalence of *R. anatipestifer* among the examined samples based on bacteriological examination:

Type of breed	No of examined samples	No of positive samples	% of positive sample
Muscovy	58	1 (diseased duck)	1,72 %
Pekin	22	1(freshly dead)	4,54 %
Cross breed	20	0	0 %
Total	100	2	2 %

% calculated according to the No. of samples examined

Out of 62 bacteriologically positive duck samples, 14 were mixed infection of all bacterial isolates and 50 were single infection (Table 7)

Table (7): Mixed infection of bacterial species isolated from examined duck samples

positive mixed isolates (14)	*E.coli+ R. anatipestifer*		*Staphylococcus+ R. anatipestifer*		*E.coli+ Staphylococcus*	
	No.	%	No.	%	No.	%
	1	7.1	1	7.1	12	85.7

% calculated according to the No. of mixed positive samples examined

Direct examination of 19 clinical samples from freshly dead ducks of different species using PCR test revealed that only two samples from Muscovy and Pekin ducks gave positive result to *R. anatipestifer* in an incidence of 7.69% and 20% respectively and overall incidence of 10.5% (Table 8).

Table (8): Molecular characterization of *R. anatipestifer* from 19 randomly selected clinical samples

Type of breed	No of examined samples	No of positive samples	% of positive sample
Muscovy	13	1 (freshly dead)	7.69 %
Pekin	5	1(freshly dead)	20%
Cross breed	1	0	0 %
Total	19	2	10,52%

Serotyping of *E. coli* strains

As shown in Table (9) serological typing of *E. coli* isolates revealed that 37 isolates were belonged to seven different serovars O:126, O:86, O:157, O:44,O:25,O:55, and O:146.

able (9) Serological identification of 37 *E.coli* isolates

No.	*E.coli* serogroups/serotypes	Ducks species						Total
		Muscovy		Pekin		Cross breed		
		No.	%	No.	%	No.	%	
1	O126: K71	4	10.8	2	5.4	1	2.7	7
2	O86: K61	3	8.1	-	-	1	2.7	4
3	O157: K-	5	13.5	2	5.4	-	-	7
4	O44: K74	4	10.8	1	2.7	1	2.7	6
5	O25: K11	4	10.8	-	-	2	5.4	6
6	O55: K59	2	5.4	2	5.4	-	-	4
7	O164: K-	2	5.4	1	2.7	-	-	3
Overall	7	24	64.9	8	21.6	5	13.5	37

3-Molecular characterization of *R. anatipestifer*:

By Screening of 19 clinical samples showing clear features of septicemia as well as 2 positive *R. Anatipestifer* isolates using PCR based identification,only two clinical samples from Muscovy and Pekin ducks respectively were positive for *R. Anatipestifer*, in additionthe two *Riemerella* cultures gave positive result (table 8) and photo (1,2). The four strains were subjected for further examination for detection of virulence genes and results were illustrated in table (10) and photo (3, 4). All *R. Anatipestifer* isolates were found to harbor *ompA* and *dnaB* virulent genes.

Table (10) Molecular characterization of virulence genes.

Sample	ompA	dnaB
1	+	+
2	+	+
3	+	+
4	+	+

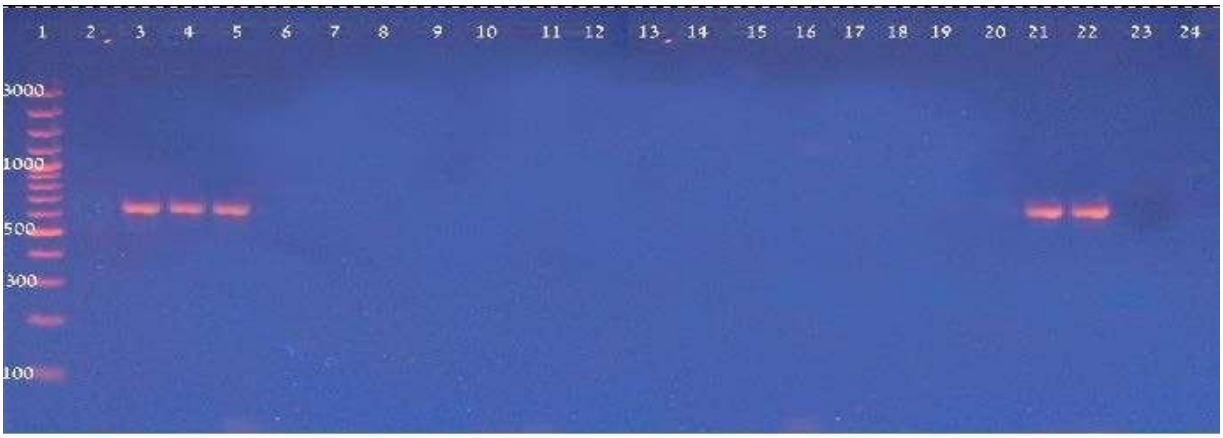
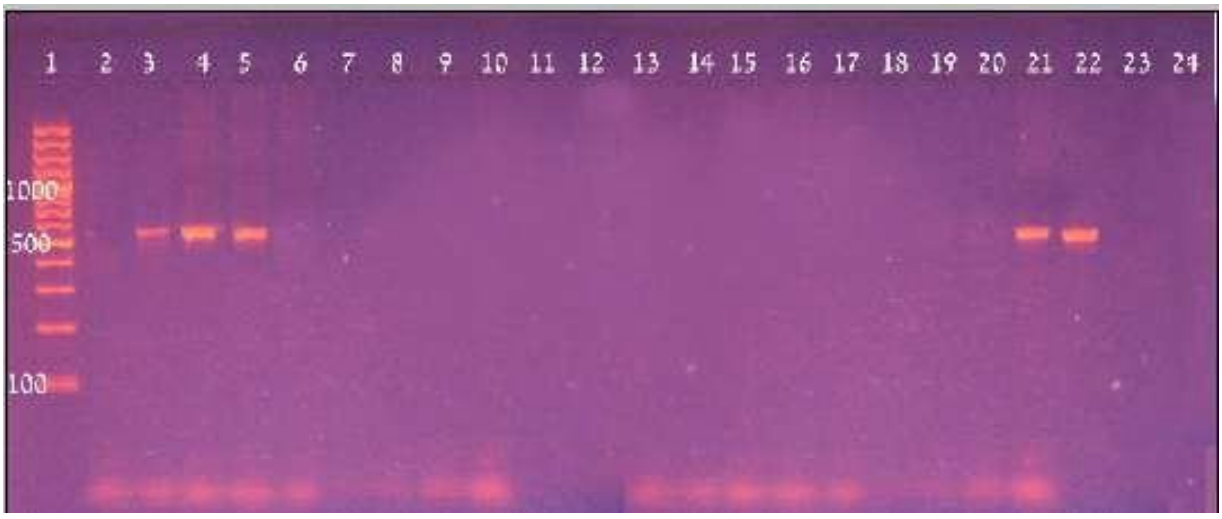


Photo (1): 16srRNA based PCR of*R. anatipestifer* gene product at665 bp.Lane 1: (100 – 3000 bp DNA ladder)Lane 2: control Negative . Lane 3: controlPositive.Lanes (4, 5):*R. anatipestifer*isolates .Lanes (6-24): clinical samples.Lanes 4,5 ,21 and 22 were positive for*R. anatipestifer.*



Photo(2): specific PCRfor amplification of*R. anatipestifer* gene product at 546 bp.Lane 1: (100 – 1000 bp DNA ladder). Lane 2: control Negative.Lane 3: control Positive. Lanes (4, 5) :*R. anatipestifer*isolates .Lanes (6-24): clinical samples. Lanes 4,5 ,21 and 22 were positive for *R. anatipestifer.*

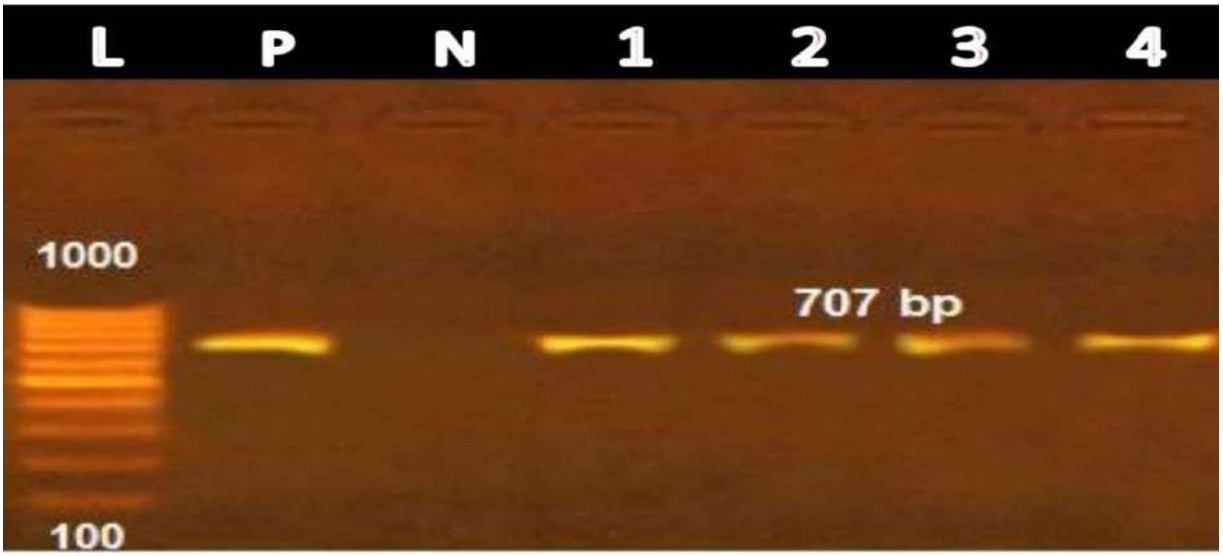


Photo (3): Agarose gel electrophoresis of PCR products after amplification of *ompA* gene products at 707 bp. lane L: molecular weight marker MWM (100 – 1000 bp DNA ladder), lane positive: Positive control, lane negative: negative control, lanes (1,2,3,4) : *R. anatipestifer* isolated from ducks. All isolates were positive to *ompA* gene.

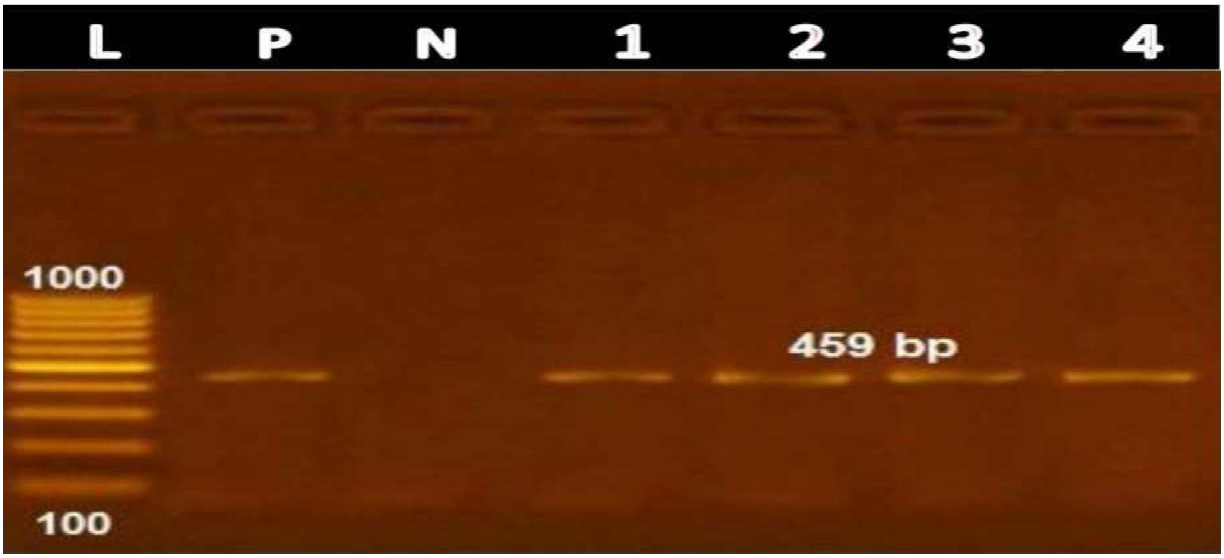


Photo (4): Agarose gel electrophoresis of PCR products after amplification of *dnaB* gene products at 459 bp. lane L: molecular weight marker MWM (100 – 1000 bp DNA ladder), lane P: positive control, lane N: negative control, lanes (1,2,3,4) : *R. anatipestifer* isolated from ducks. All isolates were positive to *dnaB* gene.

Antibiotic sensitivity:

Azetreonam (ATM_{30}), Cefadroxil (CDX_{30}), Cephradine (CRD_{30}), Amikacin (AK_{30}), Amoxicillin (AML_{25}),Amoxycillin+ Clavulinic acid (AMC_{30}), Colistin suphate (CT_{25}), Oxytetracyclin (OT_{30}), Ampiclox (AX_{30}), Ofloxacin (OFX_5) and Imipenem (IPM_{10}) were used for antimicrobial pattern.

Table (11) : Antimicrobial susceptibility pattern of *E.coli, S.aureuse* and *R.anatipestifer* against 11 antibiotics using disc diffusion test

Isolates / Antibiotics	*E.coli* (37)				*S.aureuse* (23)				*R.anatipestifer* (2)			
	S		R		S		R		S		R	
	No	%	No	%	No	%	No	%	No	%	No	%
Azetreonam (ATM_{30})	28	75.7	9	24.3	16	69.6	7	30.4	2	100	0	0
Cefadroxil (CDX_{30})	31	83.8	6	16.2	20	87	3	13	0	0	2	100
Cephradine (CRD_{30})	29	78.4	8	21.6	23	100	0	0	0	0	2	100
Amikacin (AK_{30})	31	83.8	6	16.2	0	0	23	100	2	100	0	0
Amoxicillin (AML_{25}),	13	35.1	24	64.9	0	0	23	100	0	0	2	100
Amoxycillin+ Clavulinic acid (AMC_{30})	17	45.9	20	54.1	21	91.3	2	8.7	0	0	2	100
Colistin suphate (CT_{25})	27	73	10	27	21	91.3	2	8.7	0	0	2	100
Oxytetracyclin (OT_{30})	26	70.3	11	29.7	19	82.6	4	17.4	2	100	0	0
Ampiclox (AX_{30})	6	16.2	31	83.8	5	21.7	18	78.3	0	0	2	100
Ofloxacin (OFX_5)	30	81.1	7	18.9	20	87	3	13	2	100	0	0
Imipenem (IPM_{10})	37	100	0	0	23	100	0	0	2	100	0	0

Discussion

E. coli infection in poultry is usually related to high economic losses and this is because of high morbidity and mortality rates, decrease food conversion rate, body weight loss **(Salama et al. 2007).**

The dissemination of *E. coli* in different organs such as liver, lungs and heart is verified by postmortem lesions including fibrinous perihepatitis, pericarditis, enteritis and pneumonia indicating that *0* led to septicemia and followed by death of the birds **(Kabir. 2010).**

R. anatipestifer infection causes new duck disease which is an epizootic infectious disease in poultry resulting in serious economic losses, especially in the duck industry through high mortality, reduced growth rate, increased condemnations and high

treatment costs **(Pala et al. 2013).**

The obtained results in Table (4) showed that the total percentage of *E. coli* in examined ducks was 37%.These results were agreed to those obtained by **Na et al. (2019)** who isolated *E. coli* from diseased ducks with percentage of 36.13% in South Korea , while higher than **Eid et al. (2019), Heba et al., (2012)** and **Elsamie et al. (2019)** who isolated *E. coli* from diseased ducks with percentage of (22,8%) , (27,2%) and (34,5%) respectively, while lower than **Liu et al. (2018)** who could isolated *E. coli* from ducks with percentage (47.1%) in China , **Singh et al . (2012)** isolated *E. coli* from ducks with percentage (66,66%), **Kissinga et al. (2018)** isolated *E. coli* from diseased ducks with percentage of (91%) and **Marwa hassan et al. (2020)** who isolated *E. coli* from diseased ducks with percentage of (96,7%).

Staphylococci, including *Staphylococcus aureus* are known to cause various diseases from acute septicemia to chronic osteomyelitis in poultry **Amen et al. (2019).** Table (5) showed that the total percentage of *Staphylococcus spp.* in examined ducks was 23%.These results were nearly similar to **Eman Farghaly et al. (2015)** who could isolated *Staphylococcus spp* from imported duck flocks with a percentage of 24.2%while higher than **Amen et al. (2019)** who isolated *S. aureus* from ducks with a percentage of 6,6 % and similar to (**Eid et al. 2019)** who isolated *S. aureus* from ducks with a percentage of 12,2 % and nearly agreed with **Bisgaard (1981)** who isolated 18% *S.aureus* due to arthritis in duck. However, **Ismail (2013)** stated that the percentage of Staphylococci species isolated from duckling in Egypt not exceed 0.9%.

On the other hand, the obtained results in table (6) showed that the total percentage of *R. anatipestifer* detected in examined ducks by bacteriological examination was 2 %table (6) and photo (1, 2).These results were nearly similar to those obtained by **Soliman et al. (2018)** who isolated of *R. anatipestifer* from diseased duck with percentage of 2.1 %while lower than **Surya et al. (2016)** isolated *R. anatipestifer* from diseased ducks by percentage of 4.86 % , **Heba et al. (2015)** isolated *R. anatipestifer* from diseased duck and duckling by percentage of 16.7 %, **Sarker et al. (2017)** who isolated *R. anatipestifer* from sick and dead ducks by percentage of 61.6 %, **Abd El Hamid et al. (2019)** who detected *R. anatipestifer* from diseased duck by PCR with percentage of 8 % while **RitamHazarika et al. (2020)** reported that13 isolates (13 from 98 samples) confirmed as *R. anatipestifer* by PCR from diseased duck and duckling by percentage of 13.2 %.

Heba et al. 2012 typed 150 *E.coli* srains isolated from different organs of ducks and revealed that, 84 strains could be identified serologically. They belonged to 15 different serogroups. The most commonly isolated Ogroups in ducks were O158, O103, O125, O44, O114, O91, O111, and O78. These results may differe from those obtained by our study which typed 37 *E.coli* from different breeds of ducks and found that they belonged to 7 serogroups, which are: O126, O86, O157, O44, O25 and O146.

Unlike our results, **Wang et al. 2010** identified 53 O serogroups from 254 *E.coli* strains from ducks, among which, the predominant serogroups were O93, O78 and O92. Moreover, O24, O38, O44, O50, O56, O69, O84, O85, O102, O121, O130, O132, O133, O139 and O146 were found for the first time in ducks in China.

Molecular characterizations of the suspected isolates of *R. anatipestifer* and clinical samples were done

using *R. anatipestifer* 16 srRNA based PCR *and R. anatipestifer* specific PCR which revealed that the suspected isolates were confirmed *and R.anatipestifer was* detected from clinical samples directly by PCR with percentage of10.52% (2 samples from 19) as showed in Table (7) and photo (1,2), and this indicate that PCR is more rapid , sensitive and species-specific method for identification of *R. anatipestifer* comparing with cultural methods which is difficult due to absence of selective media for isolation and occurrence of over growth by other bacteria and this agree with **Kardos et al. (2007)** who used a novel PCR assay for identification of*R. Anatipestifer* from pure cultures as well as clinical samples from birds and **Soman et al. (2014)** who reported that the ability of PCR assay to detect *Riemerella* organisms directly in clinical specimens is an added benefit and suggests that PCR assay can facilitate fast and proper identification of *R. anatipestifer* infection in ducks and can replace the traditional methods of differentiation which are difficult and time-consuming.

Among the 21 serotype of *R. anatipestifer*, OmpA of *R. anatipestifer* is major immunogenic protein, OmpA is a conserved and strong antigenic determinant and hence is suggested to be a valuable protein for the sero detection of *R. anatipestifer* infections, **(Subramaniam et al. 2000)** and plays an important role in virulence of the organism where mutated OmpA strains become attenuated and shows some sort of loss of adhesions to Vero cells which indicates that OmpA is an important virulence factor of *R. anatipestifer* **(Hu et al. 2011).** Four (4) isolates were subjected for further examination by PCR for detection of virulence genes as (ompA and dnaB genes) and results were illustrated in table (8) and photo (3,4).*OmpA* gene and *dnaB* genes were detected in all isolates indicating that all isolates were virulent and pathogenic strains. These results agree with **Heba et al. (2015)** who reported that *Omp*A gene specific for *R. anatipestifer* was detected among all isolates by PCR ,and **Abd El Hamid et al. (2019)** who reported that using PCR and sequence analysis of *Omp A* gene is considered a highly sensitive, rapid and an alternative method for serotyping of *R. anatipestifer*.

Antimicrobial sensitivity pattern of bacterial isolates was performed against 11 antibiotics. The most effective antibiotics on all isolates were Imipenem (IPM_{10}) and Ofloxacin, while they showed resistace to Amoxicillin (AML_{25}). These results are nearly agreed with **Simona 2014** who tested the antimicrobial avtivities of *E.coli* against 19 antibiotics. The test revealed low sensitivity to Ampicillin (19.6%), Tetracycline (29.5%) and Amoxicillin (37.5%).

Kibret and Abera (2011), studied the resistance pattern of *E.coli* at the period from 2003 to 2010. They reported that the resistance rate of *E.coli* to erythromycin and amoxicillin increased more than 80% comparing with studies obtained by **Khan et al.2002,** while the resistance rate to tetracycline increased more than 60% comparing with the same study.

In our study, the resistance percentage of *E.coli* to amoxicillin and amoxicillin +clavulinic acid was relatively high (64.9%) and (54.1%) respectively.

Moreover, **Daoud et al. 2020** studied the antimicrobial susceptibility profile of *E.coli* and stated that, the pattern was changing constantly due to modifications in the antibiogram interpretation criteria and antibiotic prescription habits. Isolates were characterized by high acquired resistance to beta lactams and trimethoprim/sulfamethazole, they found that, the highest sensitivity percentage was to fosfomycin (99.3%).

In addition, **Onifade et al. 2015** observed that there is a high level of antibiotic resistance in the *E.coli* isolates. This antimicrobial resistance, most importantly to the frontline antibiotics such as cirprofloxacin, ofloxacin, tetracycline,

chloramphenicol and others, is of major concern. However, in our study, a good susceptibility percentage was observed to ofloxacin and cefadroxil 81.1% and 83.8% respectively.

Akanbi et al.2017 studied the resistance of *S.aureus* to commonly used antibiotics. This resistance is linked to their ability to acquire and disseminate antimicrobial resistance determinants in nature. The resistance of *S.aureus* to antibiotics varied considerably with the highest resistance recorded to ampicillin and penicillin (96.7%), rifampicin and clindamycin (80%), oxacillin (73.3%) and erythromycin (70%), while showing various susceptibility to imipenem (96.7%), levofloxacin (86.7%), chloamphenicol (83.3%). These results nearly agreed with our results which observed the resistance of *S.aureus* to betalactam group ampicillin,amoxicillin (100%), while that showing high susceptibility to imipenem (100%) and ofloxacin (87%).

(Baba et al.1987 and **Pathanasophon et al. 1994)** reported the high sensitivity of *R. anatipestifer* to penicillin G, erythromycin and polymyxin B, **Chang et al. (2003)** revealed that penicillin, ceftiofur, chloramphenicol, flumequine and kanamycin are the effective antibiotic and **Gyuris et al. (2017)** reported that the majority of the strains were susceptible to ampicillin (95.1%), penicillin (93%) , sulphamethoxazole–trimethoprim (92.4%) and spectinomycin (86.5%).

The variations in the resistance pattern of the *R. anatipestifer* isolates with previous studies could be attributed to the variations in geographical origin of the strains, and drug resistance profiles of *R.anatipestifer* were found to vary with time. In order to reduce the irresponsible use of antibiotics, disc diffusion analysis should be done for effective antibacterial treatment as recorded by **(Zhong et al. 2009).**

Conclusion:

Multiple bacterial pathogens including *Escherichia coli, R. anatipestifer* and *Staphylococci* become the major threats of duck health globally. Since both *E. coli* and *R. anatipestifer* infections occur at about the same age and are not distinguishable by clinical signs or lesions resulting in high mortality rates in ducks farms. Colibacillosis continues to be a major cause of mortality in many duck farms. *R. anatipestifer* is a major bacterial pathogen affecting ducks and ducklings worldwide, causing ducks septicemia (new duck disease) which resulting in high morbidity and mortality rates in ducks. In this study *Escherichia coli, R. anatipestifer* and *Staphylococci* could be isolated and identified from diseased and freshly dead ducks. On gross examination, the most affected organs were liver and heart. PCR assay was used for confirmation of *R. anatipestifer* isolates and direct detection of *R. anatipestifer* from clinical samples as well as characterization of virulence genes (OmpA and dnaB genes).Antibiotic sensitivity of isolates revealed that the isolates were sensitive to imipenem and ofloxacin and resistant to amoxicillin. Combination of genotypic and phenotypic characterization is more valuable as an epidemiological tool for identification of bacterial pathogens affecting ducks thus help in early treatment and control strategies.

References:

M.I. Abd El Hamid, D.A.M Abd El-Moaty, E.F. El-Sergany, H.M. Salem El-Sawy and A.M. Abbas, " Utility of molecular biology tools for identification and characterization of Egyptian *Riemerella anatipestifer* duck isolates. *Inter J Vet Sci*, 8(4): 335-341,2019.

O.E. Akanbi, N.A. Herny, J. Fri , A.C. Otigbu, and A.M.Clarke, " Antimicrobial susceptibility of Staphylococcus aureus isolated from recreational waters and beach sand in Eastern Cape Province of South Africa. *Int. J.Environ.Res.Public Health*. 14,1001, 2017.

A. Amara, Z. Ziani, K. Bouzoubaa, "Antibioresistance of *E.coli* strains isolated in Moroco from chickens with colibacillosis. *Vet.Microbiol*.43:325-330, 1995.

O.Amen, G.H. Abeer, M.S. Ashgan. S.I. Ragab, "Detection of antibiotics resistance genes in *staphylococcus aureus* isolated from poultry farms. *Assiut Vet. Med. J.* Vol. 65 No. 163, 1-9, 2019.

T. Baba, Y. Odagiri, T. Morimoto, T. Horimoto and S. Yamamoto, "An outbreak of *Moraxella (Pasteurella) anatipestifer* infection in ducklings in Japan. *Jpn. J. Vet. Sci*. 49: 939-941, 1987.

H.J. Barnes and W.B. Gross, "Colibacillosis: Calnek, B.W.(Ed), *Disease of Poultry*. 10ª Ed. University Press, Ames, Lowa " Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45(4): 493-496, 1966.

M. Bisgaard, "Arthritis in ducks. I. Aetiology and public health aspects. *Avian Pathol.*, 10(1):11-21,1981.

C.F.Chang, P.E. Hung and Y.F. Chang, "Molecular characterization of a plasmid isolated from Riemerella anatipestifer. *Avian Pathol*. 27, 339–345, 1998.

C.F. Chang, W.H. Lin, T.M. Yeh, Chiang TS and Chang, Y.F, "Antimicrobial susceptibility of *Riemerella anatipestifer* isolated from ducks against the effi cacy of ceftriafur treatment. *J. Vet. Diagn. Invest*. 15: 26-29, 2003.

T.Chikuba, H.Uehara, S.Fumikura, K.Takahashi, Y.Suzuki, K. Hoshinoo and Y.Yamamoto, "Riemerella anatipestifer infection in domestic ducks in Japan, *J .Vet Med Sci*, 78: 1635-1638, 2014.

Clinical and Laboratory Standard Institute (CLIS) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement.Vol.31,No.1, 2012.

W.S. Darwish, W.F. Saad Eldin and K.I. Eldesok, "Prevalence, molecular characterization and antibiotic susceptibility of *Escherichia coli* isolated from duck meat and giblets. *J. Food Safe*. 35: 410-415, 2015.

N. Daoud, M. Hamdoun, H. Hannachi, C. Gharsallah, W. Mallekh and O. Bahri, "Antimicrobial susceptibility pattern of *Escerichia coli* among Tunisian outpatients with community-Acquired urinary tract infections (2012-2018). *Curr Urol*, 14:200-205, 2020.

P.R.A. Edwards and W.H.Ewing, "Identification of Enterobacteriaceae. Burges Publication Company, Minneapolis, Minnesota, 1972.

H.M. Eid, A.M. Algammal, W.K. Elfeil, F.M. Youssef, S.M. Harb and E.M. Abd-Allah, "Prevalence, molecular typing, and antimicrobial resistance of bacterial pathogens isolated from ducks. *Vet.World.* 12(5): 677-68, 2019.

M. El-Demerdash, H. Abdien, D. Mansour and W.Elfeil, "Protective efficacy of synbiotics in the prevention of Salmonella Typhimurium in chickens. *Glob.Anim. Sci. J.,* 2(2): 78-85, 2015.

W.M.K. Elfeil, "Duck and Goose PRRs Clone, Analysis, Distributions, Polymorphism and Response to Special Ligands. College of Animal Science and Veterinary Medicine, Jilin University, China. p140, 2012.

L.K.A. EL-Samie, A.M. Abd-Elmoaty, N.A. Ibrahim and W.S. Darwish, "Prevealnce and molecular characterization of avian pathogenic *E. coli* in ducks Adv. *Anim. Vet. Sci*. 7(s2): 163-168, 2019

M. Eman, E. Farghaly, G.S.h. Azhar and B. Heba Identification and Molecular Characterization of *Staphylococcus Aureus* From Newly Hatched Imported Poultry. *SCVMJ*, XX (2). 317-330, 2015.

I. Geornaras, J.W. Hasting, A.Von Holy," Genotyping analysis of *E.coli* strains from poultry carcasses and their susceptibility to antimicrobial agents. *Appl. Environ.Microbiol*.67:1940-1944, 2001.

S. Gomis, L. Babiuk, D.L. Godson, B. Allan, T. Thrush, H. Townsend, P. Willson, E. Waters, R. Hecker and A.Potter, "Protection of chickens against *Escherichia coli* infections by DNA containing CpG motifs. *Infect. Immun*., 71(2): 857-863, 2003.

Gyuris Éva, Enikő ,Wehmann, Katalin czeibert, Tibor Magyar. "Antimicrobial susceptibility of *Riemerella anatipestifer strains isolated from* geese and ducksin Hungary. *Acta*

Veterinaria Hungarica, 65 (2), pp. 153–165,2017.

D. Heba, A. Samir, K.F. Mohamed, J.El-Jakee, "Identification of Duck Septicemia in Egypt. *Global Vet,* 15: 397-400, 2015.

R. Heba, S. Abd El-Aziz, M. Refai, "Incidence of *E. coli* in chickens and ducks in different governorates in Egypt .*1st Conf. of An. Health Res. Inst. Assoc.* pp. 420 – 426, 2012.

Q. Hu, X. Han, X. Zhou, C. Ding, Y. Zhu and S.Yu, "OmpA is a virulence factor of Riemerella anatipestifer. *Vet. Microbiol*. 150, 278–283, 2011.

Q. Hu, J. Tu, X. Han, Y. Zhu, C. Ding and S.Yu, "Development of multiplex PCR assay for rapid detection of Riemerella anatipestifer, Escherichia coli, and Salmonella enterica simultaneously from ducks. Journal of Microbiological Methods 87 : 64–69, 2011.

R.H.I..Ismail, "Some studies on bacterial causes of respiratory troubles in ducklings. M.V.SC.Thesis, Fac. Vet. Med., Zagazig University, 2013.

S.Kabir, "Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control andpublic health concerns. *Int. J. Environ. Res. Publ. Health*. 7: 89-114, 2010.

N.A. Kan, N. Saba, S. Abdus, A.Q. Ali, "ncidence and antibiogram pattern of *E.coli* isolates from various clinical samples from patients at NIH Islamabad. *Pak J.Biol.Sci*. (1):111-113, 2002.

G. Kardos, J. Nagy, M. Antal, A. Bistyak, M. Tenk and I. Kiss, Developmentof a novell PCR assay specific for *Riemerella anatipestifer*. *Lett Appl*. *Microbiol*.44: 145-148, 2007.

L. Kaul, L.P Kaul and N.M. Shah, "An outbreak of colibacillosis in chicks at an organized poultry farm under semi-arid zone of north Gujarat, Indian Vet.J. 69: 373-374, 1992.

M. Kibret, B. Abera, "Antimicribial susceptibility pattern of *E.coli* from clinical sources in northeast Ethiopia. African Health Science 11(1):40-45, 2011.

H.D. Kissinga, F. Mwombeki, K. Said, A.A.S. Katakweba, H.E. Nonga, A.P. Muhairwa, "Antibiotic susceptibilities of indicator bacteria *Escherichia coli* and *Enterococci* spp. isolated from ducks in Morogoro Municipality, *Tanzania. BMC Research Notes,* 11(87): 1-6, 2018.

M. Liu, Y. Huang, J. Liu, F. Biville, D. Zhu, M. Wang, R. Jia, S. Chen, X. Zhao, Q Q. Yang, Y. Wu, S. Zhang, X. Chen, Y. Liu, L. Zhang, Y. You, Y. Yu and A. Cheng, " Multiple genetic tools for editing

the genome of *Riemerella anatipestifer* using a counter selectable marker. *Appl Microbiol Biotechnol*. 102(17):7475-7488, 2018.

J. MacFie, "Current status of bacterial translocation as a cause of surgical sepsis. *Br. Med. Bull*. 71: 1-11, 2004.

E.H. Marwa, K.M. Mohamed, M. Rana El-Fattah and E.E.Mohamed, "Molecular Detection of Some Virulence Genes of *E. Coli* Isolated from Broiler Chickens and Ducks at Ismailia Governorate. *SCVMJ,* XXV (1). 1-19, 2020.

P.R. Murray, E.J.O. Baron, M.A. Pfaller, J. Jorgensen, R.H. Yolken, " *Manual of clinical microbiology*. 8th Ed., Vol.1, ASM press, Washington D. C, 2003.

S.H. Na, D.C. Moon, M.J. Choi, S.J. Oh, D.Y. ung, E.J. Sung, H.Y. Kang, B.H. Hyun, S.K. Lim,"Antimicrobial resistance and molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from ducks in South Korea. *Foodborne Pathog. Dis.* 16(12):799-806, 2019.

A.K. Onifade, M.A. Oladoja and D.O. Fadipe, "Antibiotics sensitivity pattern of *Escerichia coli* isolated from children of school age in Ondo state, Nigeria. 7(2):73-76, 2015.

S. Pala, U.R. Nair, C. Somu and M. Mahendran, "Molecular diagnosis of new duck disease in India by 16S rRNA gene based PCR. *Adv. Anim. Vet. Sci.*1 (5): 140 – 142, 2013.

P. Pathanasophon, T. Sawada and T.Tanticharoenyos, "Physiologic characteristics, antimicrobial susceptibility and serotypes of *Pasteurella anatipestifer* isolated from ducks in Thailand. *Vet. Microbiol.* 39: 179-185, 1994.

M. Pattison, P. McMullin and J.M.Bradbury, Poultry Diseases. *Elsevier Health Sciences,* Philadelphia, PA, 2008.

P.J. Quinin, M.E. Carter, B. Markey, G.R.Carter, "Clinical veterinary microbiology printed in Spain by Grafos, S.A. Arte Sobre Pape J., 2004.

P.J. Quinn, M.E. Carter, B. Marker and G.R.Carter, *Pasteurella*. In: *Clinical Veterinary Microbiology*. Wolfe publication. Mosby Year Book Europe Ltd, Spain, pp: 254-258, 1994.

Y.K. Reddy, A. Koteeswaran and N. Dorairajan, "Characterization of E.coli isolates from pathological condition of poultry in Namakkal, *Indian Vet.J.,*71:209-212, 1994.

H. Ritam, M.K. Doley, H. Sarmah, P. Deka and R.K.Sharma, Isolation and Molecular Identification of *Riemerella anatipestifer* from Ducks in Assam . *Int.J.Curr.Microbiol.App.Sci.* 9(3): 318-327, 2020.

D. Rubbenstroth, M. Ryll, J.K. Knobloch, B. Kohler and S.Rautenschlein, "Evaluation of different diagnostic tools for the detection and identification of *Riemerellaanatipestifer*. *Avian. Pathol*., 42(1): 17-26, 2013.

S.S. Salama, A.A. Kheder, E.A. Elebiary, M.M.Taha Molecular strategies for the differentiation and identification of local *Escherichia coli* isolated from chicken: I. Characterization of protein profile. BS. *Veterinary Medicine Journal* 17(1): 25-28, 2007.

M. Salehi and R. Ghanbarpour, "Phenotypic and genotypic properties of *Escherichia coli* isolated from coli septicemic cases of Japanese quail. *Trop. Anim. Health Prod*. 42(7): 1497-1504, 2010.

R.R. Sarker, M.S. Rahman, M.E. Haque, U.K. Rima and M.Z. Hossain, " Identification of Ribonuclease Z Gene from an Outbreak of *Riemerella anatipestifer* Infection in Ducks of Bangladesh. *Poult Fish Wild Sci* 5: 185, 2017.

M.E. Sedeik, A.M. Awad, , H. Rashed, W.K. Elfeil, "Variations in pathogenicity and molecular characterization of infectious bursal disease virus (IBDV) in Egypt. *Am. J. Anim. Vet. Sci*., 13(2): 76-86, 2018.

P. Segers, W. Mannheim, M. Vancanneyt, K. De Brandt, K.H. Hinz and K. Kersters, "*Riemerella anatipestifer* gen. nov., comb.nov., the causative agent of septicaemia and serum exsudativa, and its phylogenetic affiliation within the Flavobacterium-Cytophaga rRNA homology group. *International Journal of Systematic and Evolutionary Microbiology*, 43, 768_776, 1993.

A. Singh, M.S.R. Khan, S. Saha, J. Hassan and U.Roy "Isolation and detection of antibiotic sensitivity pattern of *Escherichia coli* from ducks in Bangladesh and Nepal. *Microbes and Health*,1(1): 6-8, 2012.

R. Singh, B. Remingto, P. Blackall and C.Turni, "Epidemiology of fowl cholera in free range broilers. *Avian Dis*. 58 (1): 124-128, 2013.

H.M. Soliman, H.N. Deif, A.M. Elsawah and J. El-jakee, "A trial on preparation of bivalent vaccine against *Riemerella anatipestifer* and *Escherichia coli* in ducks. *Bioscience research*, 15(4):3598-3605, 2018.

M. Soman, S.R. Nair, M. Mini, B.K. Mani, S.Joseph, "Isolation and polymerase chain reaction-based identification of *Riemerella anatipestifer* from ducks in Kerala, India, *Veterinary World* 7(9): 765-769, 2014.

S. Subramaniam, B. Huang, H. Loh, J. Kwang, H.M. Tan, K.L. Chua, J. Frey, "Characterization of a predominant immunogenic outer membrane

protein of *Riemerella anatipestifer*. *Clin.Vacc. Immun*. 7: 168-174, 2000.

N. Sun, J.H. Liu, F. Yang, D.C. Lin, G.H. Li, Z.L.Chen and Z.L.Zeng, "Molecular characterization of the antimicrobial resistance of *Riemerella anatipestifer* isolated from ducks. *Vet. Microbiol.*158, 376–383, 2012.

P.S. Surya, P.M. Priya and M.Mini, "Microbiological Communication Biotyping and antibiogram of Riemerella anatipestifer from ducks in Kerala. Biosci Biotech Res Comm, 9: 457-462, 2016.

H.J. Tsai, Y.T. Liu, C.S. Tseng and M.J. Pan, "Genetic variation of the ompA and 16S rRNA genes of *Riemerella anatipestifer*. *Avian Pathology* (February) 34 (1), 55- 64, 2005.

X.P. Wang, D.K. Zhu, M.S. Wang, A.C. Cheng, R.Y. Jia, S Chen, X.Y. Chen and T.Tang, "Development and application of specific polymerase chain reaction assay targeting the *gyrB* gene for rapid detection of *Riemerella anatipestifer*. *Poultry Science*, 91:2450–2453, 2012.

J.N. Weiser and E.C. Gotschlich, "Outer membrane protein A (OmpA) contributes to serum resistance and pathogenicity of *Escherichia coli* K-1. *Infect Immun*, 59: 2252-2258, 1991.

C.Y. Zhong, A.C. Cheng, M.S. Wang, D.K. Zhu, Q.H. Luo, C.D. Zhong, L. Li and Z. Duan, "Antibiotic susceptibility of *Riemerella anatipestifer* field isolates. *Avian Dis* 53: 601-607, 2009.

دراسة عن بعض البكتيريا الممرضة المسببة للتسمم الدموي و النفوق في البط مع إشارة خاصة لميكروب الريميريللا أناتيبستيفير

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تعتبر عدوي الريميريللا اناتيبيستيفير و الإيشيريشيا كولاي من أهم الميكروبات الممرضة التي تصيب الطيور المائية مسببة خسائر إقتصادية فادحة في تربية وصناعة البط حيث أنهم من المسببات لتسمم الدم و النفوق في البط. يهدف هذا البحث إلي عزل وتصنيف بعض الميكروبات الممرضة والتي تسبب تسمم الدم و النفوق في البط. تم فحص عدد 100 بطة من أنواع (مسكوفي- بيكيني- سلالات أخري) تعاني من أعراض تسمم الدم, أعراض تنفسية وعصبية والتي تم تجميعها من مختلف المزارع والتربية المنزلية في محافظة البحيرة. أظهرت النتائج أن نسبة عزل ميكروب الإيشيريشيا كولاي و الاستافيلوكوكاي و الريميريللا اناتيبيستيفير كانت 37%, 23%, 4% علي التوالي. تم التأكيد علي معزولات الريميريللا اناتيبيستيفير عن طريق إختبار تفاعل إنزيم البلمرة المتسلسل وكذلك الكشف عن الميكروب مباشرة في العينات والكشف عن بعض جينات الضراوة لهذا الميكروب المرتبطة بضراوة) و قد أظهرت النتائج dnaB و ompAالميكروب في البط مثل (جينات أن كلا من نوعي الجينات متواجدة في جميع المعزولات. كما أظهرت نتائج الفحص السيرولوجي لعترات الإيشيريشيا كولاي (37) أن هذه العترات تنتمي بواقع (O126, O86, O175, O44, O55, O164 إلي أنواع 3,4,6,6,7,4,7) معولات علي التوالي. بإجراء إختبارات الحساسية للمضادات الحيوية تبين أن جميع المعزولات حساسة إلي إيميبنيم و أوفلوكساسين. يعتبر تفاعل إنزيم البلمرة المتسلسل إختبارا دقيقا وحساسا وسريعا للكشف عن ميكروب الريميريللا اناتيبيستيفير من المعزولات والعينات مباشرة.