Prevalence of *eae*A and *qac*EΔ1 genes in *Escherichia coli* isolated from omphalitis in baby chicks

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A B S T R A C T

A total of two hundred diseased Saso chicks with omphalitis were examined (1400 samples) for the isolation of *E. coli* from different organs (liver, ceacum, spleen, heart, lung, yolk sac and cloacal swab).Results showed that 64 cases were positive with an incidence of 32%. Fifty isolates (25%) of *E.coli* were recovered from chicks could be serogrouped in 19 O groups with the most predominant serotype was O_{91} 20 % (10 out of 50 isolates), O_{26} 10% (5 strains out of 50) O_{78}8% (4 strains out of 50), and 7 strains untypable *E. coli* 14%. Among *E. coli* O groups were found to be resistant to Amoxicillin 93.02% followed by Tetracycline 74.42%, Enrofloxacin 46.51%, Erythromycin 30.32%, Ciprofloxacin 27.91%, Norofloxacin and Streptomycin 20.93% and Gentamycin 6.98%. While they were found to be sensitive for Gentamycin, Streptomycin, Ciprofloxacin, Tetracycline, Erythromycin, Norofloxacin, Enrofloxacin, and Amoxicillin as the following: 88.37%, 46. 51%, 34.89%, 23.26%, 18.60%, 9.30%, 9.30% and 6.98%, respectively. The incidence rate of *eae*A gene of *E.coli* was 15.79%.Quaternnary ammonium compound (*qac*EΔ1) gene also was detected in *E. coli* with incidence rate 63.16%.

Keywords: Omphalitis, *E. coli*, Virulence gene, Antibiotic resistance, Disinfectant resistant gene

1. INTRODUCTION

Omphalitis is an infectious and non- contagious condition of yolk sac accompanied by unhealed navels in chicks. Affected chicks appear normal until a few hours before death Kahn *et al*., (2008). Yolk sac infection caused chick mortality during the first week of the post-hatching period Yassin *et al*., (2009). *Proteus spp*., *Enterobacter spp*., *Pseudomonas spp*., *Klebsiella spp*., *Staphylococcus spp*., *Streptococcus spp*., *Clostridium spp*., *Bacillus cereus* and *Enterococcus spp.* were bacteria that have been isolated from yolk sac infections in chicks in different locations all over the world. *Escherichia coli* (*E. coli*) was frequently observed to cause omphalitis Ulmer Franco,(2011).

The occurrence of a specific serotype and its role in disease production depends upon the health status of the birds, climatic conditions, geographical situations and management strategiesSrinivasan*et al.,* (2013).

Salehi *et al.*,(2007) conducted a study to determine the presence of virulence genes in 12 isolates of Avian Pathogenic *Escherichia coli* (APEC) in Iran. All 12 isolates were tested for the presence of *eae*A gene by multiplex polymerase chain reaction, 2 isolates possessed *eae* sequence.

A certain degree of *association* over time between bacterial resistance to antiseptics and antibiotics has been reported. It has been observed that some bacteria which express increased resistance to antiseptics are generally less susceptible to antibiotics. Outer membrane changes have been believed to be one of the mechanisms responsible for such increased non-specific cross-resistance Russell, (2000).

The *qac*E gene (including its attenuated variant *qac*EΔ1) is widely spread in Gram negative bacteria, mainly in *Enterobacteriaceae* and *Pseudomonas spp* (Chang *et al*., 2007; Wang *et al*., 2008a and Mak *et al.,* 2009).

Disinfectants including quaternary ammonium compounds (QACs) have been introduced into farm environments. particular concern was that repeated usage of disinfectants may results in the selection and persistence of bacteria with reduced susceptibility not only to the antiseptics but possibly to antibiotics as well Randall *et al.,* (2004b).

Quaternary ammonium compound (QAC) based disinfectants are often used in environments where antibiotics are used Hegstad *et al*.,(2010). Antibiotic resistance gene and QAC are together carried on class 1 integrons, increasing concerns that QAC exposure resistance may select for antibiotic resistance by selecting for class 1 integrons. Gaze *et al* ., (2005).

Therefore the aim of this study was to investigate the prevalence of omphalitis and the predisposing factors associated with the occurrence of yolk sac infection in poultry farm by isolation and identification of *Escherichia coli* associated with yolk sac infection, antimicrobial sensitivity tests and application of Polymerase Chain Reaction for detection of *eae*A and *qac*EΔ1 genes.

2.MATERIAL AND METHODS

2.1 Samples Collection

A total of 200 chicks (1400 samples) from diseased chickens from one to seven days old of Saso breed were collected from different farms at Dakahlia Governorate were subjected to clinical and postmortem (P.M) examination as well as for isolation and identification of *Escherichia coli* from different organs including liver, caecum, spleen, lungs, heart, yolk sac and cloacal swab. All samples were collected and handled aseptically to prevent cross contamination.

2.2. Bacterial Isolation:

Isolation of *E.coli* was carried out according to *Quinn et al*.,(2002).

2.3. Diagnostic E. coli antisera

The isolates were serologically identified according to Kok *et al.,* (1996) by using rapid diagnostic *E.coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

Polyvalent and monovalent diagnostic *E.coli* antisera were used for serogrouping of *E.coli* isolates according to somatic (O) and capsular (K) antigen.

2.4. Antibiotic Sensitivity test

The antimicrobial susceptibility testing was done according to Finegold and Martin (1982) using the agar disc diffusion method on Mueller Hinton agar and using 8 antibiotic discs included Amoxicillin 10mcg, Enrofloxacin 5mcg, Tetracycline 30mcg, Gentamycin 10mcg, Erythromycin 15mcg, Ciprofloxacin 5mcg , Streptomycin10mcg andNorfloxacin10mcgfrom Oxoid (1998). The interpretation of inhibition zones of tested culture was done according to CLSI (2011).

2.5. DNA Extraction

DNA was done according to Simonelli *et al*., (2009). . Oligoneucleotide primers were designated according to Integrated DNA Technology and were used for amplification of the Ettaching and effacing mechanisms gene (*eae*A) and Quaternary ammonium compound *qac*ED1gene. Theprimers were received in lyophilized form and resuspended in Tris/EDTA (TE) buffer to reach a final concentration of 100 pmol/µl. These primers suspected to amplify specific segment of 248 and 362 bp.as shown in table (1).The DNA extraction for the selected isolates was performed using ABIOpure Genomic DNA extraction kit. The Oligonucleotide Primers

which provided from Metabion (Germany) are listed in table (1). The primers were utilized in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of template. The reaction was performed in a Biometra thermal cycler. The products of PCR were separated by electrophoresis on 1-1.5% agarose gel (ABgene) in 1x TBE buffer at room temperature. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100 bp DNA Ladder (Qiagen, USA) was used to determine the fragment sizes. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

3. RESULTS

Omphalitis was detected in chicks from one day to seven days as following; day 1 (20%), day 2 (26.67%), day 3 (37.5%), day4(30%), day 5(51.4%), day 6 (26.67%) and day 7 (15%). Table (2).

Internal organs from each chicks were bacteriologically examined to reveal the incidence of *E. coli* in different organs. *E. coli* was recovered from different internal organs as the following ,14% from liver, 10% from caecum, 11% from spleen, 10% from heart, 9% from lung, 11.5% from yolk and 8% from cloacal swab. Table (3).

The serological examination of 50 *E. coli* isolates resulted in detection of different serogroups including O_{91}, O_{26}, O_{78} ,O_{125} ,O_{151} ,O_{55},O_{86} ,O_{128}, O_{1}, O_{27}, O_{158}, O_{166},O_{28}, O_{103},O_{142}, O_{144}, O_{159}, O_{6}, O_{02} ,while 7 strains were untyped. Table (4).

Sensitivity test was done using 8 antibiotics. *E. coli* O groups was found to be resistant to Amoxicillin antibiotic (93.02%) followed by Tetracycline 74.42%, Enrofloxacin 46.51%, Erythromycin 30.32% , Ciprofloxacin 27.91%, Norofloxacin and Streptomycin 20.93% and Gentamycin 6.98%. While they were found to be sensitive for Gentamycin, Streptomycin ,Ciprofloxacin, Tetracycline, Erythromycin, Norofloxacin, Enrofloxacin, and Amoxicillin as the following : 88.37%, 46. 51%, 34.89%, 23.26%, 18.60%, 9.30%, 9.30% and 6.98%, respectively. Table (5).

PCR was used for detection of *eae*A gene that play an important role in virulence of *Escherichia coli.* Figure (1). The gene was present in 3 out of 19 isolates. Also detection of

*qac*EΔ1gene that play a role in resistance of *Escherichia coli* to disinfectant. Figure (2). The gene was present in 12 out of 19 isolate.

4.DISCUSSION

Yolk sac infection (YSI) is a major cause of mortality in broilers during the first week of life (Bains, 1979 , Coutts, 1981 and Jordan 1996).

As shown in table (2), out of 200 chicks (1400 samples) examined, *E. coli* 50 (25%). These results agreed with that of Saif *et al*.,(2008),who reported that, Escherichia coli (*E. coli*) is the most common contaminant of yolk sacs in chickens and about 70% of chicks with omphalitis had this bacterium in their yolk sacs, also agreed with Abadi Amare *et al.,* (2013).On the other hand, higher incidence of *E. coli* 83.9% was mentioned by Iqbal *et al.,* (2006). The results were different by Ahmed (2016) who examined 216 yolk sac and revealed that 152 of them were infected with *E. coli* with an incidence of 70.3%.

The gross lesions observed in chicks died of yolk sac infection included unabsorbed/ retained yolk sac and edematous yolk which was also reported by different workers (Suha *etal.,* 2008 , Ahmed *et al* ., 2009 and Kawalilak *et al.,* 2010).

The obtained results of this study revealed that the most *Escherichia coli* isolates obtained from liver of the examined chicks followed by yolk, spleen, caecum, heart , lung and cloacal swab 14%, 11.5%, 11%, 10%, 10% , 9% and 8% respectively and the explanation of these results is due to infection with APEC generally begins as a localized infection of the air sacs commonly referred to as air sacculitis or the air sac disease which in turn may spread to other internal organs resulting in systemic infection Barnes *et al.,* (1999).

These results were agreed with Sharada *et al.,* (2010) who recovered the highest percent of isolates from cases of hepatitis 44.6%, enteritis 33.8%, and pericarditis 16.9%.

The cultural characteristics of *E. coli* was similar to the findings of other authors (Choudhury *et al.*, 1993, Nazir *et al.,* 2004 , Jakaria *et al.,* 2012 and Naurin *et al.*, 2012).

In this study, 50 out of 200 *E.coli* isolates recovered from chicks could be serogrouped in 19 O groups with the most predominant serotype was *Escherichia coli* O_{91} 20 % (10 out of 50 isolates) of all isolates and these results go hand to hand with the previous studies of

(Suwanichkul and Panigrahy 1988 ; Gross 1991 and Bosch *et al.,* 1993), who reported that serogroup O_{91} was traditionally associated with colibacillosis in poultry.

Other serogroups were identified in this investigation as O_{26} 10% (5 strains out of 50), O_{78} 8% (4 strains out of 50), O_{86} & O_{01}& O_{125} 6% (3 strains out of 50), O_{166}& O_{128} 4% (2 strains out of 50), O_{27} & O_{142} & O_{158} O_{151}& O_{02} & O_{06} & O_{055} & O_{144} & O_{159}& O_{28} and O_{103} 2% (1 strains out of 50) and 7 strains untypable *E. coli*.

Among the serogroup isolated in this study is O_{86}. This serogroup is known to be highly pathogenic for 3-5 day-old chicks Burkhanova, (1970). Besides this, O_{86} and O_{26} groups isolated in this investigation are among the enteropathogenic *E. coli* known to be associated with infant haemorrhagic colitis and bloody diarrhoea Cravioto *et al.*,(1979). This is suggestive of the possible zoonotic effect of some*E. coli* serogroups associated with dead-in-shell embryos.El-Jakee *et al.,* (2012) isolated O_{06}, O_{26}, O_{27}, O_{86}, O_{111} and O_{128}. *E. coli* O_{27} was previously isolated from cloacal swabs of chickens Amira *et al* ., (2010). Isolation of *E. coli* O_{06}&O_{15}& O_{25} and O_{78} was done from oviducts of layer hens with salpingitis Salehi and Ghanbarpour (2010), *E. coli* O_{44}&O_{125}& O_{26} &O_{78} & O_{157} and O_{06} were isolated previously from chicken and ducks Heba *et al* ., (2012). The variable frequency of isolation of different serogroups from poultry is probably due to the variation of serogroups over different studies period and locations. However studying more isolates is needed to establish a correlation between certain *E. coli* serogruops and omphalitis in chicks.

Samah and Ahmed(2013)revealed that, 11 different serotypes of *E. coli* which were identified in Egypt as follows, O $_{114}$ predominates with 17.86% of the total isolates, O_{125} and O_{55} with 14.29% each, O_{111} and O_{26} with 10.71% . However, *E. coli* isolates pathogenic for poultry commonly belong certain serotypes, particularly serotypes O_1, O_2 and O_{78} and to some extent O_{15} and O_{55} (Gross, 1994 and Chart *et al.,* 2000), only 4 strains belong to O_1 and O_{55} in the present study.

As shown in Table (5),*E. coli* O groups was found to be 93.02% resistant to Amoxicillin antibiotic followed by Tetracycline 74.42%, Enrofloxacin 46.51%, Erythromycin 30.32% , Ciprofloxacin 27.91%,Norofloxacin and Streptomycin 20.93% and Gentamycin 6.98%. While was found to be sensitive for Gentamycin, Streptomycin ,Ciprofloxacin, Tetracycline,

Erythromycin, Norofloxacin, Enrofloxacin, and Amoxicillin as the following : 88.37%, 46. 51%, 34.89%, 23.26%, 18.60%, 9.30%, 9.30% and 6.98%, respectively.

These results were agreed with that of Ahmed (2016) who said that *E. coli* isolates were highly sensitive to Gentamycin (90%). On the other hand, *E. coli* strains in this study were highly resistant amoxicillin (93.02%) and that results were agreed with (Abd- El- Galil *et al.,* 1993, Hammoudi and Aggad 2008 and Ahmed 2016), but in some reports 223 strains of *E. coli* isolated from fowls were 89% sensitive to amoxicillin Gyurov (1985).

The present study showed resistance percentages to Enrofloxacin (46.51%). Almost similar resistance were detected byAggad *et al.,* (2010) and Zakeri and Kashefi(2012) 45% and 60% respectively.

The relatively high resistance rate of Tetracycline in the isolated *E. coli* in this study were (74.42%)may be due to the consequence of widespread and lengthy use of this group of antibiotics as feed additive, for prophylactic purposes and/or diseases treatmentRahimi,(2013). Bacterial resistance to Tetracycline is plasmid mediated, with a wide variety of genetic determinants Prescott *et al.,* (2000). This makes it more possible for a susceptible bacterium to acquire resistance factors, as was shown by Tricia *et al.,* (2006). However these results were agreed with earlier reports of Roy *et al.,* (2006 b) and Al Ghamdi *et al.,* (1999) as high reported resistance to these antibiotics (57.0-100%) in chicken isolates, it disagreed with those of Kolar *et al.,* (2005) who showed less resistant to Tetracycline about 48%.

The results of Antibiotic susceptibility of our study are invariance with some studies and in accordance with others, indicating that antibiotic susceptibility pattern varies with different isolates, time and development of multiple drug resistant *E.coli* as reported by (Holmberg *et al.,* 1984 and Sharada *et al*., 2010).

Omphalitis-derived isolates extremely are not included in APEC group because some authors had mentioned that these *E. coli* isolates are just opportunistic and non- pathogenic agents Rosario *et al.,* (2005). It had been shown that *E. coli* isolated from breeder farm, hatchery and broiler farms carried the virulence associated genes Dias da Silveira *et al.*,(2002).

In this study, (as in figure 1) the incidence rate (15.79%) of *eae*Agene of *E.coli* detection was recorded, as it was detected by PCR in 3 out of the 19 tested isolates and these results were nearly agreed with (Wani *et al.,* 2004 and Kilic *et al.,* 2007) who reported incidence rate about 2.49%and 35.71% respectively and differ from results obtained by (Samah and Ahmed 2013and Suardana *et al*., 2011) who reported the incidence rate 71.4% and 95% respectively.

In this study,the *qac*EΔ1gene was reported in *E.coli* (63.16%), as it was detected by PCR in 12 out of the 19 tested isolates (as in figure 2). These results were nearly in accordance with Amira (2016) who found the distribution of *qac*EΔ1gene was 93.1%.

The co-resistance of QAC and antibiotics could be achieved by linkage of different resistance mechanisms on the same plasmid, transposon or integrin or any combination of these Hegstad *et al.,* (2010). The localization of these QAC determinants on different mobile elements, may share in the transfer of resistance to the other bacteria Gillings *et al.,* (2009 1,2).

4. CONCLUSION

Out of 200 chicks (1400 samples) examined, *E. coli* incidence was 50 (25%). Was found to be highly resistant to Amoxicillin while was highly sensitive to Gentamycin. Incidence rate of *eae*A gene was (15.79%), while *qac*EΔ1gene was (63.16%).

Chicks should be obtained from hatcheries which adopt strict hygienic measures during the whole hatching process. Moreover, hygienic environment should be provided to the young chicks during brooding and special attention should be paid to the humidity in the brooding house.

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Table (1):Oligonucleotide primers for virulence and resistant genes

Genes	*Primer Sequences (5'-3')*	*Size (bp)*
*eae*A	*ATGCTTAGTGCTGGTTTAGG* *GCCTTCATCATTTCGCTTTC*	248
*Qac*ED1	*TAA GCC CTA CAC* *AAA TTG GGA GAT AT* *GCC TCC GCA GCG ACT TCC* *ACG*	362

Table (2): Incidence of *E. coli* infection in chicks from one day to seven days

Age	Examined chicks	Positive	Incidence	Isolated bacteria	+ve case	%
Day 1	15	3	20%			
Day 2	45	12	26.67%			
Day 3	40	15	37.5%	E. coli	50/200	25
Day 4	30	9	30%			
Day 5	35	18	51.4%			
Day 6	15	4	26.67%			
Day 7	20	3	15%			
Total	200	64	32%			

Table (3):Rate of recovery of *E coli* from internal organs

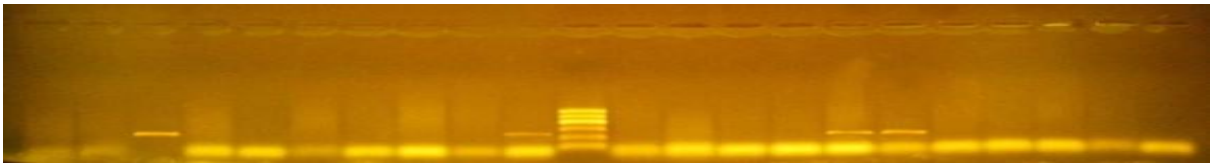
Examined organs in 200 chicks	Number of positive	Percentage of positive %
Liver	28	14
Caecum	20	10
Spleen	22	11
Heart	20	10
Lung	18	9
Yolk	23	11.5
Cloacal swab	16	8

Table(4): *E. coli* serogroupes recovered from bacteriologically examined chicks

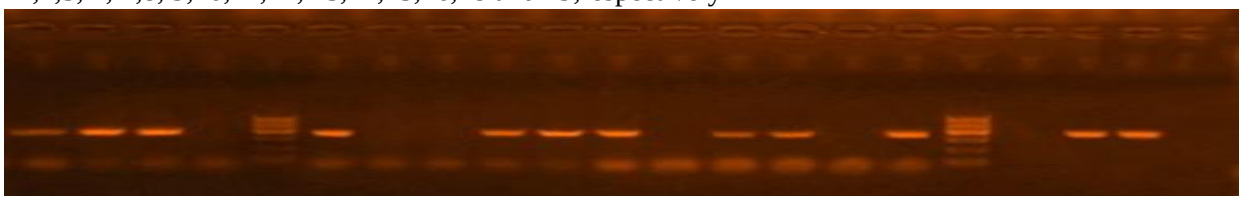
The infected E. coli serotype	Number of positive chicks	Percentage of positive %
O_{91} K -	10/50	20
O_{125} K -	3/50	6
O_{26} K $_{60}$	5/50	10
O_{151} K -	1/50	2
O_{55} K $_{59}$	1/50	2
O_{86} K $_{64}$	3/50	6
O_{128} K -	2/50	4
O_{1} K -	3/50	6
O_{27} K -	1/50	2
O_{158} K -	1/50	2
O_{166} K -	2/50	4
O_{28} K -	1/50	2
O_{103} K -	1/50	2
O_{142} K $_{86}$	1/50	2
O_{144} K -	1/50	2
O_{159} K -	1/50	2
O_{06} K -	1/50	2
O_{78} K -	4/50	8
O_{02} K -	1/50	2
Untyped	7/50	14
Total	50	100

Table (5:)Sensitivity of *E. coli* serotypes to antimicrobial agents

Antibiotics	E.coli		
Ciprofloxacin (CF)	R 12(27.91%)	I 16(37.21%)	S 15(34.88%)
Enrofloxacin (ENR)	R 20(46.51%)	I 19(44.19%)	S 4(9.3%)
Norfloxacin (NOR)	R 9 (20.93%)	I 30(69.77%)	S 4(9.3%)
Tetracycline(T)	R 32(74.42%)	I 1(2.32%)	S 10(23.26%)
Erythromycin (E)	R 13(30.23%)	I 22(51.16%)	S 8(18.61%)
Gentamycin (G)	R 3(6.98%)	I 2(4.65%)	S 38(88.37%)
Streptomycin (S)	R 9(20.93%)	I 14(32.56%)	S 20(46.51%)
Amoxicillin (AM)	R 40(93.02%)	I 0	S 3(6.98%)



Figure(1): *eae*A gene of *Escherichia coli* Amplification of248bp was observed in the extracted DNA of O_{28}, O_{103} and O_{128} in lane number 5, 6 and 17 respectively. No amplification in lane number 1,2,3, 4, 7,8, 9,10,11,12, 13,14,15,16,18 and 19,respectively



Figure(2):*QacE*Δ1 gene of *Escherichia coli* Amplification of 362 bp was observed in the extracted DNA of O_{26}, O_{91}, O_{28}, O_{151}, O_{55}, O_{86}, O_{125}, O_{166}, O_1, O_{128}, O_{78} and O_2 (in lane number2, 3, 5, 7, 8, 10, 11, 12, 15, 17, 18 and 19 respectively). No amplification in in lane number1, 4, 6, 9, 13, 14 and16 respectively .