



Prevalence of *eaeA* and *qacEΔ1* genes in *Escherichia coli* isolated from omphalitis in baby chicks

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ABSTRACT

A total of two hundred diseased Saso chicks with omphalitis were examined (1400 samples) for the isolation of *E. coli* from different organs (liver, caecum, 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 sero-grouped in 19 O groups with the most predominant serotype was O₉₁ 20 % (10 out of 50 isolates), O₂₆ 10% (5 strains out of 50) O₇₈ 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 *eaeA* gene of *E. coli* was 15.79%. Quaternary ammonium compound (*qacEΔ1*) gene also was detected in *E. coli* with incidence rate 63.16%.

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

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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 strategies (Srinivasan 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 *eaeA* 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 *qacE* gene (including its attenuated variant *qacEΔ1*) is widely spread in Gram negative bacteria, mainly in *Enterobacteriaceae* and *Pseudomonas spp.* (Chang et al., 2007; Mak et al., 2009; Wang et al., 2008a). 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 *eaeA* and *qacED1* 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, Streptomycin 10mcg and Norfloxacin 10mcg from 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). Oligonucleotide primers were designated according to Integrated DNA Technology and were used for amplification of the Attaching and effacing mechanisms gene (*eaeA*) and Quaternary ammonium compound *qacED1* gene. The primers 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 ABIO pure 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%), day 4 (30%), day 5 (51.4%), day 6 (26.67%) and day 7 (15%). Table (2). Internal organs from each chick 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 O₉₁, O₂₆, O₇₈, O₁₂₅, O₁₅₁, O₅₅, O₈₆, O₁₂₈, O₁, O₂₇, O₁₅₈, O₁₆₆, O₂₈, O₁₀₃, O₁₄₂, O₁₄₄, O₁₅₉, O₆, O₀₂, 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,

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

Genes	Primer Sequences (5'-3')	Size (bp)
<i>eaeA</i>	ATGCTTAGTGCTGGTTTAGG	248
	GCCTTCATCATTTGCTTTC	
	TAA GCC CTA CAC	
<i>QacED1</i>	AAA TTG GGA GAT AT	362
	GCC TCC GCA GCG ACT TCC	
	ACG	

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%	E. coli	50/200	25
Day 2	45	12	26.67%			
Day 3	40	15	37.5%			
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* serogroups recovered from bacteriologically examined chicks

The infected <i>E. coli</i> 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%)	

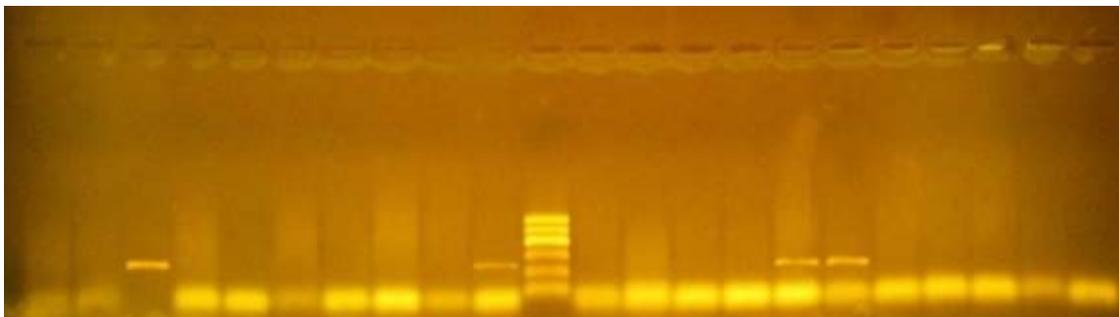


Figure (1): *eaeA* gene of *Escherichia coli* Amplification of 248 bp was observed in the extracted DNA of O₂₈, O₁₀₃ and O₁₂₈ in lane number 5, 6 and 17 respectively. No amplification in lane number 1,2,3, 4, 7,8, 9,10,11,12, 13,14,15,16,18 and 19, respectively

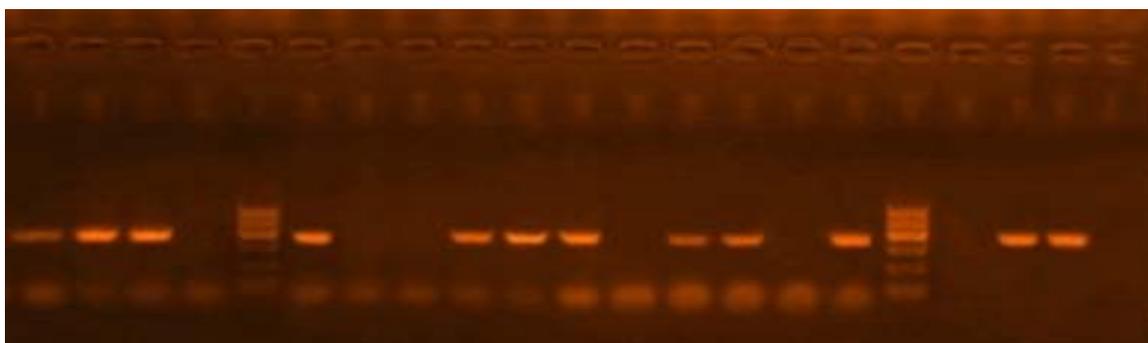


Figure (2): *QacEΔ1* gene of *Escherichia coli* Amplification of 362 bp was observed in the extracted DNA of O₂₆, O₉₁, O₂₈, O₁₅₁, O₅₅, O₈₆, O₁₂₅, O₁₆₆, O₁, O₁₂₈, O₇₈ and O₂ (in lane number 2, 3, 5, 7, 8, 10, 11, 12, 15, 17, 18 and 19 respectively). No amplification in in lane number 1, 4, 6, 9, 13, 14 and 16 respectively

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 *eaeA* 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 *qacEΔ1* gene 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; 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 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 (Ahmed, 2016; Kawalilak et al., 2010; Suha et al., 2008).

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; Jakaria et al., 2012; Naurin et al., 2012; Nazir et al., 2004). 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* O₉₁ 20 % (10 out of 50 isolates) of all isolates and these results go hand to hand with the previous studies of Gross (1991); Suwanichkul and Panigrahy (1988); van den Bosch et al. (1993), who reported that serogroup O₉₁ was traditionally associated with colibacillosis in poultry. Other serogroups were identified in this investigation as O₂₆ 10% (5 strains out of 50), O₇₈ 8% (4 strains out of 50), O₈₆ & O₀₁ & O₁₂₅ 6% (3 strains out of 50), O₁₆₆ & O₁₂₈ 4% (2 strains out of 50), O₂₇ & O₁₄₂ & O₁₅₈ O₁₅₁ & O₀₂ & O₀₆ & O₀₅₅ & O₁₄₄ & O₁₅₉ & O₂₈ and O₁₀₃ 2% (1 strains out of 50) and 7 strains untypable *E. coli*.

Among the serogroup isolated in this study is O₈₆. This serogroup is known to be highly pathogenic for 3-5 day-old chicks Burkhanova, (1970). Besides this, O₈₆ and O₂₆ groups isolated in this investigation are among the enteropathogenic *E. coli* known to be associated with infant hemorrhagic 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 O₀₆, O₂₆, O₂₇, O₈₆, O₁₁₁ and O₁₂₈. *E. coli* O₂₇ was previously isolated from cloacal swabs of chickens (Amira et al., 2010). Isolation of *E. coli* O₀₆ & O₁₅ & O₂₅ and O₇₈ was done from

oviducts of layer hens with salpingitis (Ghanbarpour and Salehi, 2010), *E. coli* O₄₄ & O₁₂₅ & O₂₆ & O₇₈ & O₁₅₇ and O₀₆ 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* serogroups and omphalitis in chicks.

Samah and Ahmed (2013) revealed that, 11 different serotypes of *E. coli* which were identified in Egypt as follows, O₁₁₄ predominates with 17.86% of the total isolates, O₁₂₅ and O₅₅ with 14.29% each, O₁₁₁ and O₂₆ with 10.71%. However, *E. coli* isolates pathogenic for poultry commonly belong certain serotypes, particularly serotypes O₁, O₂ and O₇₈ and to some extent O₁₅ and O₅₅ (Chart et al., 2000; Gross, 1994), only 4 strains belong to O₁ and O₅₅ 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); Ahmed (2016); Hammoudi and Aggad (2008), 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 by Aggad et al. (2010); 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 treatment (Rahimi, 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. (2006b) 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 Silviers et al., 2002). In this study, (as in figure 1) the incidence rate (15.79%) of *eaeA* gene 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 (2013) and Suardana et al. (2011) who reported the incidence rate 71.4% and 95% respectively. In this study, the *qacEΔ1* gene 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 *qacEΔ1* gene 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., 2009a).

5. 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 *eaeA* gene was (15.79%), while *qacEΔ1* gene 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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