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Bacteriological and Molecular Studies on Bacteria Causing Omphalitis in Chicks with Regard to Disinfectant Resistance

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Abstract: This study aimed to assess the prevalence of omphalitis and the predisposing factors associated with yolk sac infection in poultry farm in Dakahlia Governorate. Two hundred diseased Saso chicks with omphalitis were examined (1400 samples) for the isolation of different bacterial strains 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%. *Escherichia coli* (*E. coli*) 50 (25%) was the most predominant isolate followed by *Staphylococcus aureus* (*S. aureus*) 25 (12.5%), *Salmonella spp* 24 (12%) and finally *Pseudomonas aeruginosa* (*P. aeruginosa*) 5 (2.5%). 50 isolates 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). Salmonella isolates were serotyped using poly and monovalent "O" and "H" antisera. By serogrouping the most predominant serotypes were *S. typhimurium* and *S. enteritidis* 20.83%. Among *E. coli* O groups were found to be 93.02% resistant to Amoxicillin while the isolates highly sensitive for Gentamycin with 88.37%. Salmonella was resistant to Amoxicillin with 83.33% while it was highly sensitive for Gentamycin with 95.8%. *Staph aureus* was 96% resistant to Erythromycin while was highly sensitive for Gentamycin 100%. *P. aeruginosa* isolates were 100% resistant to Tetracycline while they were highly sensitive for Gentamycin 80%. The incidence rate of *eae*A gene of *E.coli*, mecA gene of *Staph aureus* and *inv*A gene of Salmonella was 15.79%, 72.22% and 85.71% respectively. Quaternnary ammonium compound (*qac*EΔ1)gene also was detected in *E. coli*, Salmonella, *Staph aureus* and *Pseudomonas aeruginosa* with incidence rate 63.16%, 57.14%, 44.44% and 100% respectively. Chicks with omphalitis harbored many different pathogens which considered source of infection during first days of life.

Key words: Omphalitis · Salmonella · *E. coli* · *S. aureus* · *P. aeruginosa* · *Virulence genes* · *Disinfectant* · *Resistant gene*

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 [1]. Yolk sac infection caused chick mortality during the first week of the post-hatching period [2]. *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*) [3] Genus Salmonella [4] *Staphylococcus* alone or with *E .coli* [5&6] and *P. aeruginosa* [7].

Virulence in microorganisms is associated with the capacity to attach and colonize at the site of infection, with subsequent damage to the host and is promoted by aggresins that interfere with the host defense [8].

Differences in virulence among salmonella serovars and in the route of salmonella infection in a variety of host species had referred to the variable virulence genes [9]. Most salmonella isolates contain the invasion gene *inv*A [10].

Methicillin-resistant *S. aureus* (MRSA) included strains that acquired a gene giving them resistance to methicillin and essentially all other beta-lactam antibiotics. This group of organisms had since emerged as a serious concern in human medicine. MRSA was first reported as a nosocomial pathogen in human hospitals. Although

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these organisms caused the same types of infections as other *S. aureus*, hospital-associated strains become resistant to most common antibiotics and treatment can be challenged [11].

Though the Quaternary ammonium compound (*qac*) genes were named after one of their substrates (QACs), they had much wider spectrum of activity [12]. Qac genes in Gram – negative bacteria were usually linked to plasmid- mediated class 1 integrons. They were found in combination with genes coding for resistance to Sulphonamides, Trimethoprim, Chloramphenicol, Aminoglycosides and ß- lactams [13].

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 bacteria associated with yolk sac inflammation, Antimicrobial sensitivity tests for the isolated bacteria and application of Polymerase Chain Reaction for detection of specific genes.

MATERIALS AND METHODS

Samples Collection: A total of 200 chicks (1400 samples) from diseased living chicks from one to seven days 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,* Salmonella, *S. aureus* and *Pseudomonas aeruginosa* from organs including liver, caecum, spleen, lungs, heart, yolk sac and cloacal swab. All samples were collected and handled aseptically to prevent cross contamination using sterile sampling materials (Bags, knifes, flasks, scissors and forceps).

Bacterial Isolation: *E.coli* isolation was carried out according to *Quinn et al*. [14] salmonella isolation was done according to ISO 6579 [15] *staph. Aureus* was isolated according to ISO 6888 [16] and *Pseudomonas aeruginosa* isolation was done according to Mo'men, [17].

Antibiotic Sensitivity Test: The antimicrobial susceptibility testing was done according to Finegold and Martin [18] using the agar disc diffusion method on Mueller Hinton agar by using 8 antibiotic discs for each microbe from Oxoid [19]. The interpretation of inhibition zones of tested culture was according to CLSI [20].

DNA Extraction: Was done according to *Simonelli et al*. [21] . Oligoneucleotide primers were designated according to Integrated DNA Technology and were used for amplification of the Ettaching and effacing mechanisms gene (*Eae*a), Methicillin resistant S.*aureus* (MRSA), Salmonella invasion protein A gene (*Inv*a), *P. aeruginosa* 16SrDNA gene and Quaternary ammonium compound *Qac*ED1gene. 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, 310, 284, 956 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.

Table 1: Oligonucleotide primers for virulence and resistant genes

Genes	*Primer Sequences* (5'-3')	Size (bp)
EaeA	*ATGCTTAGTGCTGGTTTAGG*	
	GCCTTCATCATTTCGCTTTC	248
*MRS*A	*GTA GAA ATG ACT GAACGTCCG ATA A*	
	CCAATT CCA CAT TGT TTC GGT CTA A	310
*inv*A	*GTGAAATTATCGCCACGTTCGGGCAA*	
	TCATCGCACCGTCAAAGGAACC	284
P. aeruginosa	*GGGGGATCTTCGGACCTCA*	
16SrDNA	*TCCTTAGAGTGCCCACCCG*	956
*Qac*ED1	*TAA GCC CTA CAC*	
	AAA TTG GGA GAT AT	
	GCC TCC GCA GCG ACT TCC ACG	362

RESULTS AND DISCUSSION

Results obtained in Tables (2, 3, 4) showed that incidence of omphalitis from one to seven days, isolated bacteria, sensitivity to antimicrobial agents and incidence of mixed infection.Detection of virulence and resistance genes for bacteria were detected in Figures (1-5).

In this study, the incidence rate of omphalitis was 32% in Saso breed during the first week of life. Mixed bacterial infections was the predominant cases recorded

Table 2: Incidence of bacteria in chicks from one day to seven days

Age	Examined chicks	Positive	Incidence	Isolated bacteria	Incidence	%
Day 1	15	3	20 %	*E. coli*	50/200	25
Day 2	45	12	26.67 %	Salmonella	24/200	12
Day 3	40	15	37.5 %	*Staph aureus*	25/200	12.5
Day 4	30	9	30 %	*P.aeruginosa*	5/200	2.5
Day 5	35	18	51.4 %			
Day 6	15	4	26.67 %			
Day 7	20	3	15 %			
Total	200	64	32 %			

Table 3: Incidence of mixed infection

Isolated bacteria	Number	%
E. coli +*P. aeruginosa*	2/200	1
E. coli + Salmonella	13/200	6.5
Salmonella + *S. aureus*	9/200	4.5
E. coli+ *Salmonella* + *S. aureus*	20/200	10
E. coli + *S. aureus*	12/200	*6*
E. coli+ Salmonella + *P. aeruginosa*	2/200	1
Total	58	29

Table 4: Sensitivity to different antibiotic agents

	E.coli	*Salmonella spp*	*S. aureus*	*P. aeruginosa*
Ciprofloxacin (CF)	R 12(27.91%)	R 6(25%)	R 1(4%)	R 1(20%)
	I 16(37.21%)	I 6 (25%)	I 3(12%)	I 2(40%)
	S 15(34.88%)	S 12 (50%)	S 21(84%)	S 2(40%)
Enrofloxacin (ENR)	R 20(46.51%)	R 8(33.33%)	R 10(40%)	R 4(80%)
	I 19(44.19%)	I 14(58.33%)	I 5(20%)	I 0
	S 4(9.3%)	S 2(8.34%)	S 10(40%)	S 1(20%)
Norfloxacin (NOR)	R 9 (20.93%)	R 3(12.5%)	R 1(4%)	R 1(20%)
	I 30(69.77%)	I 4(16.67%)	I 1(4%)	I 1(20%)
	S 4(9.3%)	S 17(70.83%)	S 23(92%)	S 3(60%)
Tetracycline (T)	R 32(74.42%)	R 13(54.17%)	R 18(72%)	R 5(100%)
	I 1(2.32%)	I 1(4.16%)	I 0	I 0
	S 10(23.26%)	S 10(41.67%)	S 7(28%)	S 0
Erythromycin (E)	R 13(30.23%)	R 9(37.5%)	R 24(96%)	*
	I 22(51.16%)	I 15(62.5%)	I 1(4%)	
	S 8(18.61%)	S 0	S 0	
Gentamycin (G)	R 3(6.98%)	R 0	R 0	R 1(20%)
	I 2(4.65%)	I 1(4.17%)	I 0	I 0
	S 38(88.37%)	S 23(95.83%)	S 25(100%)	S 4(80%)
Streptomycin (S)	R 9(20.93%)	R 5(20.83%)	*	R 3(60%)
	I 14(32.56%)	I 6(25%)		I 0
	S 20(46.51%)	S 1354.17%)		S 2(40%)
Amoxiciilin (AM)	R 40(93.02%)	R 20(83.33%)	R 20(80%)	*
	I 0	I 4(16.67%)	I 2(8%)	
	S 3(6.98%)	S 0	S 3(12%)	
Oxacillin (OX)	*	*	R 18(72%)	*
			I 0	
			S 7(28%)	
Doxycyclin (DO)	*	*	*	R 4(80%)
				I 0
				S 1(20%)
Naldixic acid (NA)	*	*	*	R 4(80%)
				I 0
				S 1(20%)

*: Not Applicable

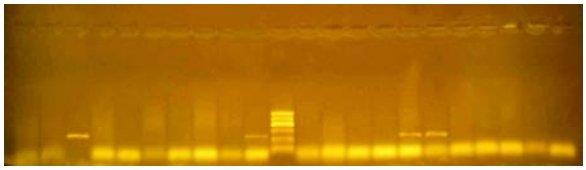

Fig. 1: *Eae*a gene of *Escherichia coli* Amplification of 248bp was observed in the extracted DNA of O_{28}, O_{103} and O_{128} in lane number 5, 6 and 17 respectively. No amplification in lane number 1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18 and 19, respectively

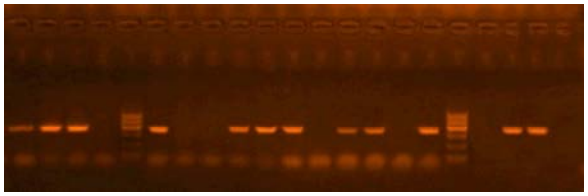

Fig. 2: QacEΔ1 gene of *Escherichia coli* Amplification of 362 bp was observed in the extracted DNA of O_{26}, O_{91}, O_{28}, O_{151}, O_{55}, O_{86}, O_{125}, O_{166}, O_1, O_{128}, O_{78} and O_2 (in lane number2, 3, 5, 7, 8, 10, 11, 12, 15, 17, 18 and 19 respectively). No amplification in in lane number1, 4, 6, 9, 13, 14 and16 respectively

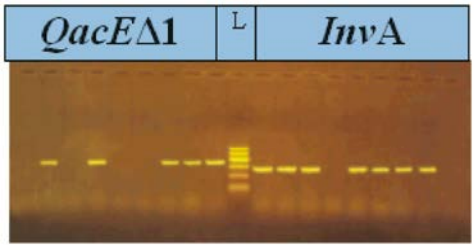

Fig. 3: Amplification of *inv*A gene of *Salmonella* strains: 284bp was observed in the extracted DNA of 1, 2, 3, 4, 6 and7. No amplification in 5. Amplification of *QacE*Δ1 gene of Salmonella strains: 362bp was observed in the extracted DNA of 1, 3, 6 and 7. No amplification in 2, 4and 5.

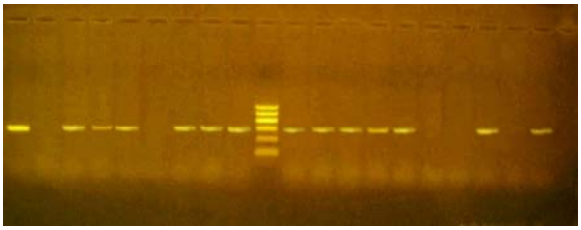

Fig. 4: MecA gene of *Staph aureus*: Amplification of 310bp was observed in the extracted DNA of 1, 3, 6, 7, 8 , 9, 10, 11, 12, 14, 15, 16 and 18 .No amplification in 4, 5, 13 and 17.

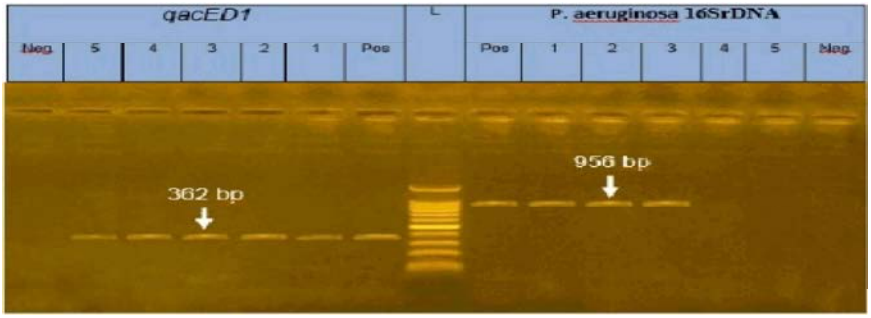

Fig. 5: *P. aeruginosa* (16SrDNA) Amplification of956bp was observed in the extracted DNA of 1, 2 and 3.No amplification in 4 and 5. *P. aeruginosa QacEΔ1* gene Amplification of 362bpwas observed in the extracted DNA of 1, 2, 3, 4 and 5.

due to *E. coli* together with any of the three species (*Staphylococcus aureus., Salmonella* spp and *Pseudomonas aeruginosa*) and these results with those of Suha *et al.* [22] and Rosario *et al.* [23].

As examined in Table (2), out of 1400 samples examined, *E. coli* 50 (25% %) was the most predominant isolate followed by *staphylococcus aureus* 25 (12.5%) followed by salmonella 24 (12%) and finally *Pseudomonas aeruginosa* 5 (2.5%). These results were agreed with those of Abadi *et al.* [24]. On the other hand, higher incidence of *E. coli* 83.9% was mentioned by Iqbal *et al.* [25] who also isolated salmonella, Staphylococcus *aureus* and *Pseudomonas aeruginosa* with a percentage of 0.5%for each one.

The incidence of yolk sac infection was summarized in Table (2), incidence was observed in 64 (32%) chicks. Maximum percentage (51.4%) of yolk sac infection was observed in chicks of 5 days old followed by age of 3 days (37.5%), 4 days (30%) , 2 days & 6 days (26.67%), 1 day (20%) and 7 days (15%).These results were agreed with those of Abadi *et al.* [24]. While disagreed with Nasrin *et al.* [26] who reported that *Salmonella* was the highest prevalence both in chicks aged 1-3 days and 4-7 days (68 and 54.3%, respectively), followed by the prevalence of *E.* coli in chicks aged 1-3 days and 4-7 days (48% and 45.71%, respectively) and the prevalence of *Staphylococcus aureus* (24% in 1-3 days old chicks and 28.6% in 4-7 days old chicks).

E. coli was found to be 93.02% resistant to Amoxicillin. While was found to be sensitive for Gentamycin, (88.37%). These results were agreed with that of Ahmed [27] who said that *E. coli* isolates were highly sensitive to Gentamycin and were highly resistant to amoxicillin (93.02%) also these results were agreed with Ahmed [27] and Hammoudi and Aggad [28]. Also resistance percentages to Enrofloxacin (46.51%) was similar to resistance detected by Aggad *et al.* [29] (45%).

Salmonella was found to be 83.33% resistant to Amoxicillin followed by Tetracycline 54.17%, Erythromycin 37.5%, Enrofloxacin 33.3%, Ciprofloxacin 25%, Streptomycin 20.83% and Norofloxacin12.5%. While it was found to be sensitive for Gentamycin, Norofloxacin, Streptomycin , Ciprofloxacin , Tetracycline, Enrofloxacin, Erythromycin and Amoxicillin as the following : 95.8%, 70. 8%, 54.17%, 50%, 41.67%, 8.33%, 0 and 0 respectively. That results partially agreed with Ahmed [27] and Hammed [30]. On the other hand, *Salmonella spp* strains in this study were highly resistant to amoxicillin (83.33%) that agreed with Kruy *et al.* [31].

Staph aureus was found to be sensitive for Gentamycin, Norofloxacin, Ciprofloxacin, Enrofloxacin, Tetracycline, Oxacillin, Amoxicillin and Erythromycin as the following: 100%, 92%, 84%, 40%, 28 %, 28%, 12% and 0, respectively. These results go hand to hand with Ahmed [27] who stated that isolates of *Staph aureus* were highly sensitive to Gentamycin and Ciprofloxacin (80% for each) while they were highly resistant to Amoxicillin (100%). Also agreed with Owuna *et al.* [32] who stated that *Staph aureus* isolates were more susceptible to Gentamycin (82.8%) and Ciprofloxacin (82.7%), while they were less susceptible to Amoxicillin (13.8%).

P. aeruginosa was found to be sensitive for Gentamycin, Norofloxacin, Ciprofloxacin, Streptomycin, Enrofloxacin, Doxycyclin, Naldixic acid and Tetracycline as the following: 80, 60, 40, 40, 20%, 20, 20 and 0%, respectively. These results were nearly similar to Ahmed [27] who stated that isolates of *P. aeruginosa* were highly sensitive to Gentamycin (100%). That agreed with Abd El-Gawad *et al.* [33] whose isolates were highly sensitive to Gentamycin. These findings were agreed with that of Kurkure *et al.* [34] whose isolates were susceptible to Gentamycin (88.57%) and Ciprofloxacin (62.85%). On the contrary strains in this study were resistant to Doxycyclin (80%) and these results were nearly similar to that reported by Ahmed [27].

As in Figure 1 the incidence rate of *eae*A gene of *E.coli* detection was recorded(15.79%), as it was detected by PCR in 3 out of the 19 tested isolates and these results agreed with Wani *et al.* [35] and Kilic *et al.* [36] who reported incidence rate about 2.49% and 35.71% respectively and disagreed with Samah and Ahmed [37] who reported the incidence rate 71.4%.

As in Figure 4 the incidence rate of *mec*A gene of *Staph aureus* detection was recorded (72.22%), as it was detected by PCR in 13 out of the 18 tested isolates and these results agreed with Lee [38] and Rania [39] who reported incidence rate about 63.16% (12/19) and 58.33% (9/14) respectively.

As in Figure 3 the incidence rate of *inv*A gene of Salmonella detection was recorded(85.71%), as it was detected by PCR in 6 out of the 7 tested isolates and these result nearly was the same with Al Atfehy [40] and Mohamed [41] in isolates of different origin in Egypt.

In this study, the *qac*EΔ1gene was reported in the present study as in *E. coli* (63.16%), as it was detected by PCR in 12 out of the 19 tested isolates (As in Figure 3). In salmonella (57.14%), as it was detected by PCR in 4 out

of the 7 tested isolates (As in Figure 4). In *Staph aureus* (44.44%), as it was detected by PCR in 8 out of the 18 tested isolates. In *Pseudomonas aeruginosa* (100%), as it was detected by PCR in 5 out of the 5 tested isolates (As in Figure 5). These results were nearly in accordance with Amira [42] who found the distribution of *qac*EΔ1 was 93.1%.

CONCLUSION

This highest prevalence of *E. coli* is presumably due to the fact that *E. coli* is the commonest environmental contaminants of poultry farms. The presence of these bacteria was indicative of substandard conditions at hatcheries and poultry farms. Since the unabsorbed yolk in the young chicks provides a suitable medium for the multiplication of bacteria. Therefore, suggested that chicks should be obtained from those 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.

The occurrence of multi-drug resistance in bacteria in chicks suffering from omphalitis is alarming as this resistance may gain access to man and animals, which might result in difficulties in treatment of bacterial infection.

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