Detection of Disinfectant resistant aerobic bacteria in unhatched chicken eggs

Aml A. bakheet[1]; Naglaa M. Ali[1]; Sayed H. Al. Habaty[2] and Soad A. Nasef [3]

[1]*Animal Health Research Institute, Poultry Diseases, Assiut Regional Laboratory.*
[2] *Animal Health Research Institute, Bacteriology, Assiut Regional Laboratory.*
[3]*Reference Laboratory for Veterinary Quality Control on Poultry Production, Poultry Diseases Dept., Animal Health Research Institute, Dokki, Giza.*
Email:d.aml_vet@yahoo.com

ABSTRACT

A total of 100 dead in shell chicken eggs and 20 swabs from hatcheries were examined for aerobic bacteria. The isolated bacteria were identified as Escherichia coli 26 isolates (21.7%); (out of them 18 isolates from unhatched eggs and 8 isolates from hatcheries). Pseudomonas aeruginosa 22 isolates (18.3%) was isolated only from unhatched eggs. While we isolated 16 (13.3%) coagulase positive Staphylococci*; 12* isolates from unhatched eggs and 4 isolates from hatcheries. Proteus spp.13 isolates (10.8%); 8 isolates from unhatched eggs and 5 isolates from hatcheries. finally Salmonella spp.11 isolates (9.2%) and we couldn't isolate any of them from hatcheries. The serological examination of E coli strains revealed that there were 9 serotypes , the most predominant serotype was O91 : H21(7isolates) , followed by O78 (4 isolates), O2 : H6 (2 isolates) , O163 : H2 (2 isolates) , O128 : H2 (3 isolates), O158(2 isolates), O26 : H11(2 isolates), O121 : H7 (2isolate) and O44 : H18(2isolates).Serotyping of *Salmonella* isolates showed that belonged to S. Kentukey 3 isolates, S. Enteritidis 3 isolates , S. Molade 2 isolates , S. Tsevie 1 isolates , S. Infantis 1 isolate and S. Larochelle 1 isolate. The antibiotic susceptibility testing of E. coli isolates were studied against 15 different chemotherapeutic agents revealed that it was 100%, 100%, 100%, and 96.1% resistant to Amoxicillin, Methicillin, Sulfamethazole/Trimethoprim and Cefoxitin respectively, while *E. coli* isolates were sensitive for Colistin, Ciprofloxacin and Gentamycin with 92.3%, 88.5% and 84.6% respectively. While P. aeruginosa isolates were 100% resistant to tetracycline, Methicillin, Ampicillin, and Amoxicillin, while they were sensitive for Gentamycin (77.3 %) and Ciprofloxacin (72.7%). Coagulase Positive Staphylococci isolates were 100% resistant to Methicillin and Cefoxitin while highly sensitive for Gentamycin and Proteus and Pseudomonas Ciprofloxacin in percentage of 93.7%and 87.5%. In case of Proteus isolates they were found to be 100% resistant to Tetracycline, Methicillin and Cefoxitin while they were sensitive for Ciprofloxacin and Streptomycin 92.3 % and 84.6 %.Finely Salmonella spp. were resistant to Methicillin and Cefoxitin with 100% and Amoxicillin 90.9% while sensitive for Ciprofloxacin and Gentamycin with 90.9%.and 81.8%. Determination of multi-drug resistance index (MDRI) for bacterial isolates recorded 0.633 in *E. coli*, 0.781 in Ps. aeruginosa, 0.612 in Staphylococci, 0.579 in *Proteus* spp. and 0.593 in Salmonella. Quaternary ammonium compound resistant gene *(qacED1)* was detected by PCR in *E. coli, Salmonella,* Coagulase Positive Staphylococci, aeruginosa with incidence rate 100% in all isolates. The study concluded that the presence of the *qac* resistance gene and multi-drug resistance bacteria of the isolated strains definite a link between antibiotic and disinfectant resistance is possible.

Key words: unhatched chicken eggs, aerobic bacteria, PCR, QAC gene resistance.

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1. INTRODUCTION

Hatchery hygiene is recognized as an important factor and common concern in healthy poultry production (Thermote, 2006). The development and maintenance of an effective hatchery sanitation program is essential for the successful operation of a poultry hatchery, so hatchery sanitation plays a crucial role in prevention and control of pathogens (Gehan *et al.*, 2004).The hatchery is the greatest source for spread the diseases within the poultry industry. The problem usually starts with contaminated eggs which are incubated under ideal condition for microbiological growth. Numerous bacterial pathogens that contaminate hatcheries have been isolated from egg shell, egg content as well as from dead in shell embryos. These pathogens included Salmonella spp., E. coli, Klebsiella spp., Proteus spp. Staphylococcus aureus and Streptococci (Al-Khalaf *et al.,* 2010 and Kirunda *et al.,* 2010). Poultry bacterial pathogens are mainly controlled by using chemotherapeutic drugs. The long term ,extensive and misuse of antibiotics for veterinary purpose may

eventually results in selection for the survival of resistant microbial species (Aarestrup, 1999). Genes encoding for this resistance also can be transferred to other formerly susceptible bacteria, thereby causing a threat to both animal and human health (Montagne *et al*., 2003). The use of disinfectants could possibly be the last line of defense for the poultry industry. Quaternary ammonium compounds (QACs) based disinfectants are frequently used in environments were antibiotics are used thus fuelling the concern of a link between QAC and antibiotic resistance (Hegstad *et al.,* 2010). The quaternary ammonium compounds (QACs) are cationic surface active detergents widely used in the poultry industry because of their low relative toxicity, good antibacterial properties non-irritating, non-corrosive, low toxicity and reasonably effective in the presence of organic matter. Therefore, it makes a disinfectant of choice for equipment like incubators and hatching trays (Haynes and Smith, 2003). Unfortunately, concerns have arisen regarding the potential emergence of cross-resistance and co-resistance between widely used disinfectants and antibiotics (Reverdy, *et al.,* 1993). Mamman *et al*. (2008) showed that Gram negative bacteria were generally more resistant to effects by disinfectants than Gram positive bacteria probably due to their having a more complex cell wall. The widespread and unrestricted use of antibiotics in animal and poultry production has led to a surge in antibiotic resistant bacterial strains, thus fuelling the search for alternative treatments for bacterial infections. One of these alternative treatments is the use of quaternary ammonium compound (QAC) based disinfectants, Reverdy *et al.,* (1993). Sheldon (2005) reported that the mechanism of bacterial resistance to biocides can be intrinsic (as in the case of spores, mycobacteria, and Gram-negative bacteria), or acquired by means of plasmids or transposons, or by genetic mutation (Cabrera et al., 2007). Exposure of microorganisms to sub-MIC concentrations could result in the emergence of clones resistant to QACs (Hegstad *et al.,* 2010). Disinfectants are generally used at very high concentrations but there is always the possibility that some bacteria are exposed to sub-MIC concentrations which could result in the development of resistance. Reverdy *et al.* (1993) suggested that the widespread use of QACs might impose a selective pressure and contribute to the emergence of disinfectant-resistant microorganisms in these environments. QACs resistance genes fall into two families. The *qac*A/B genes belong to the major facilitator super family and are only found in staphylococci on multi-resistance plasmids; Other QACs resistance genes belong to the small multidrug resistance family and include *qac* C/genes (Paulsen *et al.* 1995 and 1996). QAC genes in Gram-negative bacteria were most frequently found in combination with genes coding for resistance to Aminoglycosides, Chloramphenicol, Sulphonamides, Trimethoprim and β-lactams Colinon *et al.* (2010) and Zhao *et al*. (2012). QACs Reports on QAC resistant bacteria have been on the increase in the food industry and veterinary practice and thus studies on bacterial resistance to QACs are on the increase.

Aim of work: Keeping in view the above facts the present research was done with the following objectives: isolation and identification of bacteria associated with unhatched chicken eggs; determination of antibiotic susceptibility pattern of isolated bacteria with detection of multidrug resistance and detection of *qac* resistance gene in isolated bacteria.

2. MATERIALS AND METHODS

2.1. Collected Samples

A total of 100 fully grown failed to pip out dead in shell embryos and 20 swabs from different hatcheries were collected (cracked and piped out eggs not collected to avoid external contamination), samples were collected from (liver, yolk sac ,heart ,shell surface of the collected dead in shell embryonated eggs) . All samples were handled aseptically and were examined microbiologically.

2.2. Bacteriological examination

Swabs from dead in shell embryos egg shell and different hatcheries were inoculated onto nutrient broth, Rappaport-Vassiliadis Soy broth and Tryptic soya broth containing 70 mg/ml Na Cl. All cultured media were incubated at 37 °c for 24 hours. The broth were streaked onto MacConkey agar, Xylose lysine-deoxycholate agar (XLD),Pseudomones agar, Mannitol Salt agar and Eosin methylene blue (EMB) agar media then incubated at 37 °c for 24hours.The collected colonies were identified morphologically using colony characters, Gram staining and biochemically according to MacFaddin (2000); Alexander (2001) and Leboff and Pierce (2011). Triple Sugar Iron agar (TSI) and IMVC tests were applied for identification of Enterobacteriaceae and applying of enzymatic reactions such as: Oxidase, Catalase, Coagulase and Urease tests for the other microorganisms according to Quinn *et al*. (2002).

2.3. Serological identification

The isolated *E. coli* and *Salmonella* strains were serotyped in clinical microbiology unit in Faculty of Veterinary Medicine, Benha University. Serological identification of *Salmonella* was carried out by slide agglutination technique according to Kauffman (1974) for the determination of Somatic (O) and Flagellar (H) antigen using Salmonella antiserum (DENKA SEIKEN Co., Japan). *E. c*oli isolates were serologically identified according to Kok *et al.,* (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for detection *E. coli* serotypes.

2.4. Antibiotic susceptibility testing

The antimicrobial susceptibility testing was done according to Finegold and Martin (1982) using agar disc diffusion method on Mueller Hinton agar. The isolated strains were tested against 15antibiotic discs of commonly used chemotherapeutic agents, from Oxoid Hampshire, U K. The interpretation of inhibition zones of tested culture was carried out according to CLSI (2015).

2.5. Multidrug resistant Index (MDRI)

Determination of multi-drug resistance index (MDRI) for bacterial isolates: Resistance to more than three antibiotics was taken as multidrug resistance (MDR). MDR index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed (Chandran *et al.,* 2008). Isolates with MDRI values of more than 0.2 or 20% were considered highly resistant.

$$\text{MDR index} = \frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotics used}} \times 100$$

2.6. Polymerase Chain Reaction (PCR) for identification of qacED1gene

The isolated E. coli, Salmonella *spp.,* Pseudomonas spp., Proteus *spp. and* Staphylococci strains were sent to the Reference Laboratory for Veterinary Quality Control on Poultry Production in Animal Health Research Institute, Dokki, Giza, Egypt, for detection of *qacED1*gene as follow:

2.6.1. DNA extraction:

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56 °c for 10 min. After incubation, 200 µl of 100% ethanol was added to the DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with few modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56 °c for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

2.6.2. Oligonucleotide Primers:

Primers used were supplied from Metabion (Germany) are listed in Table (1).

2.6.3. PCR amplification:

Primers were utilized in a 25- µl master mix reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

2.6.4. Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gel pilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

3. RESULTS

A total of 88 isolated bacterial agents were obtained and identified as E. coli (26 isolates), Pseudomonas aeruginosa (22 isolates), only coagulase positive Staphylococci (16 isolates), Proteus spp. (13 isolates) and Salmonella spp.(11isolates) with incidences of isolation 21.7%, 18.3 %, 13.3%, 10.8% and 9.2% respectively, and an overall incidence of (73.3%). Out of the 26 *E. coli* isolates18 were isolated from unhatched dead in shell embryonated eggs while 8 were isolated from hatcheries. While all the isolates of Ps. aeruginosa were obtained from unhatched

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension
				Secondary denaturation	Annealing	Extension	
qacED1	5' TAA GCC CTA CAC AAA TTG GGA GAT AT '3 3' GCC TCC GCA GCG ACT TCC ACG '5	362	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.
Reference	Chuanchuen et al. (2007)						

eggs (egg room, egg dish and other equipments), 12 isolates of the 16 coagulase positive Staphylococcus spp. were obtained from unhatched eggs while 4 isolates were obtained from hatcheries. Concerning Proteus species 13 isolates were obtained from both unhatched eggs, (8 isolates) and hatcheries (5 isolates), finally, Salmonella spp.11 isolates were isolated from unhatched eggs and couldn't be isolated from hatcheries as showed in Table (2).

Serotyping of *E. coli* isolates by slide agglutination technique revealed the distribution of isolates in 9 different serotypes, the most predominant serotype was O91:H21 (7isolates), followed by O78 (4isolates),O2:H6 (2isolates), O163:H2 (2isolates), O128:H2(3isolates), O158 (2isolates), O26:H11(2isolates), O121:H7 (2isolates) and O44:H18(2isolates) were obtained as shown in Table (3). Salmonella isolates were serotyped using poly and monovalent "O" and "H" antisera. By serotyping ,the most predominant serotypes were S. kentukey and S. Enteritidis, 3 isolates for each in percentage of (27.3 %) followed by 2 isolates S. Molade(18.2 %), and 1 isolate of each of S. Tsevie, S. Infantis and S. Larochelle in percentage of (9.1 %) as showed in Table (4).

The results of antibiotic susceptibility testing of the isolated bacterial agents against 15 different antibiotics (table 5) revealed that *E. coli* were completely resistant to Amoxicillin, Methicillin, Sulfamethazole/Trimethoprim and highly resistant to Cefoxitin (96.1% resistance), while the isolates were highly sensitive for Colistin, Ciprofloxacin and Gentamycin with 92.3% 88.5% and 84.6% respectively. *Ps. aeruginosa* isolates were 100% resistant to Tetracycline, Methicillin, Ampicillin, Amoxicillin, Neomycin, Erythromycin and Sulfamethazole/Trimethoprim while they were sensitive for Gentamycin (77.3 %) and Ciprofloxacin (72.7%). Coagulase Positive *Staphylococci* was 100% resistant to Methicillin and Cefoxitin while resistant to Amoxicillin and Sulfamethazole/Trimethoprim at 93.7 %, while it was highly sensitive for Gentamycin and Ciprofloxacin in percentage of 93.7% and 87.5%. In case of *Proteus* spp*.,* they were found to be 100% resistant to Methicillin, Cefoxitin and Tetracycline, while they were sensitive for Ciprofloxacin and Streptomycin at percentage of 92.3% and 84.6%. Finely *Salmonella* spp. were resistant to Methicillin and Cefoxitin with 100% and Amoxicillin and Cefadroxil with 90.9% respectively while it was sensitive for Ciprofloxacin and Gentamycin with 90.9%.and 81.8% respectively. The study recorded multidrug resistant index (MDR) among the isolated bacteria at least for 3 chemotherapeutic agents as 0.63 in E. coli, 0.78 in Ps. aeruginosa, 0.61 in Staphylococci, 0.58 in Proteus spp. and 0.59 in Salmonella,(more than 0.2). These results are shown in table (5).

In this study, the *qac*ED1 gene was detected by PCR in all representative isolates of E. coli, Salmonella spp., Coagulase Positive Staphylococci, Pseudomonas aeruginosa and Proteus spp. in percentage of (100%) (5 isolates of each bacterial strain), this explained in Figure (1 and 2).

Table (2): prevalence of bacterial isolates from dead-in- shell embryos and hatcheries:

Bacterial isolates	No. of isolates from unhatched eggs (100)	%	No. of isolates from hatcheries (20)	%	prevalence rate (120)
E. coli (26)	18	18.0 %	8	40.0 %	21.7 %
Pseudomonas aeruginosa (22)	22	22.0 %	0	0 %	18.3 %
CoagulasePositive Staphylococci (16)	12	12.0 %	4	20.0 %	13.3 %
Proetus spp.(13)	8	8.0 %	5	25.0 %	10.8 %
Salmonella spp. (11)	11	11.0 %	0	0 %	9.2 %
Total (88)	71	71.0 %	17	85 %	73.3 %

Table (3): Serological identification of *E. coli* strains

Serotype	No. of isolates	Isolation rate
O91 : H21	7 isolate	26.9 %
O78	4 isolates	15.4 %
O2 : H6	2 isolates	7.7 %
O163 : H2	2 isolates	7.7 %
O128 : H2	3 isolates	11.5 %
O158	2 isolates	7.7 %
O26 : H11	2 isolates	7.7 %
O121 : H7	2 isolates	7.7 %
O44 : H18	2 isolates	7.7 %
Total	26	100 %

Table (4): Serological identification of *Salmonella* strains

Serotype	Group	No. of isolates	Isolation rate
Salmonella Kentucky	C 3	3 isolates	27.3 %
Salmonella Molade	C 2	2 isolate	18.2 %
Salmonella Enteritidis	D 1	3 isolates	27.3 %
Salmonella Tsevie	B	1 isolate	9.1 %
Salmonella Larochelle	C 1	1 isolate	9.1 %
Salmonella Infantis	C 1	1 isolate	9.1 %
Total		11	100 %

Table (5): In vitro antibiotic resistance pattern

Antibiotic discs	*E. coli (26)*	*Pseudomonas aeruginosa (22)*	Coagulase Positive *Staphylococci (16)*	*Proteus spp.(13)*	*Salmonella spp. (11)*
Amoxicillin(25µg)	R (100%)	R (100%)	R (93.7%)	R (92.3%)	R (90.9%)
Methicillin (5 µg)	R (100%)	R (100%)	R (100%)	R (100%)	R (100%)
Ampicillin (10 µg)	R (76.9%)	R (100%)	R (43.7%)	R (84.6%)	R (81.8%)
Cefoxitin (30 µg)	R (96.1%)	R (95.4%)	R (100%)	R (100%)	R (100%)
Cefadroxil (30 µg)	R (92.3%)	R (90.9%)	R (87.5%)	R (92.3%)	R (90.9%)
Enrofloxacin (10 µg)	R (19.2%)	R (72.7%)	R (43.7%)	R (23.1%)	R (27.3%)
Ciprofloxacin(5 µg)	R (11.5%)	R (27.3%)	R (12.5%)	R (7.7%)	R (9.1%)
Colistin (25 µg)	R (7.7%)	R (50%)	R (18.7 %)	R (23.1%)	R (18.2%)
Gentamycin (10 µg)	R (15.4%)	R (22.7%)	R (6.25%)	R (23.1%)	R (18.2%)
Streptomycin(10 µg)	R (23.1%)	R (31.8%)	R (18.75%)	R (15.4 %)	R (27.3 %)
Neomycin (30 µg)	R (84.6 %)	R (100%)	R (87.5%)	R (76.9%)	R (72.7%)
Tetracycline(30µg)	R (69.2%)	R (100%)	R (68.7%)	R (100%)	R (63.6%)
Florphenicol (l5 µg)	R (73.1%)	R (81.8%)	R (62.5%)	R (15.4 %)	R (72.7%)
Erythromycin(l5 µg)	R (80.8%)	R (100%)	R (81.2 %)	R (46.1%)	R (63.6%)
Sulfamethoxazole /Trimethoprim (25µg)	R (100%)	R (100%)	R (93.7%)	R (76.9%)	R (54.5%)
MDR index	0.63	0.78	0.61	0.58	0.59

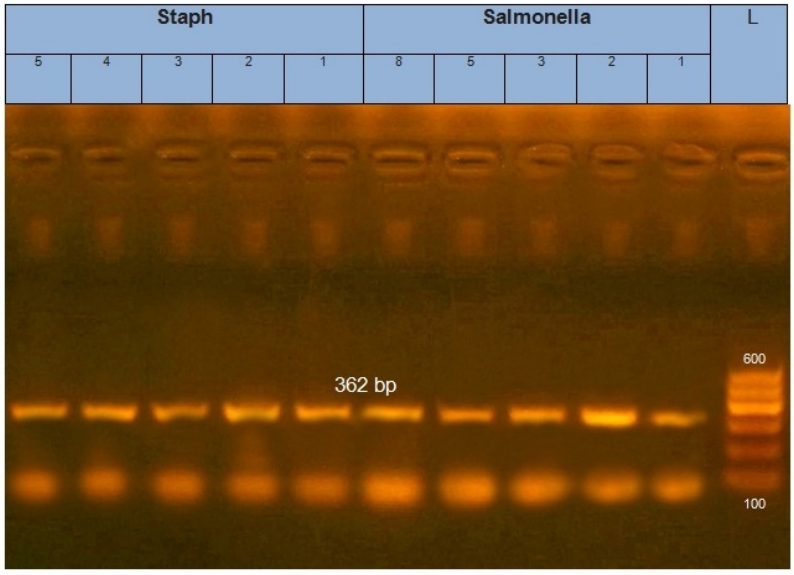

Fig. (1): PCR identification of qacED1gene in *Salmonella* and Coagulase Positive *Staphylococci*
*Qac*ED 1 gene of Salmonella Amplification of 362 bp was observed in the extracted DNA of isolates cod number 1, 2, 3, 5 and 8. *Qac*ED 1 gene of Coagulase Positive staphylococci Amplification of 362 bp was observed in the extracted DNA of isolates cod number 1, 2, 3, 4 and 5.

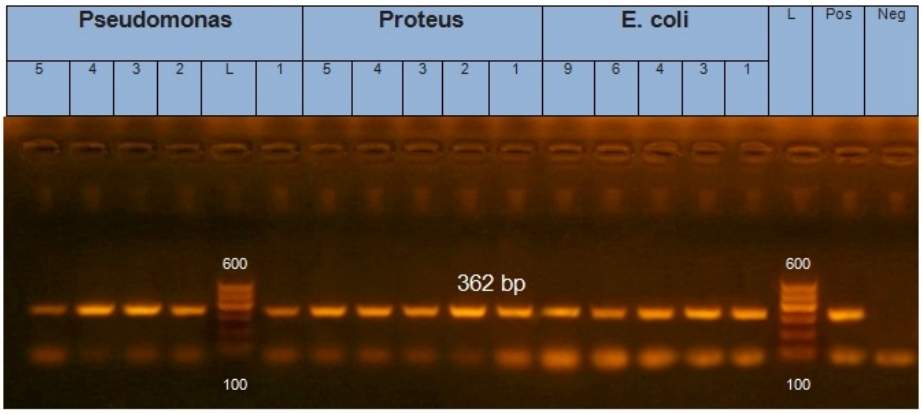

Fig. (2): PCR identification of *qac*ED1gene in *E. coli, Proteus* and *Pseudomonas*
*Qac*ED1gene of *Pseudomonas* Amplification of 362 bp was observed in the extracted DNA of isolates cod number 1, 2, 3, 4 and 5. *Qac*ED1gene of *Escherichia coli* Amplification of 362 bp was observed in the extracted DNA of isolates cod number 1, 3, 4, 6 and 9. *Qac*ED 1 gene of *Proteus* Amplification of 362 bp was observed in the extracted DNA of isolates cod number 1, 2, 3, 4 and 5

4. DISCUSSION

Bacterial agents play an important role in the hatcheries by decreasing the rate of hatchability and affecting the health of newly hatched chicks and their future performance. This is due to ignorance of the hygienic and therapeutic measures. Effective disinfection programs are vital in the poultry farms especially in hatcheries. These programs control several pathogens which causes economic losses in poultry industry. The regular use of these substances in poultry production may select for bacteria that are less susceptible to biocides and antibiotics due to bacterial adaptation. Little is known about resistance to disinfectant and antibiotics and their co-resistance in bacteria. In the present study bacteriological examination of 120 samples from dead-in- shell embryos and commercial broiler chicken hatcheries revealed that the recovered isolates were: E. coli 26 isolates (21.7%),Pseudomonas aeruginosa 22 isolates (18.3 %),Coagulase Positive Staphylococci 16 (13.3%),

Proetus spp. 13(10.8%), and Salmonella spp. were 11 (9.2%) as shown in Table (2). These results were agreed with those of Al-Khalaf *et al.,* (2010) and Kirunda *et al.,* (2010)who could isolated the same bacterial strains from egg shell, infertile eggs, dead in shell embryos and newly hatched chicks .They isolated Escherichia *coli ,* Salmonella species, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Citobacter diversus and Enterobacter cloacae. Moreover, (Walker *et al.* (2001) mentioned that numerous bacterial pathogens which contaminate hatcheries had been isolated from egg shell, egg content as well as from dead in shell chicken embryos, these isolates included *Salmonella spp., Escherichia coli,* Staphylococcus aureus, Proteus spp. *and* Pseudomonas spp. In addition, it has been described by Azmy (2010) who recorded high incidence of *E. coli* 44 (25.88%), Salmonella species 44 (25.55%), Klebsiella 42 (24.7%), Pseudomonas aeruginosa 34(20.0%), Proteus Vulgaris 18 (10.59%), *Staph. aureus*16 (9.41%), and Streptococcus fecalis 8 (4.7%), in dead-in-shell chicken breeds. In the same manner, Mamman et al. (2008) could isolate and identified 62 *E.* coli, 21 Proteus spp., 6 Pseudomonas spp., 11 Staphylococcus aureus, 8 Staphylococcus spp., 5 *micrococcus* spp., 2 Corynebacterium spp. and 1 *Bacillus* spp. from 600 dead-in-shell chick embryos and 4 commercial hatcheries. The difference in the isolation rate may be attributed to the heavy contamination of the eggs after lying and the improper handling and storage of the hatching eggs also may be due to climatic and geographic differences.

Serotyping of *E. coli* isolates by slide agglutination technique revealed the distribution of *E. coli* isolates in 9 different serotypes. The most predominant serotype was O91 (7 isolates), followed by O78 (4isolates), 3 isolates of O128, and 2 isolates of O2, O163 O158, O26, O121 and O44. The present results agreed with results of Shalaby and Abd El-Hamid (1987) who found that the most prevalent serotypes were O78, O128 and O114, and Chart *et al*., (2000) who reported that *E. coli* isolates are pathogenic for poultry commonly belong to certain serotypes, particularly the serotypes, O78, O1, and O2, and to some extent O15 and O55. Also our results partially agreed with Al-Khalaf *et al.,* (2010) whose serological typing of *E. coli* isolates from hatchery revealed 6 serotypes of *E. coli* they were O126 ,O111, O26, O119 ,O125, O55,also with Samah *et al*.,(2015)who detected,13 serotypes were,O27 ,O152, O125, O6, O159 ,O169 ,O91, O166, O145, O25, O153, O115, and O29.

The obtained data revealed that the recovered 11 salmonella isolates were seriologicaly identified to the most predominant serotypes were *S.* Kentukey and *S.* Enteritidis, 3 isolates from each in percentage of (27.3 %) followed by 2 isolates of *S.* Molade (18.2 %), and1 isolate of *S.* Tsevie, *S.* Infantis and *S.* Larochelle in percentage of (9.1%). These results partially agreed with Byrd *et al.,* (1999) who isolated a total of 11 different Salmonella serotypes from hatcheries, with S. Heidelberg (9/30) and S. kentucky (6/30) accounting for 50% of the total isolations. Also Azmy (2010) found the most prevalent types were 16 isolates *S.* Enteritidis, 8 isolates *S.* Gallinarum, 3 isolates *S.* Pullorum; 4 isolates *S. Dublin.* The results agreed with Nabin *et al*. (2010) who found that *S.* Enteritidis and *S.* Kentuky to be the most frequent serotypes among samples from chicken farms and observed that they were isolated from a wide variety of samples, including egg yolk, egg shell, boots, water, and feed. These differences in serotypes of isolated *E.* coli and salmonella might be due to the locality and to the environmental condition of isolation.

Antimicrobial resistance has become a global problem, and the huge consumption of antibiotics by both humans and animals resulted in development and spread of a large number of antibiotic resistances among bacterial populations thus creating critical public health problems. In the current study *E. coli* isolates were multidrug resistant (resistant to at least three or more classes of antimicrobial agents). *E. coli* was found to be resistant to Amoxicillin, Methicillin, Sulfamethazole/Trimethoprim (100%), cefoxitin (96.1%) and cefadroxil (92.3%),while it was found to be sensitive to Colistin, Ciprofloxacin and Gentamycin with 92.3% 88.5% and84.6% respectively. Higher susceptibility rate to Ciprofloxacin also was detected by Azmy (2010) and Hasan *e t al*.(2011) who reported a sensitivity rate of 87.1%.On the other hand lower resistance rate was recorded by (Li *et al,* 2007) who recorded 19% resistant rate to Ciprofloxacin. While sensitivity rate of *E. coli* to Gentamycin was (84.6%), these results were agreed with that of Ahmed (2016) and Asherf *et al*. (2016) who said that *E. coli* isolates were highly sensitive to Gentamycin and were highly resistant to Amoxicillin.

P. aeruginosa isolates were 100% resistant to Tetracycline, Methicillin, Ampicillin, Amoxicillin, Neomycin, Erythromycin and Sulfamethazole/Trimethoprim. These results were similar to Célia *et al.* (2005) who observed high resistance rate to the third generation Cephalosporin, as well as Cefepime and

Ceftazidime. While *P.aeruginosa* were highly sensitive to Gentamycin (77.3%) and Ciprofloxacin (72.7%), also these results were nearly similar to Asherf *et al.* (2016)who cited that *P. aeruginosa* isolates were highly sensitive to Gentamycin(80%) and Ciprofloxacin and highly resistant to Tetracycline (100%).

In the present study isolates of *Coagulase Positive staphylococci* were resistant to multiple antimicrobials, as Methicillin (100%), Amoxicillin (93.7%), cefoxitin (100%) and Cefadroxil (87.5%). while they were highly sensitive to Gentamycin (93.75%), Ciprofloxacin (87.5%) and Streptomycin (81.25%). These results were agreed with Samah *et al*., (2015) who recorded that isolated Coagulase Positive Staphylococci were 73.3% sensitive to Ciprofloxacin 70% to Gentamycin, but resistance against Trimethoprime-Tulphamethazone. Also results go hand to hand with Ahmed (2016) 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 with Asherf *et al*. (2016) who stated that isolates of Staph aureus were highly sensitive to Gentamycin (100%) and Ciprofloxacin (84%) while they were resistance to Tetracycline (72%) and Amoxicillin (80%).

In case of *Proteus* isolates they were multidrug resistant, as the isolates found to be 100% resistant to Methicillin, Cefoxitin and Tetracycline, Cefoxitin, Amoxicillin (92.3%), Cefadroxil (92.3%). These results were nearly similar to those of Aly and Mohammed (2015) who reported that approximately 50-100% of *Proteus* isolates showed resistance to Lincomycin, Oxacillin, Oxytetracycline, Chloramphenicol, Ampicillin and Ciprofloxacin.

In our study *Salmonella* spp. were resistant to Methicillin and Cefoxitin with 100% and Amoxicillin and Cefadroxil with 90.9%while it was sensitive for Ciprofloxacin and Gentamycin with 90.9%.and 81.8%.These results were agreed with that of Ahmed *et al.* (2011) *and* Asherf *et al*. (2016).

The *qac* genes are widely spread among clinical and environmental bacteria; it is obvious that their distribution is generally linked with a particular bacterial species, Jaglic and Cervinkova (2012). The association between the presence of*qacED1* and antimicrobial resistance was significant. Interestingly, all *qacED1* positive *E. coli* ,*Salmonella* spp, *Ps. aeruginosa* ,*Staphylococci* and *Proteus* isolates in our study were multidrug resistant (resistant to at least three or more classes of antimicrobial agents). So, in the present study the *qacED1* gene was detected in 100% of the tested isolates of E. coli, Salmonella spp., Ps. aeruginosa, Staphylococci *and* Proteus*, as* shown in Figure (1, 2). These results was agreed with Kucken *et al*. (2000) who demonstrated that QAC resistance genes were commonly present among *E. coli* isolates; and partially agreed with Asherf *et al*., (2016) who revealed that*qacED1*gen was reported in *E. coli* (63.6%). Also agreed with that of Likouzou *et al.*(2014) who recorded that several QAC genes were newly described in *E. coli ,* and highly associated with multidrug resistance phenotype, also, the use of QACs in the environment might select for *E. coli* strains with acquired QAC resistance that also carry genes encoding resistance to medically important antimicrobial agents. Sidhu *et al*. (2002) reported that biocide resistance *E. coli* strains exhibiting resistance to multiple antimicrobial. In case of the other isolated microbes, Asherf *et al*. (2016) recorded that *qacED1* gene was detected in Staph aureus by 44.44%, in *Salmonella* was 57.14% and 100% in *P.* aeruginosa isolates. These results were nearly in accordance with Amira (2016) who found that the distribution of *qacED 1* was 93.1%.The results were in agreement with Wang *et al*., (2008) who stated that, *qacE* gene (including its attenuated variant *qacED1*) is widely spread in Gram negative bacteria, mainly in Enterobacteriaceae and Pseudomonas spp. Although (Bjorland *et al*., 2005) recorded that, *qac* resistance genes have already been identified from Staphylococci isolated from various sources. Heir *et al.* (1995) reported that 80% of QAC-resistant staphylococci harbored *qac* genes. In another study, the QAC gene was found in 94.6% of QAC-tolerant *S.* aureus isolates (Liu *et al.,* 2009). Also Russel (2002) indicate that the presence of *QAC* genes in staphylococci results in selection of antibiotic-resistant bacteria, also speculated that disinfectant resistance might contribute to antibiotic resistance by co-resistance or cross-resistance mechanisms or co-selection.

5. CONCLUSION:

This study confirms that un hatched chicken eggs can harbor multi-drug resistance bacterial pathogens not only responsible for economic losses but also having zoonotic importance. Moreover, the study concluded that the presence of the *qac* resistance genes and multi-drug resistance bacteria of the isolated strains definite a link between antibiotic and disinfectant resistance is possible, so effective disinfection programs are vital in the poultry farms especially in hatcheries. These programs control several pathogens, which causes economic losses in poultry industry.

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