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DETECTION OF ANTIBIOTICS RESISTANCE GENES IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM POULTRY FARMS

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ABSTRACT

Poultry industry in Egypt was subjected to various problems one of them was the early chick mortalities that cause great economic losses and its investigation is the request of many poultry scientists and men. In poultry staphylococci, including *Staphylococcus aureus* are known to cause various diseases from acute septicemia to chronic osteomyelitis. Two hundred and sixty samples of chickens, ducks and turkeys were collected from different poultry farms. A survey on *Staphylococcal* infection among chickens, ducks and turkeys was carried out. It was found that out of 260 infected and dead chickens, ducks and turkeys suspected to be infected with Staphylococcosis, 54 cases (20.76%) revealed *Staphylococcus* micro-organisms. The isolated strains were typed as: *Staphylococcus aureus* (74.07%), *Staphylococcus* epidermidis (14.8 %) and *Staphylococcus saprophyticus* (11%). The results of sensitivity biogram revealed that *S. aureus* were highly sensitive to Amoxicillin, Ampicillin, Ciprofloxacine, Enrofloxacine and lincomycin and resistant to Erythromycin, Streptomycine and Chloramphenicol. The results of MIC of 6 representative coagulase positive *Staphylococci* isolates against 6 selected antibiotics commonly used in poultry farms showed that 100% of isolates were sensitive to amoxicillin, enrofloxacin, ciprofloxacin and oxytetracyclin, while 100% of 6 isolates were resistant to streptomycin and lincomycin. The incidence of isolation of *Staphylococci* from the internal organs of examined birds and from unabsorbed yolk sac, joints, liver, intestinal content and heart blood was 36.3%, 20%, 19.2%, 17.3% and 16.9% respectively. Ten of each detected isolates were examined by cPCR for resistance genes blaZ and aac(6') aph (2''). The isolates harbored these resistance genes with percentage of 100% for *S. aureus.* In experiment, the pathogenicity of the isolated strains of *S. aureus* for 7 day- old chicks was studied. *S. aureus* injected subcutaneously, oral and intranasal caused death of 100%, 100% and 26.7% of the used chicks respectively.

Key words: *Staphylococcus aureus*, MIC, cPCR, blaZ and aac (6') aph (2") genes resistance.

INTRODUCTION

Constant increase in daily growth, especially in broiler chicks, makes the length of the fattening period shorter, and today's birds eat less and produce more. To achieve this, the first few days of a chick's life are important because this is the basis that laid for optimum growth and health. Staphylococcus aureus (S. aureus) is receiving wide spread attention, due to multi-resistant strains, diminishing the usefulness of antibiotics in human and animal medicine and, thereby limiting therapeutic options. *Staphylococcal* infections were a worldwide problem in chicken and turkey, and cause economic losses due to decrease of egg production and weight gain (Adayel, 2005). Out of 120 isolates, 77 (64.1%) *Staphylococcal* strains were recovered from diseased and dead chickens (Aml and Samah, 2014). Pesavento *et al.* (2007) observed that 23.8% occurrence of coagulase positive *S. aureus* from poultry sample analysed during a one-year survey in Italy. Suleiman *et al.* (2013) recorded that *S. aureus* is associated with many clinical syndromes including tenosynovitis, omphalitis, femoral head necrosis, infected hock and stifle joints secondary to coccidiosis and "bumblefoot". Abdellatif *et al.* (2018) mentioned that (7.5%) occurrence of *S. aureus* was collected from 30 flocks. Popy *et. al.* (2011) reported that *Staphylococcus sp.* (41.4%) was isolated from trachea and nasal sinuses of chickens and clinical signs of these affected birds were depression, conjunctivitis, frothy oculo-nasal discharge, conjunctivitis, facial oedema, and respiratory rales. Onaolapo *et al.* (2017) showed that *S. aureus* high resistance was observed against tetracycline, ciprofloxacin, oxacillin and cotrimoxazole while the isolates showed significant susceptibility to cefoxitin, amoxiclav and gentamicin. White *et al.* (2003) found that *S. aureus* isolates were commonly resistant to tetracycline (40% MIC_{90} > 32 µg/ml), lincomycin (19% MIC_{90} > 32 µg/ml),

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erythromycin (12% MIC_{90} > 8 µg/ml), and kanamycin (8%MIC_{90} < 128 µg/ml) and all S. aureus isolates were susceptible to chloramphenicol, gentamicin, streptomycin, nitrofurantion, linezolid, quinupristin/dalfopristin, vancomycin. Aml *et al.* (2018) found that the results of MIC showed that 100% of isolates were resistant to streptomycin, spectinomycin, ampicillin, cefotaxim, trimethoprim-sulphamethazole, gentamycin, and methicillin with MIC >128 µg/mL, while 100% of isolates were sensitive to tylosin, lincomycine, enrofloxacin, ciprofloxacin and oxytetracyclin. Mohammed *et al.* (2015) mentioned that *S. aureus* strains show resistance to Cephalosporins; Tetracyclines; Macrolides (Erythromycin); Cloramphenicol; Quinolones (Nalidixic acid) and Aminoglycosides (Neomycin, Gentamycin). Abdellatif *et al.* (2018) found that (100%) bla Z was detected in *S. aureus* while aac(6') aph (2") was detected in *S. aureus* isolates (80%). Nabila (1982) made three routes of infection for 3-day old chicks. *Staphylococcus aureus* injected subcutaneously, oral or by scarification caused death of 100%, 100% and 23.33% of the used chicks respectively.

This work aims to study the incidence of *S. aureus* in poultry farms, detection of antibiotics resistance genes in the isolates, evaluate the different methods of infection by *S. aureus* divided to 3 groups each one inject by one method (subcutaneously– oral– intranasal) and studying the antimicrobial susceptibility and resistance of bacterial isolates by in vitro disc susceptibility and by using minimum inhibitory concentration (MIC).

MATERIALS AND METHODS

1. Samples collection:
A total of 260 samples from diseased and freshly dead chickens, ducks and turkey of different ages and breeds were collected from private farms in Assuit, Sohag and Almnya Governorates, Egypt. Samples were for isolation heart blood, lungs, intestinal contents, liver, spleen, yolk sac and swollen joints of dead and infected birds showing lesions suggestive of *Staphylococcal* infection. All samples were taken under aseptic conditions.

2. Isolation and identification:
The collected samples and swabs were inoculated into nutrient broth and incubated at 37 °C for 24 h. A loopful of the inoculated broth was subcultured on nutrient agar, blood agar and Baird Parker agar medium and incubated at 37°C for 24-48 hours. Tentatively identified according to morphological features, pigment production, type of haemolysis produced, gram staining, catalase test, coagulase test (in tubes), sugar fermentation tests including mannitol, maltose, xylose and sucrose and characteristic growth on Mannitol salt agar which used as selective as well as differential medium for isolation and identification of Staphylococci according to the methods Quinin *et al.* (2004).

3. Antibiotics susceptibility testing:
The isolated *Staphylococci* were tested for their antimicrobial susceptibility using the disk diffusion technique on Mueller-Hinton agar (Difco, Sparks, MD). The results were recorded after 24 h of incubation at 37º C. The test was performed according to the method described in the guidelines of the Clinical and Laboratory Standards Institute (CLSI-2013). The zone of inhibition of each antibiotic disc was recorded. Amoxicillin; Ax (25 µg/disk), Ampicillin; Am (10 µg/disk), Streptomycine; S (10 µg/disk), Cephalothin; KF (30 µg/disk), Oxytetracycline; T (30 µg/disk), Erythromycin; E (15 µg/disk), Ciprofloxacine; CIP (5 µg/disk), Chloramphenicol; C (30 µg/disk), Enrofloxacine; ENR (5 µg/disk) and Lincomycin; MY (10 µg/disk) were also tested.

4. Determination of minimum inhibitory concentration (MIC):
MICs of antibiotics were evaluated using the broth microdilution method in Mueller-Hinton broth (MHB) with an initial inoculum of $5x10^5$ cells in non-treated polystyrene microtiter plates CC7672-7596; (CytoOne) in accordance with the Clinical and Laboratory Standards Institute (CLSI. 2007). Bacteria were prepared in phosphate-buffered saline (PBS) until a McFarland standard of 0.5 was achieved. The solution was subsequently diluted 1:300 in MuellerHinton broth (MHB) to reach a starting inoculum of $5x10^5$ colony-forming units (CFU/mL). Bacteria were then transferred to a 96-well microtiter plate. Antibiotics were added (in triplicate) to wells in the first row of the microtiter plate and then serially diluted along the vertical axis. The plate was incubated at 37°C for 22–24 hours before the MIC was determined. MIC was defined as the lowest concentration which inhibited the visible growth of bacteria. To determine the (MIC) for the isolated microorganisms used six of different antimicrobial drugs are (Streptomycin - Oxytetracycline- Ciprofloxacine- lincomycin- amoxicillin- enrofloxacine).

5. Detection of Antimicrobial Resistance genes for bacterial isolates:

5.1. Extraction of DNA: A rapid boiling procedure was used to prepare template DNA from bacterial isolates according to Duran *et al*. (2012). Two to 5 loops of bacteria taken from the Baird Parker agar agar plate were collected and suspended in 200 µl of lysis buffer comprised of 1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA. After boiling for 10 min, the suspension was centrifuged for 2 min to sediment bacterial debris. The supernatant was aspirated, and from which 5 µl was used directly for PCR amplification.

5.2. Primers: Two pairs of primers were supplied from metabion (Germany) and Biobasic (Canada). They have specific sequence and amplify specific products

Table 1: Oligonucleotide primers sequences.

Microorganism	Gene	Primer sequence (5'-3')	Length of amplified product	Reference
Staphylococcus	*blaZ*	ACTTCAACACCTGCTGCTTTC	173 bp	**Duran *et al.*, 2012**
		TGACCACTTTTATCAGCAACC		
	aac(6')aph (2'')	GAAGTACGCAGAAGAGA	491 bp	
		ACATGGCAAGCTCTAGGA		

6. Experimental infection:
A total number of 60 one day old baladi chicks were obtained from private farm**.** Chicks were proved to be free from bacterial pathogens after bacteriological examination. Divided into four groups (15 chicks each) (A, B, C and D). Group (A, B and C) each one inject with *S. aureus* by one method (subcutaneously–oral– intranasal) about 10^5, $5x10^6$ and $5x10^6$ CFU/ml respectively and group D kept as control. The infected chickens were observed twice daily to record their general health condition and to notice any clinical signs. Post mortem examination was performed on chickens which died after infection to record any lesions of the internal organs and bacteriological reisolation.

RESULTS

Based on the identification methods used in our study, a total of 54 (20.76%) Staphylococcal isolates were recovered from 260 chickens, ducks and turkeys samples. Out of these isolates, as a result of morphological and biochemical tests, 50 isolates were from chickens (24.75%), 2 strains from ducks (6.6%) and 2 strains turkeys (10.71%) as noted in table (2). Bacteriological identification revealed that colonies of Staphylococci on Baird Parker agar medium were black, shiny and convex surrounded by clear zone. Also there was a characteristic golden yellow growth on Mannitol salt agar. The *Staphylococcal aureus* isolates showed beta-heamolysis on blood agar medium, positive coagulase and fermented mannitol, maltose and sucrose but not fermented xylose as shown in table (4).

Microscopic characters were gram positive cocci arranged on clusters. The incidence of isolation of *Staphylococci* from the internal organs of examined birds and incidence of *S. aureus* in relation to age are summarized in figure (1 and 2). Results of antibiotic Susceptibility profile of 20 staphylococcal strains against 10 antimicrobial agents are noted in table (5).

Table 2: Prevalence rate of isolations recovered from examined birds.

	Chicken			Duck			Turkey			Total		
	No. of samples	**+ ve**	**%**	**No. of samples**	**+ ve**	**%**	**No. of samples**	**+ ve**	**%**	**No. of samples**	**+ ve**	**%**
Staphylococcal species	**202**	**50**	**24.75%**	**30**	**2**	**6.6%**	**28**	**2**	**10.71%**	**260**	**54**	**20.76%**

Table 3: shows distribution of *Staphylococcal* species isolated from poultry.

	Staph. aureus	**Staph. epidermidis**	**Staph. saprophyticus**	Total
No. of cases	**40**	**8**	**6**	**54**
Total %	15.4%	3.07%	2.3%	20.76%

Table 4: Biochemical and fermentation reactions of suspected *Staphylococci* isolated from poultry.

		Staph. aureus	**Staph. epidermidis**	**Staph. saprophyticus**
haemolysis on blood agar		+ ve	-ve	-ve
Coagulase production test		+ ve	-ve	-ve
Acid production from	**mannitol**	+ ve	-ve	+ ve or - ve
	maltose	+ ve	+ ve	+ ve
	sucrose	+ ve	+ ve	+ ve
	xylose	-ve	-ve	-ve

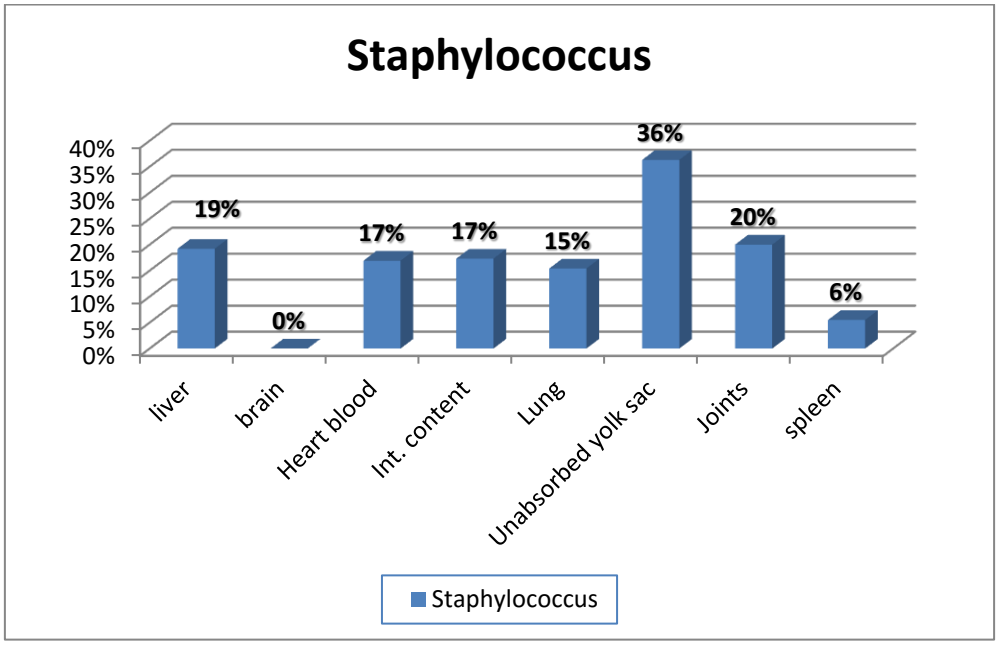

Figure (1): The incidence of *Staphylococcus* in different organs of examined Chicken, Duck and Turkey

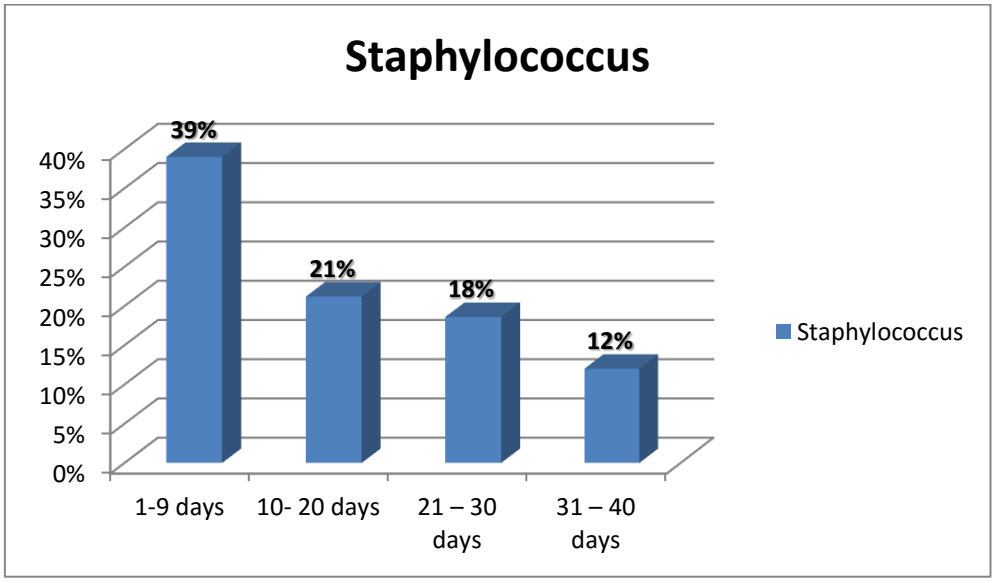

Figure (2): Total incidence of *Staphylococcus* infection in relation to age.

Table 5: Susceptibility testing of *Staphylococcus.*

Antimicrobial agent	Disk potency(µg)	No. of isolates	Sensitive isolates		resistant isolates	
			Number	%	Number	%
Amoxicillin	(Ax_{25})	20	20	100	0	0
Ampicillin	(Am_{10})	20	20	100	0	0
Streptomycine	(S_{10})	20	5	25	15	75
Cephalothin	(KF_{30})	20	0	0	20	100
Oxytetracycline	(T_{30})	20	12	60	8	40
Erythromycin	(E_{15})	20	0	0	20	100
Ciprofloxacine	(CIP_5)	20	18	90	2	10
Chloramphenicol	(C_{30})	20	5	25	15	75
Enrofloxacine	(ENR_5)	20	20	100	0	0
Lincomycin	(MY_{10})	20	19	95	1	5

The results of MIC of 6 representative coagulase positive staphylococci isolates against 6 selected antibiotics commonly used in poultry farms were illustrated in table (6), results showed that 100% of isolates were sensitive to amoxicillin, enrofloxacin, ciprofloxacin and oxytetracyclin, while 100% of 6 isolates were resistant to streptomycin and lincomycin.

Genotypic identification of 10 representative coagulase positive Staphylococcus isolates was performed by cPCR assay. Simultaneously, Ten of each detected isolates were examined by cPCR for resistance genes blaZ and aac(6') aph (2'). The isolates harbored these resistance genes with percentage of 100% and 100% respectively for *S. aureus* respectively in Figure (3, 4).

Table 6: Showing results of susceptibility of 6 isolates of *Staphylococcus aureus* to antibacterial agents.

Antibiotic	No. of isolates with MIC (µg/ml) of:											Break point of drug sensitive	No. of sensitive isolates	% of sensitive isolates
	0.125	0.25	0.5	1	2	4	8	16	32	64	128			
Streptomycin	-		-	-	-	-	-	-	4	2	-	0.6-16	0	0%
Amoxicillin	-	-	-	-	4	2	-	-	-	-	-	0.3-128	6	100%
Oxytetracycline	-	-	-	-	-	5	-	1	-	-	-	0.6-128	6	100%
Lincomycin	-	-	-	-	-	-	2	4	-	-	-	1-2	0	0%
Ciprofloxacine	2	4	-	-	-	-	-	-	-	-	-	0.6-1	6	100%
Enrofloxacine	3	3	-	-	-	-	-	-	-	-	-	0.5-1	6	100%

Experimental infection

The incubation period varied from 24 hours up to 14 days according to the type of bacteria and the route of infection. The results obtained are shown in table (7 and 8) and in picture (1, 2 and 3). *S. aureus* injected subcutaneously caused death of all birds (100%) within 3–5 days post-inoculation. Also, administration of the organism orally caused death of all birds (100%) within 2–8 days post-inoculation, while intranasal route caused death of only 26.7%. No symptoms or post-mortem findings were observed in control chickens during the 4 week observation period.

Table 7: Respond of chicks to ***S****. aureus* by various routes of infection.

Group No.	Subgroup No.	Bacterial inoculated	Route of infection	No. of inoculated chicks	Cumulative death losses at (days after infection)											Accumulated value			
					8	9	10	12	14	15	18	20	22	24	28	No. Dead	No. Survived	Mortality Ratio	Mortality (%)
I	A	*Staph. aureus*	S/C	15	-	-	5	4	5	1	-	-	-	-	-	15	0	15/15	100%
	B		orally	15	-	2	3	5	2	1	2	-	-	-	-	15	0	15/15	100%
	C		Intrasal	15	-	-	-	-	2	1	1	-	-	-	-	4	11	4/15	26.7%

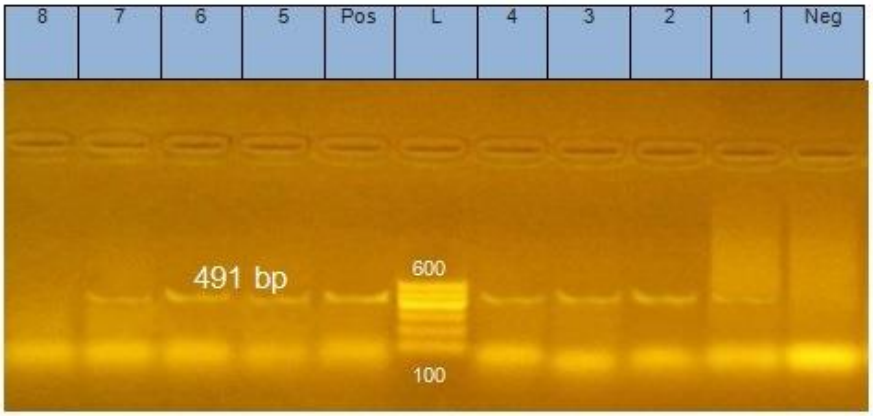

Figure (3): showing result of PCR for resistance gene aac (6') aph (2") for *Staphylococcus.*

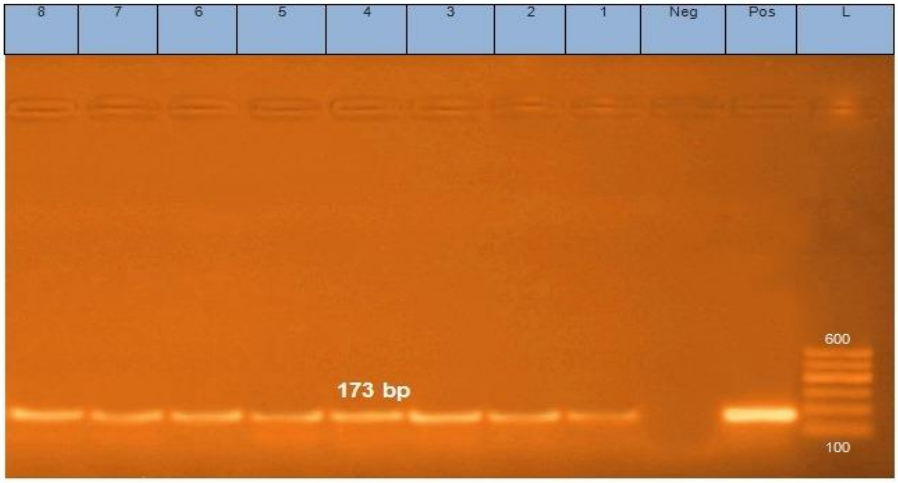

Figure (4): showing result of PCR for resistance gene blaZ for *Staphylococcus*

Table 8: Show clinical signs and gross lesion of ***Staphylococcus aureus.***

Staphylococcus aureus	Route of infection	Signs
Clinical signs	**subcutaneously**	Depression, dropping of wings, dullness, ruffled feathers, inability to stand, lameness and swelling of joints.
	Orally	Orally infected chicks showed similar symptoms. In addition on, diarrhea was observed.
	Intra nasal	Nasal infected chicks developed gasping, depression and dropping of wings.
The postmortem findings		Typical septicemia, congestion and hemorrhages of the subcutaneous blood vessels, muscles of the breast and thigh, congestion of the lungs, liver and kidney and caseous masses in the joints. Meanwhile unabsorbed yolk sac and enteritis were observed in chickens infected orally.

Picture (1): On the left picture show chick inoculated S/C with *Staph. aureus* and showing depression, dullness, inability to walk and dropping wings. **On the right picture** show chick inoculated intranasal with *S. aureus* and showing depression, difficult to stand, gasping and dropping of wings.

Picture (2): Chick inoculated S/C with *Staph. aureus* and showing air saculitis and pericarditis, congestion of subcutaneous tissue, congestion and enlargement of the liver.

Picture (3): On the left picture show chick inoculated orally with *Staph. aureus* and showing congestion of subcutaneous tissue, congestion of internal organs of the liver, heart and intestine. **On the right picture** show chick inoculated intranasal with *Staph. aureus* and showing congestion of subcutaneous tissue, congestion of internal organs of the lung and kidney.

DISCUSSION

In recent years, *Staphylococcus* infections have been considered of importance in the causation of certain problems in intensive poultry farms, extensive investigations have been carried out for nature and incidence of these infections among poultry in Egypt.

The incidence of *Staphylococci* among chickens, turkeys and ducks was determind. The clinical signs observed in chickens, turkeys and ducks from which *staphylococcus* micro-organisms could be isolated were: depression, dropping of the wings, dullness, ruffled feathers, inability to stand these results agree with Lowder (2011). On the other hand, Popy *et al*. (2011) and Onaolapo *et al*. (2017) showed respiratory symptoms in natural out breaks of *staphylococcal* infection in fowls, ducks and turkeys. These symptoms were not observed in naturally infected birds examined in the present work.

Post-mortem examination of birds naturally infected with *Staphylococci* revealed congestion and hypertrophy of liver and spleen, unabsorbed yolk sac and congestion of the breast and thigh muscles. These findings were more or less similar to observations of Popy *et al*. (2011) recorded that *Staphylococcus sp.* (41.4%) was isolated from chickens and gross lesions of upper respiratory tracts were catarrhal tracheitis and rhinitis.

Staphylococcus micro-organisms with recovery rate 20.76%. These results agree with previous studies reported by Pesavento *et al.* (2007). In contrast Aml

and Samah (2014) reported that 64% *Staphylococcal* strains were isolated from diseased and dead broiler chickens samples.

S. aureus was the most dominant species recovered (15.4%), followed by *S. epidermidis* (3.07%) and *S. saprophyticus* (2.3%). These results agree with previous studies reported by Nabila (1982) while Abdellatif *et al.* (2018) found that *S. aureus* was isolated from 15 (7.5%) cases from 200 broiler samples.

The incidence of isolation of *Staphylococci* from the internal organs of examined birds from unabsorbed yolk sac, joints, liver, intestinal content and heart blood was 36.3%, 20%, 19.2%, 17.3% and 16.9% respectively. These results were less similar to previous studies reported by Nabila (1982).

In the present study, the incidence of *Staphylococcal* organism was high (39%) in birds aged 1-9 days, while a lower number of strains were recovered from samples of birds (21.2%) in birds aged 10-20 days and (18.6%) in birds aged 21-30 days.

The sensitivity of isolated *Staphylococcus aureus* strains to various drugs was studied. It was found that most isolates were highly sensitive to Amoxicillin, Ampicillin, Ciprofloxacine, Enrofloxacine and lincomycin and were resistant to Erythromycin, Streptomycine and Chloramphenicol and similar findings were reported by Emilia (2008) and contradictory to that reported by Jamali *et al*. (2015) stated that S. *aureus* resistant to tetracycline, chloramphenicol, and gentamicin but low incidence in case of erythromycin, kanamycin, streptomycin, penicillin G, and oxacillin.

The results of MIC of 6 representative coagulase positive *staphylococci* isolates against 6 selected antibiotics commonly used in poultry farms showed that 100% of isolates were sensitive to amoxicillin, enrofloxacin, ciprofloxacin and oxytetracyclin, while 100% of 6 isolates were resistant to streptomycin and lincomycin. These results were similar to previous studies were observed by Aml *et al*. (2018).

Ten of each detected isolates were examined by cPCR for resistance genes blaZ and aac (6') aph (2''). The isolates harbored these resistance genes with percentage of 100% and 100% respectively for *S. aureus.* These results were similar to previous studies were observed by Abdellatif *et al*. (2018).

In experiment, the pathogenicity of the isolated strains of *Staphylococcus aureus* for 7 day- old chicks was studied. *Staphylococcus aureus* injected subcutaneously, oral and intranasal caused death of 100%, 100% and 26.7% of the used chicks respectively. Similar findings were reported by Nabila (1982).

Chicks infected subcutaneous with *S. aureus* showed signs of depression, dropping of wings, dullness, ruffled feathers, inability to stand and lameness. Diarrhea was detected only in chicks infected orally. Chicks developed gasping was detected in chicks infected intranasal. Nearly similar findings were reported by (Awan, 1998) and (Lowder, 2011).

Postmortem lesions observed among chicks infected with *S. aureus* were mainly typical of septicemia, congestion and hemorrhages of the subcutaneous blood vessels, muscles of the breast and thigh, liver and kidney and caseous masses in the joints. Meanwhile unabsorbed yolk sac and enteritis were observed in chickens infected orally and congestion of the lungs was observed in chickens infected intranasal. These lesions are similar to those observed by Popy *et al*. (2011).

In Conclusion, this study confirms that *Staphylococcal* infection responsible for economic losses and zoonotic importance. The high incidence of antibiotic resistant of *S. aureus* among poultry farms which were detected in our study could mean the antibiotics misuse and abuse especially in farms which will accelerate the development and spreading of antibiotics resistant bacteria not only between chickens but also in the environment or even to the human being. Moreover, the study concluded that the presence of the blaZ and aac (6') aph (2'') resistance genes bacteria of the isolated strains.

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الكشف عن الجينات المقاومة للمضادات الحيوية في المكورات العنقودية الذهبية المعزولة من مزارع الدواجن

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تم إجراء بحث لعدوى الميكروب العنقودي في الطيور وقد امكن فحص ٢٦٠ حالة من الدجاج والبط والرومي وكانت الاعراض والصفات التشريحية لها تدل علي الاصابة بالميكروب العنقودي ومن هذة الحالات اثبت بكتريولوجيا ان نسبة الاصابة الاجمالية بالميكروب يصل إلي ٥٤ حالة (٢٠.٧٦ ٪) وتم تصنيف العترات المعزولة وكانت تصنف فى المجموعات الأتيه: الميكروب العنقودي الذهبي (٧٤.٠٧ ٪) ، الميكروب العنقودي الابيض (١٤.٨ ٪) الميكروب العنقودي السابروفيتيكس (١١ ٪). وتم أجرء اختبار حساسية العترات للمضادات الميكروبية المختلفة واتضح أن الميكروب العنقودي الذهبي كانت حساسة للغاية لمركبات الأموكسيسيلين والأمبسيلين والسيبروفلوكساسين والإنروفلوكساسين والليلنكومايسين ومقاومة للإريثروميسين والستربتومايسين والكلورامفينيكول وايضا عترات الميكروب السبحي شديدة الحساسية للأموكسيسيلين ، الأمبيسلين ، الإينروفلوكساسين والينكوميسين ، تليها الأوكسيتيتراسيكلين والكلورامفينيكول ومقاوم للستربتومايسين والاريثروميسين والسيبروفلوكساسين والسيفالوثين واخيرا الليستريا مونوسيتوجينز شديدة الحساسية للإنروفلوكساسين والستربتومايسين والكلورامفينيكول ، تليها الأموكسيسيلين والأمبيسيلين ومقاومة لباقي المركبات. وتم تحديد مضادات الحساسية للميكروبات وأنماط مقاومة الميكروبات المعزولة باستخدام الحد الأدنى من التركيز المثبط (MIC). والكشف عن جينات المقاومة للمضادات للميكروبات البكتيرية المعزوله بواسطة cPCR للميكروب العنقودي هي (blaZ وaph (6') Aac). وتم عمل العدوى التجريبية لكتاكيت بلدي عمرها ٧ أيام للميكروبات العنقوديه بواسطة العدوي بطرق مختلفة للحقن.