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Molecular Detection of Antibiotic Resistance Genes in Identified Coagulase Negative Staphylococci from Chickens Flocks and Hatcheries in Egypt.

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THE COLLECTED 942 samples (6 organ/bird) from 157 birds taken from 34 broiler chicken farms aged 2- 31 days including 13 and 21 clinical diseased and apparently health flock as well as 221 hatchery samples. Samples were tested for isolation of Coagulase negative staphylococcus (CoNS).

Results of isolation from chicken flocks with clinical signs are 9/13(69.23%) were positive. Positive samples (11/354, 3.11%) including : 3liver, 4 kidney, 2 intestine, 1 air sac and 1 nasal sinus. Out of apparent healthy flocks 8/21 (38.95%) were positive. Positive samples (15/ 588, 2.55%) including 3 liver, 2 kidney, 4 intestine, 3 lung, 2 air sac and 1 nasal sinus. Results showed that 15 positive flocks out of 34 flocks (34.09%) with 26 Staphylococcus isolates from 942 samples (2.77%) including 6 liver, 6 kidney, 6 intestine, 4 lung, 2 air-sacs and 2 nasal sinus with rate of 3.82%, 3.82%, 3.82%, 2.55%, 1.27% and 1.27%; respectively. Also12 isolates out of 26 (46.15%) were CoNS include 8 S. xylosus (75 %), 2 S. scuiri (16.67%) and 2 S. lentus (16.67%).

Hatchery samples reveals the isolation of 26 staphylococcus isolates (11.76%). The tested 108 fertile eggs and dead in shell embryos resulted in 14 and 12 isolates in rate of 12.96% and 13.79%; respectively. Ten isolates were CoNS (38.46%) and represented 4.52% out of total samples including 8 S. xylosus and 2 S. scuiri. Eight S. xylosus was 6 (5.55%) from infertile eggs and 2 (2.29 %) from dead in shell, While the 2 S. scuiri (1.85%) were obtained from infertile eggs.

The tested CoNS isolates showed 100% resistance to Oxytetracycline 30 µg/ml (T30), Trimethoprime + Sulphamethexole 2.25/23.75 µg/ml (SXT) , Calindamycin 2 µg/ml (DA) and Oxacillin 30 µg/ml (OX). All isolates were 100% susceptible to Vancomycin 30 µg/ml (VA) and 90% susceptibility to Enrofloxacin 5 µg/ml (ENR). Multidrug resistance was detected in form of resistance to 9, 4 and 5 out of tested 13 antibiotics in 2 S.lentus , 5 S. scuiri and 15 S. xylosis; respectively.

Ten isolates were tested for the presence of 7 resistance gens including: mecA , tetK, blaZ , kan , ermC, icaD, bab gene. Seven isolates from the tested 10 (70%) having 4 resistance gens. The most detected gens are mecA tetK, blaZ and ermC where it was detected in 90, 80, 60 and 90% respectively. Kan , icaD and bab gens were detected in rate of 30.0 and 0 %; respectively.

In conclusion: CoNS could isolated from healthy and diseased chicken flocks as well as from chicken hatchery. The obtained isolates were multidrug either phenotypic and /or genotypic resistant. Good hygienic measures in both chicken farms and hatchery with monitoring of drug resistance of CoNS those act as source for resistance genes to bacterial pathogens and their importance to the poultry and public health are recommended.

Keywords: Broilers, Hatcheries, CoNS, Antimicrobial susceptibility, PCR. resistance genes.

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Introduction

Bacterial organisms of the genus Staphylococcus are non-motile, non-spore forming, glucose fermenting, and catalase producing [1] .Staphylococcus is one of the most prevalent pathogens in both humans and animals [2]. Coagulase-negative staphylococci (CoNS) , including many species such as *S. hyicus* [3], *S. gallinarum* [4], *S.xylosus* and *S. epidermidis* [5,6], and commonly been isolated from the nares and skin of healthy chickens, and their taxonomic positions were discussed apart from the pathogenicities. CoNS had been isolated from frozen and chilled industrialized, uncooked chicken parts or entire carcasses [7] , raw chicken's meat [8, 9] , meat product [10] , cooked chicken products [11] , breast, neck and wing of chickens [12] , chicken carcasses herd-wise pooled neck skin samples [13] as well as poultry bioaerosol [14] .

Although CoNS in chickens have generally been accepted as harmless inhabitants, it has gradually become clear that they manifest pathogenicity under suitable conditions. From dermatitis and tenosynovitis, CoNS species of *S. hyicus*, *S.sciuri*, *S. simulans* and *S. epidermidis* were isolated. Those CoNS infections in chickens appear to be opportunistic. CoNS infections in chickens are considered to be opportunistic [15,16] .

In the last decade, CoNS have developed resistance to multiple antibiotics [17,18] . Strains of CoNS of both animal and human origins are believed to serve as important reservoirs of antimicrobial resistance genes [19] . Genes encoding antibiotic resistance are usually located on mobile genetic elements, allowing their horizontal transfer to pathogenic staphylococci [20].

The present study is an attempt for isolation, identification and study Antibiotic resistance phenotype and Molecular detection of resistance genes in CoNS from chickens flocks and hatcheries in Egypt

Material and Methods

Samples

Samples were collected from El-Fayoum, Bani Suief, El-Minia and Giza Egyptian governorates during 2016-2017. Total number of 157 birds were collected from 34 broiler chicken farms (13 clinical diseased and 21 apparently health) aged 2- 31 days. Organs including kidney, intestine, liver, nasal sinus, lung and air sac were collected from each bird with total number of 942 organs. Diseased chickens were suffered from signs varied from whitish diarrhea, nasal discharge, sneezing, coughing, gasping to swollen head as well as retarded growth. A total number of 221samples were collected including 26 swabs from hatcheries and incubators, 108 non fertile eggs and 87 dead in shell embryo. The samples were labeled, transported in sterile plastic bags to the laboratory and kept in refrigerator at 2-5 °C till examination.

Isolation and identification

Cultivation of samples for isolation of Staphylococci species was performed according to El Seedy et al. [21]. Colonial morphology on different media including; tryptone soya broth and agar, mannitol salt agar, blood agar, Congo red and Baird-Parker media was observed after incubation at 37°C for 24-48 hours [22] . Morphological identification of Staphylococci species was done using Gram's stain [23] . Biochemical identification of Staphylococcus sp. using INTEGRAL SYSTEM STAFILOCOCCHI kit [24] *(*. Further confirmation was achieved by the API (BioMerieux SA) kit.

Antimicrobial susceptibility test

In vitro antibiotic sensitivity test for Staphylococci strains was performed using Mueller Hinton agar (Oxoid) plates and antibiotic discs of 13 chemotherapeutic agents by disc diffusion technique [25]. The strains were cultivated on Mueller Hinton agar, and then the antibiotic discs were located by means of a dispenser. After incubation at 37°C for 24 hrs, the strains were evaluated as sensitive, intermediate and resistant by measuring inhibition zones diameters around the antibiotic discs [25,26] .

Chemotherapeutic agents

The used antimicrobial agents and their corresponding concentrations were as follows: Cefatoxaime 30 µg/ml (CTX), Enrofloxacin 5 µg/ml (ENR), Amoxicillin+Clavulanic acid 30 µg/ml (AMC), Vancomycin 30 µg/ml (VA), Oxacillin 30 µg/ml (OX), Kanamycin 30 µg/ml (K), Calindamycin 2 µg/ml (DA), Ceperazone 2 µg/ml (CFP), Trimethoprime+Sulphamethexole 2.25/23.75 µg/ml (SXT), Chloramphenicol 30 µg/ml (C30), Cefapime 30 µg/ml (FEP), Oxytetracycline 30 µg/ml (T30) and Gentamycin 10 µg/ml (CN) [26].

Detection of antibiotic resistance genes PCR

Molecular detection of antibiotic resistance genes of the isolates was done. Extraction of DNA from samples was performed using the QIAamp DNA Mini Kit (Catalogue number 51304). The QIAamp DNA Mini Kit provided silica-membrane-based nucleic acid purification from different types of samples. The spin-column procedure did not require mechanical homogenization, so total hands-on preparation time was only 20 minutes. Ethanol 96% (Applichem) was added to the lysate and vortexed. The sample was washed and centrifuged according to the manufacturer's instructions. DNA was eluted with 100 µl of elution buffer supplied in the kit. Used primers for identification of resistance genes in CoNS are presented PCR in Table 1. Temperature and time of amplification conditions of the primers during PCR are shown in Table 2. Analysis of the PCR products in Agarose gel electrophoresis was done as *Sambrook et al.* [27].

Results and Discussion

Staphylococcus is one of the most prevalent pathogens in both humans and animals [2]. CoNS in chickens gradually become clear that they manifest pathogenicity under suitable conditions. The bacteriologically tested organ samples revealed the presence of staphylococci those showed typical morphological characters on used sold media and Biochemical reaction [1, 28] as well as Gram- positive stain [29].

Total positive samples were 26 from 942 samples (2.77%) including 6 Liver, 6 Kidney, 6 Intestine, 4 Lung, 2 Air-sacs and 2 Nasal sinus with percentage of 3.82%, 3.82%, 3.82%, 2.55%, 1.27% and 1.27%; respectively. The result agree with the obtained 197 isolates were identified from 50 coetaneous specimens from 5-week-old normal broilers by Scanlan and Hargis [16].

Results of isolation from chicken flocks with clinical signs (Table3) showing 9/13(69.23%) flocks were positive, with 11/354 (3.11%) samples were positive . It was reported that Staph was isolated from different disease conditions in chickens including celluilitis lesions in broiler chickens [30], clinical respiratory signs in layer [31] and ducklings exhibiting tremor [32].

Positive samples including : 3liver, 4 kidney, 2 intestine, 1 air sac and 1 nasal sinus. Previous work showing that CoNs was recovered from nares, blood, liver, and hock joint [33] from poultry carcasses (35 liver, 35 skin, and 30 intestine).

Isolation from healthy chicken flocks (Table3) showing 8/21 (38.95%) flocks were positive, 15/588 (2.55%). Similar results as CoNS isolates from healthy [34]. , cutaneous specimens from 5-week-old normal broilers [16] from 1- to 8-week-old healthy chickens in three flocks [35]. Positive samples including 3 liver , 2 kidney, 4 intestine, 3 lung , 2 air sac and 1 sinus . The result agree with the reported isolation from chicken organs including the nares, nasal swabs and sinus [36,37] ; liver [33,37] ; intestine and cloacal swabs [37,38] and internal organs [39,40]). While Nawaz et al .[39] and Aarestrup et al. [40] reported isolation from hock joints.

Hatchery samples (Table 3) showing the isolation of 26 staphylococcus isolates (11.76%). The tested 108 fertile eggs and dead in shell embryos resulted in 14 and 12 isolates in rate of 12.96% and 13.79%; respectively. staphylococcus was isolated (13.3%) from surfaces and contents of Japanese quail eggs [41], 75% of isolates from egg shell and yolk [42] also, isolated in rate of 21.67% (78/360) including 23.12% from 160 dead in shell and 20.5% from 200 one day old chick [18].

Identification of CoNS species from broiler chicken flocks and hatchery biochemically [28] INTEGRAL SYSTEM *STAPHYLOCOCCI* KIT was used [24]. All *Staphylococci* isolates were oxidase negative, catalase positive [28] and coagulase negative [8, 43].

Staphylococcus isolated from chicken's organs 12/26 proved to be CoNS in rate of 46.15%. This result agree with Awan and Matsumoto [33] reported the prevalence of CNS in broiler farms. Isolates from the blood, liver, and hock joint were 79 Staphylococcus, 77 among of them were CoNS. *Kaszanyitzky et al.* [44] recovered 61.7% CNS out of recovered strains while Lazarovich et al. [45] investigated 10% CoNS. The incidence of CoNS species from chicken's (Table 4) showing that the 12 CoNS include 8 *S. xylosus* (75 %), 2 *S. scuiri* (16.67%) and 2 *S. lentus* (16.67%). Our result agree with 1*S. sciuri* and 7 *S. lentus* were isolated from healthy and sick poultry [34]. Two *S. lentus* were identified from scabby-hip lesions in broiler chickens [16], CNS were 19% *S. lentus*, 18% *S. simulans*, 13% *S. cohnii10% S. gallinarum* and 7 % *S. captis* [33] .

TABLE 1. Primers used in molecular identification of resistance genes in CoNS isolates using PCR

Primer	Sequence	Amplified product	Reference
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure et al., 2006
ermC	ATCTTTGAAATCGGCTCAGG CAAACCCGTATTCCACGATT	299 bp	Schlegelova et al., 2008
tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	Duran et al., 2012
blaZ	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173 bp	Duran et al., 2012
Kan	GTGTTTATGGCTCTCTTGGTC CCGTGTCGTTCTGTCCACTCC	621 bp	Frana et al., 2001
icaD	AAA CGT AAG AGA GGT GG GGC AAT ATG ATC AAG ATA	381 bp	Ciftci et al., 2009
bab	CCCTATATCGAAGGTGTAGAATTG GCTGTTGAAGTTAATACTGTACCTGC	971 bp	Cucarella et al., 2001

TABLE 2. Temperature and time conditions of the primers for Staphylococci identification during PCR according to Emerald Amp GT PCR Master Mix (Takara) kit.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
mecA	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
ermC	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
tetK	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
blaZ	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
Kan	94˚C 5 min.	94˚C 30 sec.	54˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
icaD	94˚C 5 min.	94˚C 30 sec.	49˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
bab	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.

TABLE 3. Incidence of Staphylococci species from chicken organs (n=157 each) and hatchery samples (n=221).

Chicken					Hatcheries			
Samples Type	No of positive samples		Total		Samples		No of isolates	
	Diseased flocks	healthy flocks	No. of Isolates	%	Type	Total number	no	%
Liver	3	3	6	3.82	Walls	26	0	
Kidney	4	2	6	3.82	Infertile eggs	108	14	12.96
Intestine	2	4	6	3.82	Dead in shell	87	12	13.79
Lung	2	3	4	2.55				
Air-sacs	1	2	2	1.27				
Nasal sinus	1	1	2	1.27				
Total	11	15	26			221	26	
Over all total	11/354 (3.11%)	15/ 588 (2.55%)	26/942= 2.77		26/221			11.76

TABLE 4. Incidence of CoNS isolates species from chicken organs (n=157 each) and hatcheries.

Chicken organs							Hatcheries				
Samples Type	CoNS species						Samples		CoNS species		
	S. xylosus		S. scuiri		S. lentus		Type	No	S. xylosus		S. scuiri	
	No.	%	No	%	No	%			No	%	No	%
Liver	4	3.18	0	0	0	0	Walls	26	0	0	0	0
Intestine	0	0	2	1.27	2	1.27	Infertile eggs	108	6	5.55	2	1.85
Lung	2	1.27	0	0	0	0	Dead in shell	87	2	2.29	0	0
Air-sacs	2	1.27	0	0	0	0						
Total	8		2		2			221	8		2	
Over all total	12/942 (1.27%)							10/221 (4.42%)				

TABLE 5. Rate of CoNS susceptibility to used antibiotics.

Species and No	State	% of Chemotherapeutic agent reactions												
		CTX	ENR	AMC	VA	OX	K	DA	CFP	SXT	C30	FEP	T30	CN
2 S.lentus	Sensitive	0	0	100	100	0	0	0	100	0	0	0	0	0
	Resistant	100	0	0	0	100	100	100	0	100	100	100	100	100
	Intermediate	0	100	0	0	0	0	0	0	0	0	0	0	0
5 S. scuiri	Sensitive	0	40	40	100	0	20	0	80	0	20	20	0	20
	Resistant	60	0	60	0	100	60	100	20	100	80	80	100	80
	Intermediate	40	60	0	0	0	20	0	0	0	0	0	0	0
15 S.xylosis	Sensitive	0	53.33	80.0	100	0	6.67	0	66.7	0	3	6,67	0	6.67
	Resistant	73.3	1.33	20.0	0	100	73.3	100	6.67	100	26.6	80.0	100	100
	Intermediate	26.7	33.3	0	0	0	33.3	0	33.3	0	13.3	13.3	0	0

Eight *S. xylosus* strains were isolated as 4 , 2 from lung and 2 from air-sacs in rate of 3.18%, 1.27% and 1.27% out of tested samples [24,31]. Two *S. scuiri* as well as 2 *S. lentus* were isolated only from intestine 2 out of 157 samples (1.27%) [24,38]**.** From a total 26 samples; 10 isolates were CoNS (38.46%) and represented 4.52% out of total samples [33,45]**.** CONS including 8 *S. xylosus* and 2 *S. scuiri*. Eight *S. xylosus* were 6 (5.55%) from infertile eggs and 2 (2.29 %) from dead in shell, While the 2 *S. scuiri* (1.85%) were obtained from infertile eggs (Table 4) [18,41,42] **.**

The tested CoNS isolates susceptibility against 13 different available antimicrobial using disk diffusion method (Table14) [10,46]**.** Results of antibiotic susceptibility showed 100% resistance to Oxytetracycline 30 µg/ml, Trimethoprime + Sulphamethexole 2.25/23.75 µg/ml (SXT) , Calindamycin 2 µg/ml. and Oxacillin 30 µg/ml. The tetracycline resistance can be supported by those reported as 23 CNS were tetracycline resistant [40], coagulase positive and negative Staphylococci isolated from broiler farm in Ismailia province (Egypt) Complete antibiotic resistance to oxytetracycline [47] and high levels of resistance towards tetracycline, oxytetracycline [48]**.**

Different levels of resistance were also detected 67·8% [49] 22% of *S. xylosus* [20,50] 29.1% [12] 18.4 % *S. scuiri, S. lentus, and S. epidermidis* isolates [13] and 21.4% out of 84 *S. xylosus* isolates from poultry bioaerosol were resistant to tetracycline [14]. While *Aslantaş et al.* [51] investigated that 89 isolates of (CNS) susceptible to Tetracycline (100%). Osman et al. [52] reported that 36 CNS isolates recovered from chicken meat showed complete resistance to Sulfamethoxazole/Trimethoprim. Aslantaş et al. [51] investigated 69.2% out of 89 isolates CNS were resistant while 2.6% of *S. scuiri, S. lentus, and S. epidermidis* were resistant by Huber et al. [11]. Resistance to Clindamycin was reported [52, 53]. Complete resistance to Oxacillin was also reported [14, 18, 52] while Piessens et al. [54] found 42.9% resistance.

All isolates were 100% susceptible to Vancomycin 30 µg/ml (VA) and showed 90% susceptibility to Enrofloxacin 5 µg/ml (ENR). While the tested isolates showed variable susceptibility to the other used antibiotics. The 100% susceptibility to Vancomycin was determined [33,32, 55]. Susceptibility of CoNS to Enrofloxacin was found to be 100% [18,33] while *Youssef and Hamed* [47] detected sensitivity of the isolates to Enrofloxacin was 60%. Drug susceptibility (Table 5) of the tested CoNS isolate multidrug resistance was detected as 2 *S.lentus* , 5 *S. scuiri* and 15 *S. xylosis* were resistant to 9, 4 and 5 out of tested 13 antibiotics; respectively. This result indicated that the isolates are multiresistant to antibiotics. Same results CoNS isolates recovered from chickens were reported including *S. sciuri*, *S. lentus* and *S. xylosus* [53,56]. Moreover, Chah et al. [57] reported that 81.3% of the CoNS were multi-drug resistance.

Phenotypic resistances were verified by PCR amplification and could be traced back to the genes [10,58]**.** Results of PCR tested isolates proved that 7 isolates from the tested 10 (70%) each having 4 resistance genes (Table 6). This result proved that drug resistance can depend on other factors rather than the genetic one. This result can agree with Zdolec et al. [59] in assessment of antimicrobial susceptibility of CNS 23.6% CNS isolates were found to be resistant to oxacillin. All isolates phenotypic resistant to oxacillin did not have the mecA gene, which was only found in 14.6% of the isolates. Also, Osman et al. [52] detected that 85.7% of CNS species phenotypic resistant to oxacillin expressed the mecA gene.

The most genes are mecA, tetK, blaZ and ermC (Fig 1-7). Results agreed with Zdolec et al. [59] assessed the antimicrobial susceptibility of CNS Isolates were tested for sensitivity to Vancomycin, Ampicillin, Erythromycin, Tetracycline, Gentamycin and Oxacillin. PCR was used for the detection of resistance genes mecA, erm B, tet K and tet M. Molecular evaluation of resistance determinants revealed tet K or Tet M genes in 8 S. epidermidis strains. Vela et al. [14] confirmed the presence of tetK, ermB, and blaZ genes in S. xylosis isolates that found resistant to tetracycline, erythromycin, and β-lactam antibiotics. Chah et al [57] found that resistance genes detected were: blaZ, tet (K), tet (M), tet (L), erm (B), lnu (A), aacA-aphD, aphA3, str, dfr (G), cat pC221,and cat pC223.

TABLE 6. Resistance genes in MDRCoNS using PCR.

Sample	Gene							No of gens
	mecA	tetK	blaZ	Kan	ermC	icaD	bab	
1	+	-	-	+	+	-	-	3
2	+	+	+	-	+	-	-	4
3	+	+	-	+	+	-	-	4
4	+	+	+	-	+	-	-	4
5	+	+	+	-	+	-	-	4
6	+	+	+	-	+	-	-	4
7	+	+	-	-	+	-	-	3
8	+	+	+	-	+	-	-	4
9	-	+	+	+	+	-	-	4
10	+	-	-	-	-	-	-	1
no of +ve	9	8	6	3	9	0	0	
%	90	80	60	30	90	0	0	

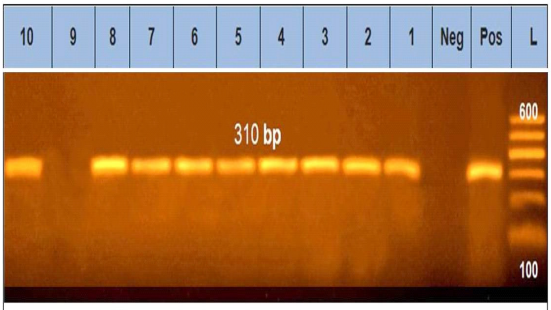

Fig. (1): Lane L: Molecular size marker (100-600 pb). The expected amplicon size of *mecA* gene 310 pb. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 10 are positive except lane 9 is negative.

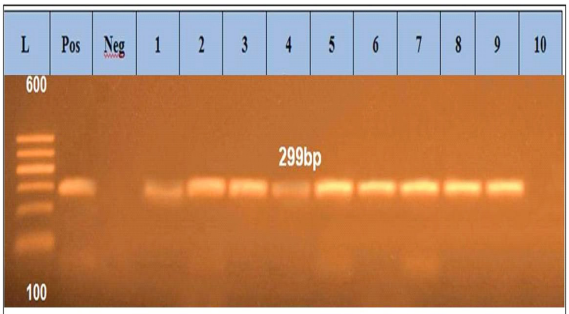

Fig. (2): Lane L: Molecular size marker (100-600 pb). The expected amplicon size of *ermC* gene at 299 pb. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 (10) are positive except lane 10 (2) is negative.

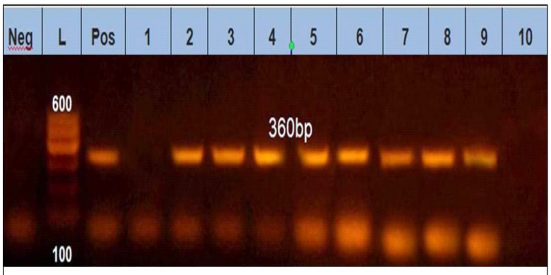

Fig. (3): Lane L: Molecular size marker (100-600 pb). The expected amplicon size of *tetK* gene at 310 pb. Lanes 1 and 10 are negative while lanes 2, 3, 4, 5, 6, 7, 8 and 9 are positive.

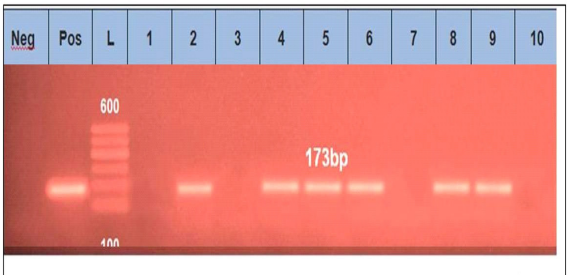

Fig. (4): Lane L: Molecular size marker (100-600 pb). The expected amplicon size of *blaZ* gene at 173 pb.Lanes 1, 3, 7 and 10 are negative, but lanes 2, 4, 5, 6, 8 and 9 are positive.

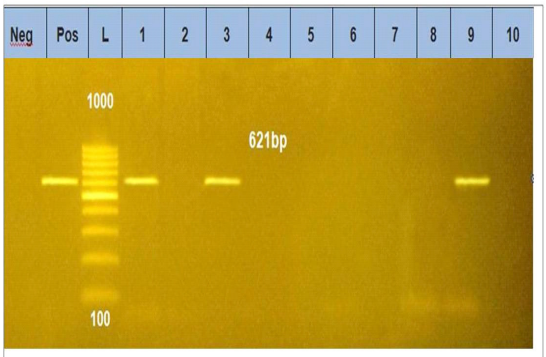

Fig. (5): Lane L: Molecular size marker (100-1000 pb). The expected amplicon size of *kan* gene at 621 pb. Lane 1, 3 and 9 are positive, while lanes 2, 4, 5, 6, 7, 8 and 10 are negative.

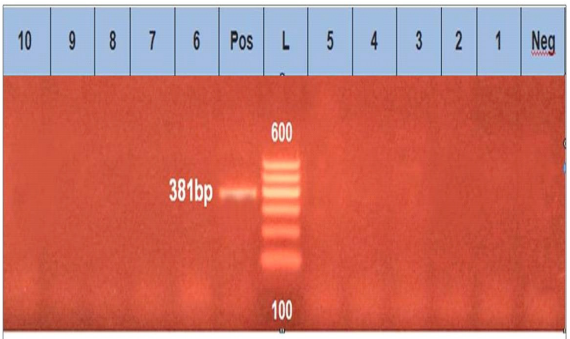

Fig. (6): Lane L: molecular size marker (100-600 pb). The expected amplicon size of *icaD* gene at 381 pb. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 are negative.

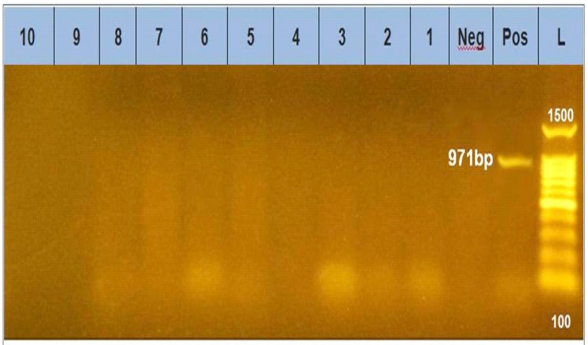

Fig. (7): Lane L: molecular size marker (100-1500 pb). The expected amplicon size of *bab* gene at 971 pb. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 are negative.

Rate of detected genes are mecA, tetK, blaZ and ermC where it was detected in 90, 80, 60 and 90%; respectively. Kan, icaD and bab genes were detected in rate of 30.0 and 0 % respectively. The detected 90% mecA was reported by Wang et al. [38] identified 95% oxacillin-resistant isolates harbored the mecA gene, also erm (C), erm (B), and aacA-aphD were detected. Al-Muhanna [60] and Chajęcka-Wierzchowska et al. [53] detected that 100% isolates of CNS carried mec A gene and 85.7% [52]. While, lower rates 68·7% by Bhargava and Zhang [49] , and 6.7% by Han et al. [53] could be assessed in oxacillin resistant isolates. Tetracycline resistant gene etKwas detected in 80%. This result is close to 100% [50, 40] where all tetracycline resistant CoNS contained the tet (K) gene. While Podkowik et al. [61] reported 60% and 61% tet(M) by Bhargava and Zhang [49]. Penicillin resistance blaZ was detected in 60% of isolates while Podkowik et al. [61] reported 92% penicillin resistance blaZ. Regarding 90% erythromycin resistance ermC the available literature proved rates of 100% [40] , 83.33% [39] 56.2 % [49] and 42% [61].

This study pointed out that monitoring antibiotic resistance in specific bacteria from broiler could provide useful insight to public health and when considering that antimicrobial resistant bacteria can be disseminated in the air during poultry transportation [62].

In conclusion: CoNS were isolated from healthy and diseased chicken flocks as well as from chicken hatchery . The obtained isolates were multidrug either phenotypic and /or genotypic resistant. We recommended good hygienic measures in both chicken farms and hatchery with monitoring of drug resistance of CoNS those act as source for resistance genes to other bacterial pathogens and their importance for both poultry and public health.

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الكشف الجزيئي للجينات المقاومة للمضادات الحيوية في المكورات العنقودية سلبية التخثر Coagulase من قطعان الدجاج والمفرخات في مصر.

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تمت الدراسه هذه علي عدد ٩٤٢عينة تم جمعها من عدد ١٥٧طائر من ٣٤ مزرعة دجاج (١٣مزرعة مصابة و ٢١ غير مصابة) تتراوح أعمارها بين ٢ - ٣١ يومًا بالاضافه الى ٢٢١ عينة من المفرخات. تم اختبار جميع العينات للكشف عن وجود المكورات العنقودية السلبية لانزيم التخثر (CoNS).

نتائج العزل من قطعان الدجاج المصاحبة الأكلينيكيا هي ١٣/٩ (٦٩,٢٣ ٪). العينات إيجابية (٣٥٤/١١ ، ٣,١١ ٪). من قطعان صحية ٢١/٨ (٣٨,٩٥ ٪) كانت إيجابية. عينات إيجابية (٥٨٨/١٥ ، ٢,٥٥. أظهرت النتائج وجود ١٥ قطيع ايجابي من ٣٤ قطعة (٣٤,٠٩٪) مع ٢٦ معزولة للمكور العنقودى من ٩٤٢ عينة (٢,٧٧٪) بما في ذلك ٦ كبد و ٦ كلى و ٦ معوية و ٤ رئة و ٢ حويصلات هوائية و ٢ جيب انفي بمعدل ٣,٨٢ ٪ ، ٣,٨٢٪ ، ٣,٨٢٪ ، ٢,٥٥٪ ، ١,٢٧٪ و ١,٢٧٪ ؛ على التوالي. أيضا ١٢ معزولة من أصل ٢٦ (٤٦,١٥ ٪) كانت CoNS تشمل ٨ S. xylosus (٧٥ ٪) و ٢ لكل من S. scuiri و S. lentus اي ١٦,٦٧ ٪ لكل منهما. كشفت عينات المفريخات عن عزل ٢٦ معزولة من المكورات العنقودية بنسبة (١١,٧٦٪). و لقد نتج عن فحص ١٠٨ من البيض المخصبة و الكابس ١٤ و ١٢ معزولة بنسبة ١٢,٩٦٪ و ١٣,٧٩٪. على التوالي وكانت عشر معزولات منها (٣٨,٤٦٪) مكورات سالبه التخثر وبمعدل ٤,٥٢٪ من مجموع العينات بما في ذلك ٨ S. xylosus و ٢ S. scuiri. الثمانية *S. xylosus* كان ٦ (٥,٥٥٪) من بيض عقيم و ٢ (٢,٢٩٪) من بيض كابس ، بينما تم الحصول على ٢ S. scuiri (١,٨٥٪) من بيض غير مخصب .

أظهرت المعزولات المختبرة للمضادات الحيويه مقاومة ١٠٠٪ للأوكسي تتراسكلين ٣٠ ميكروغرام / مل ، تريميثوبريم + سولفاميثيزول ٢,٢٥ / ٢٣,٧٥ ميكروغرام / مل ، الكالنداميسين ٢ ميكروجرام / مل وأوكساسيلين ٣٠ ميكروغرام / مل. كان معدل حساسيه المعزولات ١٠٠ ٪ الي الفانكومايسين إلى ٣٠ميكروغرام / مل و ٩٠ ٪ للانروفلوكساسين ٥ ميكروغرام / مل . تم الكشف عن المقاومة للعقاقير المتعددة في شكل مقاومة ٩ و ٤ و ٥ من ١٣ مضادات حيوية تم اختبارها في ٢ S. lentus و ٥ S. scuiri و ١٥ S. xylosis؛ على التوالي. تم اختبار ١٠ معزولات لوجود ٧ جينات مقاومة تتضمن: mecA، tetK، blaZ، kan، ermC، icaD، bab gene. سبعة معزولات من الـ ١٠ (٧٠٪) المختبرة لها ٤ جينات مقاومة. أكثر أنواع الجين التي تم الكشف عنها هي mecA tetK و blaZ و ermC حيث تم تحديدها في ٩٠ و ٨٠ و ٦٠ و ٩٠٪ على التوالي.اما الجينات icaD ، kan و bab gens تم الكشف عنها بمعدل ٣٠,٠ و ٠ ٪. على التوالي.

من نتائج الدراسة يتضح انه تم عزل CoNS من قطعان الدجاج الصحية والمريضة وكذلك من المفرخات. كانت العزلات التي تم الحصول عليها متعددة المقاومه للمضادات وبها عوامل وراثيه . لذا نوصي بالتدابير الصحية الجيدة في مزارع الدجاج والمفرخات مع مراقبة مقاومة CoNS للأدوية والتي تعمل كمورثات للمقاومة للمكروبات الاخرى وأهميتها لصناعة الدواجن والصحة العامة. كما ان الامراضيه هذه المعزولات والعوامل التي تؤثر علىها تحتاج إلى الدراسه.