Detection of species-specific gene thermonuclease (nuc) in *Staphylococcus aureus* from chickens by real- time PCR assay.

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ABSTRACT

Accurate and rapid detection of *S. aureus* is crucial to control its infections and minimize its leakage to the food chain. Therefore, the primary purpose of this research is to isolate *S. aureus* and characterize the isolates by coagulase production from cloacal, tracheal and nasal swabs of apparently healthy broiler & layer chickens and from different organs (joints, liver, lungs, ovaries, foot abscesses and heart blood) of diseased broiler & layer chickens. A total of 318 samples were collected as follows: 108 sample from different farms of apparently healthy broiler & layer and 210 samples from diseased layer & broiler chickens. The samples were examined bacteriologically, 164 (51.6%) *Staphylococcal* isolates were recovered from the 318 sample. 37/164 (22.6%) coagulase positive *S. aureus* were isolated. Upon confirmation by Real Time PCR for thermonuclease (*nuc*) gene using SYBR Green, isolates showed amplification at C_T value 19, 20 and 21 with Melting temperature (Tm) values in the range of 76–77 ^oC. Dissociation curve between 75-80^oC which confirmed as *S. aureus*. The isolated *S. aureus* was highly sensitive to vancomycin, amoxicillin + clavulinic acid and cephalothin by 84.5%, 83.8% and 78.4 respectively and highly resistant to ampicillin, oxacillin and penicillin by 75.7%, 73% and 70.2% respectively.

Keywords: *Staphylococcus aureus*, *nuc* gene, Real time PCR, SYBR Green.

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1. INTRODUCTION

S. aureus is the only staphylococcal species in poultry considered to be pathogenic. Typical pathogenic *S. aureus* strains are Gram-positive, coccoid in shape, found in clusters, aerobic, facultative anaerobic**,** non-spore forming and non-motile belong to the family *Micrococcaceae* (Willett, 1992). *S. aureus* is an important cause of disease in poultry. It can be involved in a wide range of clinical conditions such as septicemia, bone and joint infections, foot abscesses and dermatitis (Jordan and Pattison, 1996). Proximal femoral degeneration (PFD), mediated mainly by *S. aureus*, is the most common cause of lameness in broiler**,** such diseases compromise animal welfare and cause economic loss through death, reduced productivity and carcass condemnations at the processing plan (McNamee *et al*., 1998). Modern molecular biological techniques for the detection and differentiation of pathogens gain more and more importance in food hygiene (Pimbley and Patel, 1998). Amplification of Thermonnusclease (*nuc)* gene is used to detect and quantify *S. aureus* and known as *S. aureus* species specific gene (Studer *et al*., 2008). Real-time PCR for detection of pathogenic bacteria as *S. aureus* and others has properties of high specificity and

sensitivity, and can be completed in one day (Ching-Yang *et al*., 2012).

2. MATERIALS AND METHODS

2.1. Samples Collection:

A total number of 318 samples from chickens were collected as follows: Those were 108 pooled samples (36 cloacal swabs, 36 tracheal swabs, 36 nasal swabs) from apparently healthy broiler & layer and 210 samples from different organs (joints, livers, lungs, foot abscesses, ovaries, heart blood) of diseased layer & broiler from different farms in Dakahlia Governorate under aseptic condition in ice box and transferred to the laboratory.

2.2. Bacteriological examination:

Cultivation and isolation of *S. aureus* was done according to (Sneath *et al*., 1986) by pre-enriched non selective medium (buffered peptone water) was inoculated with the collected samples at ambient temperature and then incubated at 37˚C for 24 hours under aerobic condition. Loopful of inoculated medium was inoculated on to blood agar, and then incubated for 24 hours at 37˚C. Colonies were examined for morphological characteristic appearance of *Staphylococcus species* by Gram staining (Swayne *et al*., 1998). To differentiate *S. aureus* from other *Staphylococcus species.* The suspected colonies were picked up and inoculated onto selective medium; mannitol salt agar (MSA) and Baird Parker's agar (BP) medium. MSA was incubated at 37˚C for 24 hours and BP was incubated at 37˚C for 24-48 hours. Heavy contaminated environmental samples from cloacal, nasal and tracheal swabs; where heavy background contamination exists were directly inoculated onto selective medium (Mannitol salt agar and Baird Parker agar). After plating the typical and suspected colonies on tryptic soya agar and brain heart infusion broth, each colony showing typical colonial appearance of *S. aureus* was subjected to biochemical identification and examined for catalase test, Oxidase test, coagulase test and Voges Preskauer test.

2.3.Antibiotic susceptibility testing:

Determination of the susceptibility of the isolated coagulase positive *S. aureus* to antibiotic discs was adopted using the disc diffusion technique (Koneman *et al*., 1979). The discs that used for *S. aureus* were Penicillin G, Ampicillin, Amoxycillin+ clavulinic acid, Oxacillin, Vancomycin, Bacitracin, Ceftriaxone, Cephalothin, Cefotaxime, Enrofloxacin, Lincomycin and Gentamicin.

2.4. Identification of S. aureus strains by using Real time PCR assay:

Extraction of bacterial DNA by boiling method (Croci *et al.*, 2004). 1 mL of an overnight *S. aureus* culture in enrichment broth at 37ºC centrifuged for 10 min at 10,000 g. The supernatant was discarded and the cell pellet re-suspended in 300µl DNase–Rnase free distilled water then vortexed. The re-suspension was heated for 10 min at 95–100ºC, and then immediately cooled and again centrifuged at 14,000 g for 5 min at 4ºC. The supernatant was carefully transferred to a new centrifuge tube and incubated again at 100ºC for 10 min then immediately transferred and chilled on ice. An aliquot of 5µl of the supernatant was stored at -20ºC until used as DNA template for PCR.

Real-Time PCR Amplification: Detection of *S. aureus* by SYBR Green Real time PCR according to (Martinon and Wilkinson, 2011). Oligonucleotides primers were designed against a conserved region, thermonuclease (*nuc*) gene as follows: the forward primer nuc – F "GCGATTGATGGTGATACGGT" and the reverse primer nuc – R "AGCCAAGCCTTGACGAACTAAAGC ". DNA samples were amplified in a total of 25 µl as the following: 12.5µl of 2x QuantiFast SYBR Green PCR Master Mix, 1µl of forward primer, 1µl of reverse primer, 5µl of DNA template and molecular

biology grade water till 25 µl. The Real Time PCR was performed under the following conditions:

Step	Description	Temperature	Time	cycles
1	Initial heat activation	95°C	5min.	one
2	Denaturation	95°C	30sec.	
3	Annealing	55°C	30sec.	35
4	Extension	72°C	1min.	
Thermal profile of SYBR® Green PCR.				
		95°C	1min.	
5	Dissociation curve	55°C	30sec.	one
		95°C	30sec.	

The PCR must start with an initial activation step of 5 min at 95°C to activate Hot Star Taq® Plus DNA Polymerase. The identification of the PCR product was performed by determining the melting temperature (Tm) of dissociation curve of the amplicon after PCR.

3. RESULTS

3.1. Result of cultural, morphological and biochemical characters of the isolated S. aureus:

S. aureus colonies appear circular, smooth, opaque and most colonies with β-haemolysis on blood agar, while on mannitol salt agar, they were yellow color surrounded by yellow halo with yellow colored medium. On Baird Parker medium, black small 1 mm colonies after 24 hours and large 2.5 mm after 48 hours surrounded by an opalescent ring and a clear zone. *S. aureus* appears as grapes like clusters under light microscope. For the biochemical results, *S. aureus* colonies were positive for coagulase test, catalase test, mannitol fermentation, tellurite reduction and Voges-Proskauer but were negative for Oxidase test.

3.2. Prevalence of coagulase positive S. aureus isolation from different samples:

A total number of 318 examined samples that were represented as 108 sample from different farms of apparently healthy broilers & layers and 210 sample from diseased layers & broilers. 164 *Staphylococcal* isolates were recovered from 318 samples collected from different types of chickens with a percentage of 51.6%. Thirty seven sample were found to be coagulase positive *S. aureus* from the 164 *Staphylococcal* isolates with an incidence of 22.6%.

3.3. Prevalence of coagulase positive S. aureus in different samples of diseased and apparently healthy layer chickens:

A total of 5 (45.5%), 4 (33.3%), 2 (20%) and 2 (16.7%) were found coagulase positive *S. aureus*. from joints, livers, ovaries and lungs of diseased layers respectively (Table 1). The coagulase positive *S. aureus* was 8 with the percentage of (25%) which were 4 (26.7%), 2 (25%) and 2 (22.2%) from cloacal swabs, nasal swabs and tracheal swabs from apparently healthy layer birds respectively (Table 3).

3.4. Prevalence of coagulase positive S. aureus in different samples of diseased and apparently healthy broiler chickens:

The isolated coagulase positive *S. aureus* were 3 (30%), 4 (21%), 2 (13.3%) and 1 (11.1%) from joints, livers, lungs and heart blood *of* diseased broilers respectively (Table 2). Six samples were positive for coagulase positive *S. aureus* with percentage of (20.7%) which resulted from 3 (23%) of cloacal swabs, 2 (22.2%) of nasal swabs and 1 (14.3%) of tracheal swabs from apparently healthy broilers respectively (Table 4).

3.5. Results of the sensitivity tests for the isolated coagulase positive S. aureus:

All coagulase positive *S. aureus* isolates examined against 12 antimicrobial discs revealed different percentage of sensitivity. Regarding to penicillin 26 coagulase positive *S. aureus* was resistant (70.2%) while, 11 strains were sensitive (28.8%). On the other hand 28 (75.7%) of the strains were resistant and 9 (24.3%) sensitive to ampicillin. 6 (16.2%) and 31 (83.8%) of the strains were resistant and sensitive to amoxicillin + clavulinic acid, respectively. On the other hands, 27 (73%) of the strains were resistant, while 10 (27%) were sensitive to oxacillin. 5 (13.5%) of

strains were resistant and 32 (84.5%) of the strains were sensitive to vancomycin. Twelve (32.4%) of the strains were resistant and 25 (77.6%) were sensitive to bacitracin, 10 (27%) and 27 (73%) of the strains were resistant and sensitive to ceftrixone, respectively. Eight (21.6%) and 29 (78.4%) of the strains were resistant and sensitive to cephalothin, respectively. Concerning to cefotaxime, 9 (24.3%) of the strains were resistant, while 28 (75.7%) were sensitive. Enrofloxacin, 11 (29.7%) of strains were resistant and 26 (70.3%) of the strains were sensitive. Lincomycin, 13 (35%) of the strains were resistant and 24 (65%) were sensitive. Finally 10 (27%) and 27 (73%) of the strains were resistant and sensitive to gentamicin respectively.

3.6. Detection of species specific (nuc) gene of S. aureus using Real time PCR:

Amplification and detection of Thermonuclease (*nuc*) gene of *S. aureus* was carried out using SYBR Green qPCR. The positive results showed amplification at C_T (threshold cycle) value 19, 20 and 21 while the negative one has no amplification. Melting temperature (Tm) values obtained for *S. aureus* isolates were in the range of 76–77 ºC. Dissociation curve between 75-80ºC (Photo No. 1).

The use of SYBR® Green reagents in real-time PCR is based on the exceptionally high affinity of SYBR® Green dye for double-stranded DNA. The progress of a real-time run using SYBR® reagent chemistry can be measured by monitoring this increase in fluorescence as SYBR® Green dye binds to PCR products. The software for the apparatus calculated the C_T for each reaction. C_T (threshold cycle) is cycle number at which the fluorescence passes the fixed threshold (Photo No. 1).

4. DISCUSSION

In this study, 164 *Staphylococcal* isolates were recovered from 318 samples collected from different types of chickens with a percentage of 51.6%. Thirty seven sample were found to be coagulase positive *S. aureus* from the 164 *Staphylococcal* isolates with an incidence of 22.6%. Coagulase positive *S. aureus* was recovered from 13 samples (26%) of diseased layer chickens, 10 samples (18.9%) from diseased broiler chickens, 8 samples (25%) from layer farms (apparently healthy), 6 samples (20.7%) from broiler farms (apparently healthy) and this result was nearly in coordinating with some researchers such that an incidence of *S. aureus* from a total 296 samples was (20.7%) and the prevalence among different type of chickens was 11 (16.7%) from diseased chickens, 20 (26.7%) from layer farms and 13 (18.8%) of broiler farms (Heba, 2008), while the incidence of *S. aureus* was 27.05% (Seham, 2003).

S. aureus in diseased chickens (layer& broiler) was highly recovered from joints 8 samples with incidence of 38% followed by livers, ovaries, lungs and heart blood with prevalence of 25.8% (8/31), 20 % (2/10), 14.8% (4/27) and 11.1% (1/9) respectively as shown in (Table 1 &2) which agree with (Seham, 2003) and (Heba, 2008). The isolated *Staphylococci* from chickens of apparently healthy layer farms (table, 3) were 32 (59.2%). The coagulase positive *S. aureus* was 8 (25%) which related to 4 from cloacal swabs (26.7%), 2 from nasal swabs (25%) and 2 from tracheal swabs (22.2%). Nearly the same results were obtained by (Cotter and Taylor, 1987) who found (25%) recovery of coagulase positive *S. aureus* from 294 rectal swabs obtained from healthy hens in lay**.** While (Zhu *et al.,* 1999) reported that the recoveries of *S. aureus* from the choana and trachea were significantly higher than those from the cloaca. The isolated *Staphylococci* were 29 with a percentage of 53.7% from apparently healthy broilers. 6 samples were positive for coagulase positive *S. aureus* with percentage of (20.7%) where 3 from cloacal swabs (23%), 2 from nasal swabs (22.2%) and 1 from tracheal swabs (14.3%) (Table, 4) while (Cotter and Taylor, 1987) reported

Table (1) Prevalence of coagulase positive *S. aureus* in different organs of diseased layer chickens.

Source of Collected Sample	Numbers of Examined Samples	Total Numbers of *Staphylococci*	Coagulase positive *S. aureus*		Coagulase negative *Staphylococci*	
			No.	%	No.	%
Joints	14	11	5	45.5	6	54.5
Livers	30	12	4	33.3	8	66.7
Ovaries	26	10	2	20	8	80
Lungs	30	12	2	16.7	10	83.3
Foot Abscesses	8	5	0	0	5	100
Total	108	50	13	26	37	74

Table (2) Prevalence of coagulase positive *S. aureus* in different organs of diseased broiler chickens.

Source of Collected Sample	Numbers of Examined Samples	Total Numbers of *Staphylococci*	Coagulase positive *S. aureus*		Coagulase negative *Staphylococci*	
			No.	%	No.	%
Joints	18	10	3	30	7	70
Livers	34	19	4	21	15	79
Lungs	32	15	2	13.3	13	86.7
Heart Blood	16	9	1	11.1	8	88.9
Abscess	2	0	0	0	0	00
Total	102	53	10	18.9	43	81.1

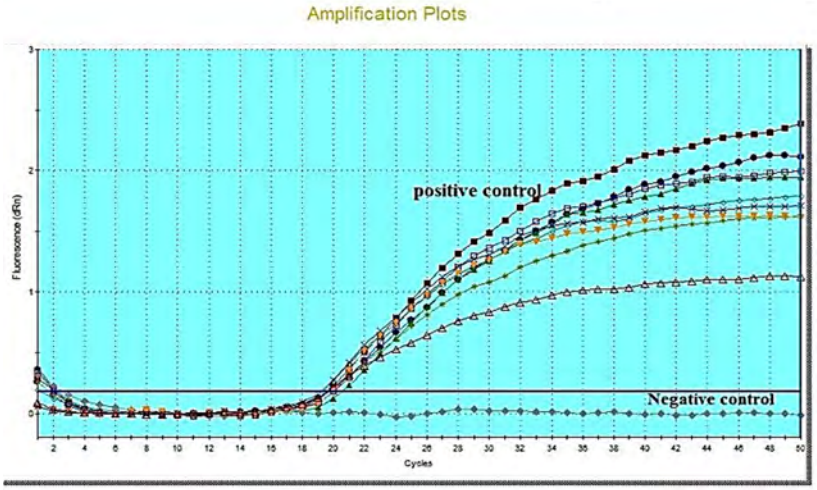
Table (3) Prevalence of coagulase positive *S. aureus* in different samples of apparently healthy layer farms.

Source of Collected Sample	Numbers of Examined Samples	Total Numbers of *Staphylococci*	Coagulase positive *S. aureus*		Coagulase negative *Staphylococci*	
			No.	%	No.	%
Cloacal Swabs	18	15	4	26.7	11	73.3
Nasal Swabs	18	8	2	25	6	75
Tracheal Swabs	18	9	2	22.2	7	77.8
Total	54	32	8	25	24	75

Table (4) Prevalence of coagulase positive *S. aureus* in different samples of apparently healthy broiler farms.

Source of Collected Sample	Numbers of Examined Samples	Total Numbers of *Staphylococci*	Coagulase positive *S. aureus*		Coagulase negative *Staphylococci*	
			No.	%	No.	%
Cloacal Swabs	18	13	3	23	10	77
Nasal Swabs	18	9	2	22.2	7	77.8
Tracheal Swabs	18	7	1	14.3	6	85.7
Total	54	29	6	20.7	23	79.3

Photo No. (1) Real Time PCR result using primer of nuc gene in chicken samples



25 % recovery of coagulase positive *S. aureus* from 294 rectal swabs obtained from healthy hens in lay which nearly similar to our result. Sensitivity test by using 12 antibiotic discs revealed different percentage of sensitivity with the highest sensitivity were to vancomycin, amoxicillin + clavulinic acid and cephalothin by percentage of 84.5%, 83.8% and 78.4 respectively while the highest resistance were to the ampicillin, oxacillin and penicillin by percentage of 75.7%, 73% and 70.2% respectively and this result was nearly similar to that of (Heba, 2008) who found that the highest resistance were to the penicillin and ampicillin by percentage of 74.6% and 72.9% respectively and the highest sensitivity were to amoxicillin + clavulinic acid and cephalothin by percentage of 98.3% and 86.4 respectively. Also (Gardini *et al*., 2003) found that Micro *Staphylococci* were generally susceptible to beta -lactams, but 12 (8.57%) strains were resistant to methicillin, 8 (5.71%) were resistant to oxacillin, and 9 (6.42%) were resistant to penicillin G. On contrary, the results of (Losito *et al*., 2005) appeared that all strains of *S. aureus* were susceptible to amoxicillin / clavulinic acid, cephalothin, oxacillin, vancomycin and bacitracin.

In this study, SYBR Green real time PCR (qPCR) assay was carried out for the detection of the thermonuclease (*nuc)* species specific gene from isolated strains has revealed that the gene was present in the isolates that was demonstrated by amplification at C_T value 19, 20 and 21 while the negative one has no amplification (Photo No1). Amplification of *nuc* gene is used to detect and quantify *S. aureus* so known as *S. aureus* species specific gene (Studer *et al*., 2008). It is considered that *S. aureus* species specific real time PCR is useful for speeding up identification of *S. aureus* by replacing the current biochemical phenotypic schemes which are time consuming. Additionally, if appropriate conditions are established, direct PCR identification of *S. aureus* from food and clinical specimens can be performed (Jos *et al*., 1996). Finally the real-time PCR assay may be recommended as a rapid method for detection of *S. aureus* (Velasco *et al*., 2014).

5. REFERENCES

Ching-Yang, C.M.H., Chiu, H.S.L.C., Huaug, C. 2012. Simultaneous

Detection of Food Pathogens, Staphylococcus aureus, Salmonella Enterica, Bacillus cereus and Vibrio parahaemolyticus by Multiplex Real-Time Polymerase Chain Reaction. Journal of Food and Drug Analysis20 (1): 66-73

Cotter, P. F., Taylor, R.L. 1987. Staph. aureus carriage in commercial layers. Poultry Science, 66(1): 86.

Croci, L., Delibato, E., Volpe, G., De-Medici, D., Pallesschi, G. 2004. Comparison of PCR, electrochemical, enzyme linked immunosorbant assayes and the standered culture method for detection of salmonella in meat products. Appl. Environ. Microbiol., 70(3): 1393-1396.

Gardini, F., Tofalo, R., Suzzi, G. 2003. A survey of antibiotic resistance in Micrococcaceae isolated from Italian dry fermented sausages. J. Food Prot., 66(6): 937-945.

Heba, B. M. 2008. Microbiological studies on Staphylococcus aureus with regard to antibiotic resistance. M .V. Sc. Thesis (Microbiology), Fac. Vet. Med. Cairo Univ.

Jordan, F. T. W, Pattison, M. 1996. Poultry diseases, 4th ED. Saunders, London, 66-69.

Jos, M.B.M., Vossen,V., Hofstra, H. 1996. DNA based typing identification and detection systems for food spoilage microorganisms: Development and implementation. Int. J. Food Microbiol., 33: 35-49.

Koneman, E.W., Allen, S.D., Sowell, V.R., Sommers, H.M. 1979. Color atlas and textbook of diagnostic microbiology. J.B. Lippincott company, Philadelphia. Toronto. J. Infect. Dis., 123: 97-98.

Losito, P., Vergara, A., Muscariello, T., Ianieri, A. 2005. Antimicrobial susceptibility of environmental Staphylococcus aureus strains isolated from a pigeon slaughterhouse in Italy .Poultry Sci., 84(11): 1802-1807.

Martinon, A., Wilkinson, M. G. 2011. Selection of optimal primer sets for use in a duplex SYBR Green-based real time PCR protocol for the detection of L. monocytogenes and S. aureus in foods. Journal of Food Safety. doi:10.1111/ j.1745e4565.2011.00301.x

McNamee, P.T., McCullagh, J.J., Thorp, B.H., Ball, H.J., Graham, D., McCullough, S.J., McConaghy, D., Smyth, J. A. 1998. Study of leg weakness in two commercial broiler flocks. Vet. Rec., 143(5):131-135.

Pimbley, D.W., Patel, P.D. 1998. A review of analytical methods for the detection of bacterial toxins. J. Appl. Microbiol. Symp. Suppl. 84, 98S–109S

Seham, M. D. 2003. Microbial studies on Staphylococcal infection in poultry. M .V. Sc. Thesis (Microbiology), Fac. Vet. Med. Alexandria Univ.

Sneath, P.H.A., Mair, N.S., Elisabeth Sharpe, M., Holt, J.G., Murray, R.G. E., Chairman, Brenner, D.J., Bryant, M.P., Krieg, N.R., Moulder, J.W., Pfennig, N., Staley, J. T. 1986. Section 12 gram-positive cocci, Bergey's Manual of Systemic Bacteriology, (2) Williams& wikins.USA.

Studer, E., Schaeren, W., Naskova, J., Pfaeffli, H., Kaufmann, T., Kirchhofer, M. 2008. A longitudinal field study to evaluate the diagnostic properties of a quantitative real-time polymerase chain reaction-based assay to detect Staphylococcus aureus in milk. J. Dairy Sci., 91: 1893-1902.

Swayne, D.E, Charman, Glisson, J.R, Jackwood, M.W, Pearson, J.E., Reed, W.M. 1998. Staphylococcosis, A laboratory manual for the Isolation and identification of avian pathogens, 4th edition. Am. Assoc. Avian Pathology.

Velasco, V., Sherwood, J.S., Rojas-Garcı´a, P.P., Logue, C.M. 2014. Multiplex Real-Time PCR for Detection of Staphylococcus aureus, mecA and Panton-Valentine Leukocidin (PVL) Genes from Selective Enrichments from Animals and Retail Meat. PLoS ONE 9(5): e97617.

Willett, H.P. 1992. Staphylococcus. In W.K. Joklik, H.P. Willett, D.B. Amos, and C.M. Wilfert (eds.). Zinsser Microbiology, 20th ed. Appleton & Lange: Norwalk, CT, 401—416.

Zhu, X.Y., Wuc, C., Hester, P.Y. 1999. Induction of the delayed footbad and wattle reaction to killed Staphylococcus aureus in chickens. Poultry Sci., 78:346-352.

الكشف عن (nuc) جين في ميكروب المكورات العنقودي الذهبي في الدجاج بواسطة تفاعل البلمرة المتسلسل حقيقي الوقت

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الملخص العربي

يتسبب ميكروب المكورات العنقودي الذهبي فى خسارة اقتصادية هائلة فى صناعة الدواجن ولذلك فقد تم فحص 318 عينة حقلية كالاتي (108 عينة من دجاج البياض والتسمين السليم ظاهريا و210 عينة من دجاج البياض والتسمين المريض والنافق حديثا) من مصادر مختلفة فى محافظة الدقهلية. تم اخذ العينات من (المفاصل، الكبد، المبايض، الرئة، دم القلب، خراج القدم) ومسحات من (الانف، القصبة الهوائية، فتحة المجمع). تم تصنيف 37 معزولة من المكورات العنقودي الذهبي من 165 من معزولات المكورات العنقودي من اجمالي 318 عينة للدواجن بنسبة ٢٢,٦٪. وقد تم التأكد بإجراء اختبار تفاعل البلمرة المتسلسل حقيقي الوقت باستخدام البادئ العام للكشف عن جين nuc وقد تبين تواجده بالمعزولات والذي اتضح بوجود الجين عند الدورة (CT) ١٩و٢٠ و٢١ ودورة درجة الانصهار (Tm) والتى تتراوح بين درجتي 76 و77 درجة مئوية . تم إجراء اختبار الحساسية للمضادات الحيوية المختلفة واظهرت المكورات العنقودي الذهبي نسب متفاوتة من الحساسية والمقاومة للمضادات الحيوية المستخدمة وكانت أعلي نسبة حساسية لمضادات الفانكومايسيين ، الاموكسيسلسن+حمض الكلافيولينيك و السيفالوثين بنسب ٨٤,٥٪ ، ٨٣,٨٪ و ٧٨,٤٪ علي التوالي بينما كانت اعلي نسبة مقاومة لمضادات الامبسيلين والاوكساسيلين والبنسيللين بنسبة ٧٥,٧٪ ، ٧٣٪ و ٧٠,٢٪ علي التوالي.

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