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Detection of methicillin-resistant *Staphylococcus aureus* from broiler carcasses in Mosul city

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Abstract

Staphylococcus (*S.*) *aureus* is deemed as one of the main pathogens in human and animals. *S. aureus* can produce various toxins that usually implicated in food poisoning. *S. aureus* could possess the *mecA* gene, which is the principle cause of β-lactam antibiotics resistance, particularly methicillin-resistant *S. aureus* (MRSA). Broiler's meat is worthy food for humans, but it may expose to contamination with MRSA during the poultry processes in the slaughterhouse. The current study aimed to assessment the spread of *S. aureus* and MRSA in the broiler carcasses via detection the *nuc* and *mecA* gene and their resistance to different antibiotics. Fifty skin swabs were taken from the broilers carcasses, during their processing in poultry slaughterhouses that scattered in various districts in the Nineveh Governorate during the period between January to April 2020. The results showed that *S. aureus* was recovered in broiler's skin swabs at a percentage of 66% (33/50) which confirmed by *nuc gene*, while MRSA isolates constitute 40% (20/50) of all *S. aureus* isolates, and distinguished as MRSA by their possessing *mecA* gene. All MRSA isolates were resistant to Ampicillin/Sulbactam, Methicillin, and Ampicillin/Cloxacillin antibiotics. The present study stressed on the reduction as much as any possible source of broiler carcasses contamination with *S. aureus* including MRSA during and post poultry processing, through applying high levels of hygienic conditions in all poultry processing premises to attain high standards of sustainability and public health standards.

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Introduction

Staphylococcus aureus is a gram-positive organism which is responsible for many different human and animal diseases. On one hand, *S. aureus* is considered as a major cause of mastitis in dairy herds, exudative dermatitis in pig, and arthritis and osteomyelitis in poultry (1,2). On another hand, *S. aureus* is also regarded as a dangerous bacterium for humans, since it causes many different diseases such as postoperative wound infections, pneumonia, nosocomial bacteremia and food poisoning due to its possessing different types of virulence factors (3). Moreover, it has the ability to transfer from animals to humans and vice versa. *S. aureus* possesses many of genes which can be able to produce various exotoxins such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) that belong to the superantigen family which causes the food poisoning for human (4). The antimicrobial usage is important for the treatment and control of bacterial diseases in humans and animals. *S. aureus* isolates are frequently resistant to many antibiotics when taken without physician prescription or through consumption of contaminated animal products with residual antibiotics. *S. aureus* has been able to adapt rapidly to some types of antibiotics which led to the production of methicillin-resistant *Staphylococcus aureus* (MRSA) (5). In the United Kingdom, MRSA has been discovered in 1961

(after production of methicillin), can be able to resist different types of antibiotics, like, β-lactams and others. After a decade, MRSA had been found in many countries that had been considered as an endemic in the mid-1970s (6). MRSA has been able to menace public health worldwide by the transmission of the MRSA strains from the animals to humans, from human to human, as well as the contamination of the hospitals, general communities, and the animal farms. Many previous studies considered some MRSA strains are epidemic strains that can spread between the hospitals and between countries. During the last years, MRSA has appeared to be increased in its spreading among animal herds resulting in meat and other animal products contamination with MRSA (7). Many authors referred to isolation of MRSA not only from chicken but also from cattle, pigs, and dogs. With the development of cultural awareness of humans worldwide, the consumers prefer to eat a low-fat with high minerals, vitamins contents, good quality protein, quickly prepared, and low expensive chicken meat compared to the other types of meat, but in the same time, human exposure to the food poisoning was increased by consuming contaminated chicken meats with MRSA (8). There are various methods to identify MRSA isolates. The classical methods are based on the morphology of MRSA colonies and traditional biochemical tests. Molecular methods used to identify MRSA isolates from the chicken carcasses are more accurate, rapid, with final results in 3-5 hours (9). There are several molecular identification methods including polymerase chain reaction (PCR), real-time polymerase chain reaction (RT-PCR), and loop-mediated isothermal amplification (LAMP). In addition, the molecular methods have been used to detect the species-specific *nuc* gene to identify the *S. aureus* organism, and the *mecA* gene to identify MRSA.

The aims of the present study were to isolate *S. aureus* from local processed broiler carcasses and detection by *nuc* gene, to distinguish MRSA isolates by detecting *mecA* gene, and finally to reveal MRSA antimicrobials resistance.

Materials and methods

Samples collection

Fifty broiler skin swabs were randomly collected from different poultry slaughterhouses distributed in various districts of Nineveh Governorate during January till April 2020. All swabs were placed in the icebox and immediately transported to the Public Health Laboratory, Department of Veterinary Public Health, College of Veterinary medicine. All swabs were incubated overnight at 37ºC in Nutrient enrichment broth (Lab M/United Kingdom). Then one loop of each sample was streaked on Blood agar plates (Lab M/United Kingdom) (nutrient agar 13 g/L containing 5% citrated sheep blood) and Mannitol salt agar (Lab M /United Kingdom), and were incubated aerobically overnight at 37ºC.

Identification of bacterial isolates

Identification of *S. aureus* isolates was based on Gram – staining, cell microscopic morphology and biochemical tests including the fermentation of mannitol using mannitol salt agar (Lab M Limited), types of hemolysis on blood agar (Lab M Limited), catalase activity, and coagulase test (using rabbit plasma).

Antimicrobial susceptibility test

The test was carried out by using three antibiotics: Ampicillin/Sulbactam (SAM 20), Methicillin (ME 10) and Ampicillin/Cloxacillin (APX 30) (Bioanalyse Company) by Adoption the Modified Kirby-Baure Method (10). Three to five purified colonies of *S. aureus* were transferred to 5 ml tubes of Nutrient Broth 23g/l (Neogen Company, UK) then incubated overnight at 37°C. Sterile cotton swab was dipped in each Nutrient Broth (containing 0.5 Macferlan concentrations) and the excess was removed by pressing the sides of the tube. Cotton swabs were then spread on the surface of Mueller-Hinton Agar 38g/l (Oxoid). After that antibiotic disks were applied to the medium using sterile forceps and left for dryness. Plates were incubated overnight at 37°C for assessing the dimeters of inhibitions to bacterial isolates.

DNA extraction and template preparation

According to the biochemical tests which applied to the suspected *S. aureus* isolates, the suspected colonies of *S. aureus* were cultivated on sheep blood agar. Based on the instructions of the manufacturer using the protocol for G^+-bacteria, Extraction of DNA for the isolates of *S. aureus* was done, using the DNeasy Blood and tissue kit (Geneaid, Biotech Ltd., Registration No. QAIC/TW/50077-, Korea). The number of *S. aureus* colonies used in this protocol were three to five colonies which were freshly cultured bacteria. All the freshly colonies were added to the 1.5 ml Eppendorf tube which contain 200 µl of the RBC lysis and incubated in the water bath for overnight at 60°C. After that, the suspension was mixed well by vortexing for 1-2 minutes. 200 µl of FABG buffer was added to each sample. Then, All the samples were vortexing for 1-2 minutes. Add 200 µl of ethanol was followed to each sample. Then, all the mixture was posed in the DNeasy Mini spin column and centrifuged at 6200 × g for 1 minute. Washing all the DNA in the spin column was carried out by adding 400 µl of AW1 buffer and centrifuged at 6200 × g for 1 minute. This step was followed by the addition of AW2 buffer in the spin column (600 µl), centrifuged at 6200 × g for 1 minute. The column spin was placed in a 1.5 ml Microcentrifuge tube. Finally, 100 µl of Elution buffer was added for harvesting the DNA. The harvested DNA was measured to estimate the concentration of DNA by using Biodrop (United Kingdom) and stored at -20°C until further use.

Amplification of the *nuc* and *mecA* Gene

The existence of the *nuc* and *mecA* gene was investigated for the identification of Methicillin-resistant *S. aureus* using the PCR assay. Amplification of the *nuc* and *mecA* gene was done using the forward and reverse primers (Table 1). The total volume mixture of PCR reaction was 25 µl. All components of the PCR reaction were placed in the PCR reaction tube (Biozym, oldenhorf, Germany). The present study used the *nuc* primer with molecular weight of 166 bp, while *mecA* primer is 533. The amount of 2×Go Taq Green Mix Master used in this reaction which including (1 unit GoldStar DNA polymerase, 400 µM dNTPs, 3 µM MgCl2, 20 µM (NH4) 2SO4, 75 µM Tris-HCl (pH 8.5), yellow and blue dyes which function as loading dye (Promega Corporation 2800 Woods Hollow Road Madison, WI 53711-5399 U.S.A.) was 12.5 µl, while the amount of nuclease-free water (Promega) was 8 µl. One µL of each forward primer and reverse primer were added (each 10 pmol/µL), (Eurofins Genomics, Ebersberg, Germany). Finally, 2.5 µl DNA template of *S. aureus* was added to each reaction tube. The PCR products were electrophoresed together with the DNA marker 100 bp ladder in 2% agarose gel (Peqlab, Erlangen, Germany).

Table 1: Oligonucleotide primers and PCR programs for amplification of *nuc* and *mecA* genes of *S. aureus*

Gene	Primer	Sequence (5- 3)	Amplicon Size [bp]	PCR Programme*	Ref.
nuc	nuc-1	5-CCTGAAGCAAGTGCATTTACGA-3	166	I	(11)
	nuc-2	5-CTTTAGCCAA GCCTTGACGAACT-3			
mecA	MEC A-1	5-AAAATCGATGGTAAAGGTTGGC-3	533	II	(12)
	MEC A-2	5-AG TTCTGCAGTACCGGATTTGC-3			

*PCR program: I: 35 times (94°C – 30s, 55°C – 30s, 72°C – 30s), II: 35 times (94°C – 30s, 54°C – 30s, 72°C – 30s)

Results

S. aureus was isolated from 33 samples out of 50 skin broiler's carcasses 66%. The phenotypic characterizations of *S. aureus* have appeared that the positive isolates were given the Gram-positive, catalase-positive, and coagulase-positive. In addition, the morphology of the positive isolates was round, golden-yellow colonies on mannitol salt agar and producing β-hemolysis on the blood agar. Furthermore, PCR results declared that all the *S. aureus* isolates possessed the *nuc* gene (Figure 1). In addition, PCR method showed that *mecA* gene in MRSA isolates had found in 20 broiler skin swab samples out of 50 total samples 40% (Figure 2). All MRSA isolates were resistant to the antibiotic methicillin, ampicillin-cloxacillin, and ampicillin-sulbactam (Figure 3).

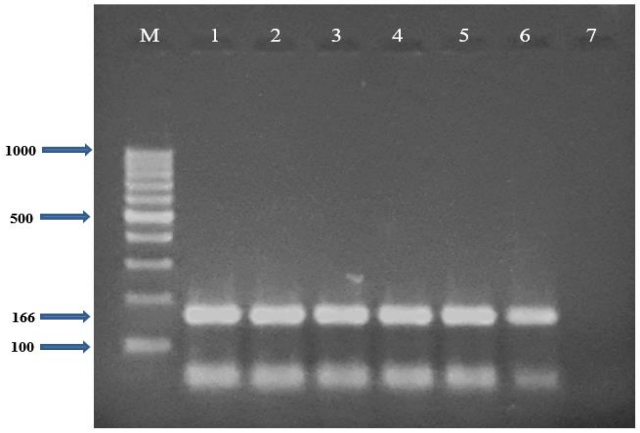

Figure 1: Identification of *nuc* gene (166 bp) in *S. aureus*. Isolates by using PCR technique.

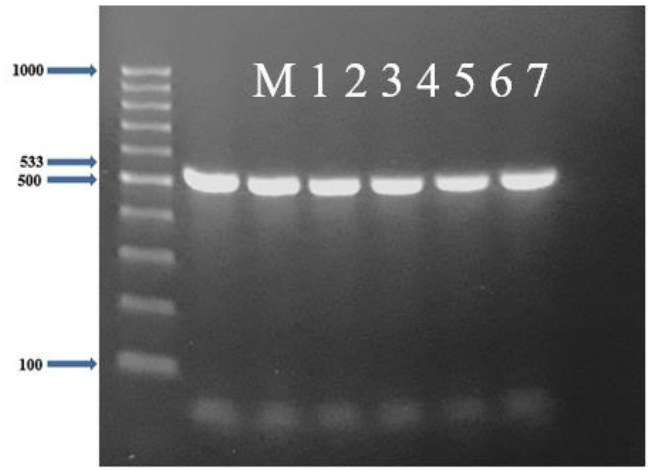

Figure 3: Identification of *mecA* gene (533 bp) in MRSA by using PCR technique.

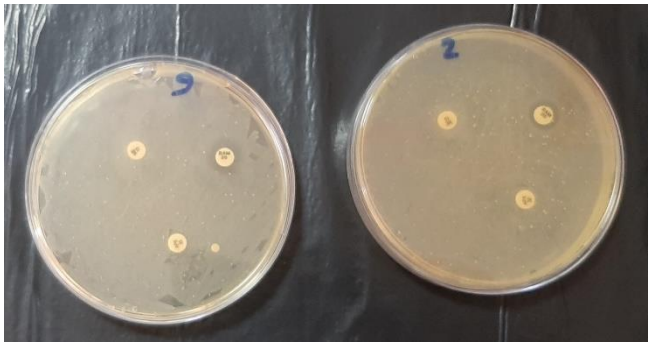

Figure 3: Antimicrobial susceptibility test of *S. aureus* resistant isolates to the β-lactam antibiotics.

Discussion

The present study was conducted to identify the distribution of MRSA among *S. aureus* isolated from broiler skin through detection of *mecA* gene in MRSA isolates, since MRSA isolates are regarded as one of the potential threats to consumer health like endocarditis. The percentage of *S. aureus* in broiler carcasses was 66% (33/50). These findings are the agreement with Kitai *et al*. (13), Buyukangaz *et al*. (14) who recorded 65.8, and 67.6% of *S. aureus* in the chicken in broiler carcass respectively, but was higher than Bounar-Kechih *et al*. (15), Marek *et al*. (16), and Igbinosa *et al*. (17) findings, who showed that the percentages of *S. aureus* in chicken carcasses were 12, 28.2, and 60%, respectively. In another side, the results of our study were lower than those obtained by Thompson *et al*. (18), Krupa *et al.* (19) who recorded the prevalence of *S. aureus* in the chicken carcasses of 97.9, and 93%, respectively. The difference in the isolation rate of *S. aureus* in the other previous studies could be attributed to exposure of broiler carcasses to several points of contamination beginning from the farms ending to the kitchen. In the farms, broilers may be infected with *S. aureus* by farmworkers which play an essential role in transmitting the pathogenic bacteria during the breeding or by transporting the broilers to the slaughterhouses. In addition, the contamination of broiler meat by pathogenic microorganism occurs during the processing of poultry in the slaughterhouses (scalding, plucking, and evisceration), as well as the broiler carcasses may exposed to cross-contamination by using unsanitary water and equipment that may increase the opportunity for contamination by these bacteria (20). Moreover, employers, and instruments which used for cutting poultry carcasses playing a significant role in the contamination of carcasses and its products by direct contact.

Based on the PCR assay, MRSA possesses the *mecA* gene which is responsible for resistant *S. aureus* to antibiotics. Our findings were higher than those found by abdalrahman *et al.* (21), and Igbinosa *et al.*, (16) who showed prevalence of 1.8%, and 20%, respectively, but lower than was reported by Bounar-Kechih *et al*. (14) in chicken carcasses of 50%. While many other studies did not isolate methicillin-resistant *S. aureus* from the poultry carcasses (22- 24). The various rates of MRSA isolated from the broiler carcasses could be related to the excessive usage of antibiotics in poultry as feed additives or growth promoters.

The use of sterilization and cleaning methods in processing plants, could reduce the microbial load added during handling and packaging steps, which play a crucial role in spreading of MRSA isolates in broiler carcasses (25). Retail meat contamination with MRSA is considered as an important vehicle for transmission MRSA to human being (26). It is interesting to note that MRSA isolated from human and poultry have genetic similarity that means broiler carcasses get contaminated through poor human sanitary conditions of slaughtering process.

In addition, MRSA isolates in the present study were resistant to all types of β-lactam antibiotics, which was in agreement with other studies (14). In recent years, the resistance of MRSA to β-lactam antibiotics had increased. The misuse of antibiotics in growth promotion or treatment of poultry and livestock lead to increase the resistance MRSA to antibiotics.

Conclusion

In conclusion, *S. aureus* was isolated from broiler carcasses, with prevalence of MRSA harboring carcasses a threat agent to consumer health. The results highlight the importance of applying HACCP program from poultry farms to the slaughterhouses.

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Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

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الكشف عن المكورات العنقودية الذهبية المقاومة للميثيسيلين من ذبائح فروج اللحم في مدينة الموصل

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الخلاصة

تعتبر المكورات العنقودية الذهبية واحدة من اهم مسببات الأمراض الرئيسية في الإنسان والحيوان. يمكن أن تنتج بكتيريا المكورات العنقودية الذهبية العديد من السموم التي عادة ما تكون متورطة في التسمم الغذائي. يمكن أن تمتلك المكورات العنقودية الذهبية جين mecA، وهو السبب الرئيسي لمقاومة المضادات الحيوية بيتا-لكتم، خاصة المكورات العنقودية الذهبية المقاومة للميثيسيلين. لحم الدجاج اللاحم طعام صالح للإنسان، ولكنه قد يتعرض للتلوث بـ المكورات العنقودية الذهبية المقاومة للميثيسيلين أثناء عمليات الدواجن في المسلخ. هدفت الدراسة الحالية إلى تقييم انتشار بكتيريا المكورات العنقودية الذهبية والمكورات العنقودية الذهبية المقاومة للميثيسيلين في ذبائح التسمين عن طريق الكشف عن الجين nuc و mecA ومقاومتها للمضادات الحيوية المختلفة. تم أخذ خمسون مسحة جلديّة من جثث الفروج أثناء معالجتها في مسالخ الدواجن المنتشرة في مناطق مختلفة في محافظة نينوى خلال الفترة من يناير إلى أبريل ٢٠٢٠. وأظهرت النتائج أنه تم العثور على المكورات العنقودية الذهبية في مسحات جلد الفروج، في نسبة ٦٦٪ (٥٠/٣٣) والتي تم تأكيدها بواسطة جين nuc، بينما شكل عزلات المكورات العنقودية الذهبية المقاومة للميثيسيلين ٤٠% (٥٠/٢٠) من جميع عزلات المكورات العنقودية الذهبية، وتم تحديدها المكورات العنقودية الذهبية المقاومة للميثيسيلين من خلال امتلاكها لجين mecA. جميع عزلات المكورات العنقودية الذهبية المقاومة للميثيسيلين كانت مقاومة للمضادات الحيوية أمبيسلين / سولباكتام وميثيسيلين وأمبيسلين / كلوكساسيللين. شددت الدراسة الحالية على الحد من أي مصدر محتمل للتلوث الناجم عن ذبائح دجاج التسمين بما في ذلك الجرثومة العنقودية الذهبية المقاومة للميثيسيلين خلال وبعد معالجة الدواجن، من خلال تطبيق مستويات عالية من الشروط الصحية في جميع أماكن تجهيز الدواجن للوصول إلى مستويات عالية من الاستدامة والصحة العامة المعايير.