Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (2) 10.29079/qjvms.2025.157137.1069

ISSN P: 1818-5746
E: 2313-4429
qjvms.qu@edu.iq

The Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Poultry Farms

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Submitted: February 02, 2025
Revised: March 10, 2025
Accepted: March 26, 2025

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Abstract
The current study aimed to isolate and identify methicillin-resistant Staphylococcus aureus (MRSA) from poultry and study its prevalence in farms in northern Iraq. Two hundred and thirty-four samples were collected from broiler farms and their environment. The samples included swabs from chicken's skin, workers' hands, ventilators, feeders, drinking water, chicken feed, bedding, soil and grass, during the period from September 2024 till December 2024.
All samples were subjected to traditional and molecular methods for detection and identification for S. aureus and MRSA. The results showed that 43.16% of the total samples were positive for S. aureus. The highest isolation rate was from workers' hands and chickens at 53.85% from the total isolates, the lowest isolation rate was from feed and water at 34.61% depending on nuc gene. The results of MRSA isolation showed that 16.24% were positive for chromogenic agar and molecular methods depending on mecA gene, the highest rat was recorded from Bedding and Chicken at 34.61% and 26.92% respectively, and the lowest percentage of isolation was from Grass, Soil and Chicken feed at 3.85% and 7.69% respectively. The presence of MRSA emphasizes the need to implement continuous monitoring and surveillance programs and studies and collect data from other environments of livestock of all kinds, society and hospitals in order to develop insights into the relationship between human and animal strains, which is important for controlling and combating the microbe.
Keyword: Staphylococcus aureus, methicillin-resistant Staphylococcus aureus,

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Introduction

Staphylococcus aureus (S. aureus) is one of the most important pathogens that threaten human life due to the high rates of infection and mortality it causes, despite its symbiotic presence with living organisms (1,2). The bacterium is highly tolerant to physiochemical environmental conditions, as it can survive in the open air for several days to weeks, Spreading for long distances, and is tolerant of drought (3,4). It also has the ability to remain alive for several weeks in dry pus (5). It can withstand a temperature of (60) °C for (30) minutes, but it dies after (60) minutes at the same temperature (6).

One of the most important sources of persistent infection with S. aureus is domesticated animals, which scientists have classified as reservoirs for the bacteria, in addition to being a potential source of infection for other animals and humans through its colonization and continuous presence on the mucous membranes of the nose, udder, teats and vagina, of various host species such as birds and humans (7,8,9). Skin and tonsils of pigs, chickens, and turkeys often harbor bacteria and are potential sources of S. aureus contamination (10), it can adapt rapidly even during selective antimicrobial pressure, which has led to the emergence of methicillin-resistant S. aureus (MRSA). Resistance to methicillin and other β-lactam antibiotics is due to the presence of the mecA gene, located in a

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mobile genetic element called the staphylococcal chromosomal cassette (SCCmec) (11).

The presence of MRSA in poultry and poultry products poses a significant concern for the poultry industry due to its potential impact on human health. The primary risk arises from the production of staphylococcal enterotoxins by MRSA strains, which can lead to staphylococcal foodborne diseases and contribute significantly to the global antibiotic resistance crisis (12,13).

It's found in the surrounding environment such as water, soil and air. It has been isolated from foods of animal origin, and is a major cause of many cases of food poisoning around the world (14,15).

The investigation of the risk of human infection following contact with animals or their products, thereof, is still neglected in Iraq. The widespread, unrestricted use of antimicrobial compounds in food-producing animals has led to the emergence of MDR, making control and eradication of MRSA difficult. MRSA strains have been isolated from several types of food products, Including poultry and its products (16).

The most important sources of contamination in poultry farms with microbes are: workers, human waste, drinking water, feed, tools used in the field, rodents and hatcheries (17,18), In addition to the environment in which poultry are raised, such as water, soil, bedding, feces, waste, sick and dead birds, eggs, and other poultry products (19,20,21,22,23).

Transmission occurs in several ways, including inhalation of air, consumption of contaminated water and food, direct contact through hands or contact with secretions or contaminated materials and vectors (24,25), several studies conducted on poultry farms have revealed the presence of S. aureus in samples collected from humans, chickens, rodents, poultry litter, and soil surrounding the farm (26,27,28).

S. aureus have been associated with a number of conditions including dermatitis, omphalitis, femoral head necrosis, arthritis, tendinitis, and Bumble foot (26,29,30), many studies conducted on poultry, including live chickens, sick chickens and dead chickens, have proven the presence of S. aureus in large quantities (28,31).

From the above and the lack of studies that evaluate the extent of contamination with methicillin-resistant S. aureus, the current study aimed to isolate and identify MRSA from poultry and study its prevalence in farms in northern Iraq.

Material and Methods

Ethical approval

The approval for conducting the research was obtained from the Institutional Animal Care and Use Committee at the College of Veterinary Medicine at the University of Mosul, No. UM.VET.2024.046, in 9/7/2024.

Study area:

This study included 26 poultry farms from different regions and distributed in three governorates in the northern part of Iraq (Duhok, Nineveh and Erbil) selected randomly, during the period from September 2024 till December 2024.

Sampling:

Two hundred and thirty-four samples were collected from broiler farms and their environment. The samples included cotton swabs from chicken's skin, workers' hands, ventilators, feeders, drinking water, chicken feed, bedding, soil and grass, (26 samples each) placed in sterile tubes or containers containing peptone water. The samples were then delivered immediately to the Scientific Research Laboratory at the College of Veterinary Medicine / University of Mosul for bacteriological analysis.

Isolation of S. aureus

Microbiological methods described by Markey et al., (32), were followed to isolate S. aureus from the different samples, including cultivation on selective Mannitol Salt Agar (MSA) medium prepared by (Himedia®/India), Gram staining and cultivation on 5% sheep blood agar were used to test the hemolytic activity of the isolates, in addition to coagulase and catalase tests. CHROMagarTM (Himedia/India) was also used to identify MRSA (9).

Molecular Identification of S. aureus

Isolation of DNA:

Accurately following microbiological testing, the DNA of S. aureus isolates were extracted and analyzed. The samples were cultivated on MSA and incubated at 37°C for 24 hours. Then DNA was extracted from S.aureus isolates using Qiagen® (Germany) DNeasy Blood and Tissue Kit, according to the instructions. The concentration of extracted DNA was then measured with the Genova Nano (Jenway®/UK) instrument, and properly kept at -20°C.

Polymerase Chain Reaction technique (PCR)

As shown in Table (1), PCR technique was utilized to amplify particular sequences of the nuc, gene for S. aureus and mecA gene for MRSA isolates. A total of 25 µl was used for the PCR reaction mixture containing 12.5 µl of Promega Corporation's (2×) GoTaq Green Mix Master, 1 µl of the forward primer, 1µl of the reverse primer, 6.5 µl of Qiagen® (Germany) DNeasy-free water, and 4 µl of extracted DNA template made up the reaction mixture. The entire mixture was placed in a PCR tube, and the total volume was adjusted to 25 µl. The PCR amplification was performed under specific thermal cycling

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conditions Table (2). These conditions, including denaturation, annealing, and extension temperatures and durations, were tailored to the PCR protocol being used and optimized for the primer set and DNA template under the study. Next, 2% agarose gel electrophoresis Peqlab (Erlangen®/ Germany) was used to visualize the target sequence amplicons. A gel from along with a 100-3000 bp ladder DNA marker. Electrophoresis was carried out to separate and visualize the amplified DNA fragments, which were then compared to the DNA ladder for size estimation

Table 1: Utilizing primers for identification of S. aureus and MRSA (mecA gene)

Gene	Primer	sequence	size (bp)	Reference
nuc	*nuc-F*	GCGATTGATGGTGATACGGTT	279	(33)
	nuc-R	AGCCAAGCCTTGACGAACTAAAGC		
mecA	*mecA-F*	GTGAAGATATACCAAGTGATT	147	(33)
	mecA-R	ATGCGCTATAGATTGAAAGGAT		

Table 2: PCR reaction program for nuc and mec A genes:

N	Steps	Temperature °C	Time min	Number of cycles
1	Initial denaturation	95	10	1
2	Denaturation	95	1	35
3	Annealing	60 (*nuc*) and 52 (*mec A*)	1	
4	Extension	72	1	
5	Final Extension	72	5	1

Results

The results of the initial isolation of S. aureus on MSA showed the growth of medium to large, round, smooth, soft, elevated and shiny yellow colonies with a change in the color of the medium from red to yellow. On blood agar, which caused beta hemolysis. Microscopically, the bacterial colonies were Gram-positive and grape-shaped, arranged in single cells or pairs, or forming irregular coccoid groups resembling grape clusters. On chromogenic medium, the bacterial colonies appeared bluish-green in color.

The isolation rates showed conformity between conventional and molecular techniques. Out of 234 samples examined, 101 samples were positive for isolation, i.e. 43.16% of the total samples included in the study (Workers hand, Chicken, Ventilator, Feeder and Water, Soil, Bedding, Grass and Chicken feed). The highest isolation rate was from workers' hands and chickens at 53.84%, and the lowest isolation rate was from feed and water at 34.61% using molecular techniques after electrophoresis of the amplification products of the nuc gene on agar gel, which were identical to the ladder size at 279 bp (Table 3 and Figure 1).

Table 3: Number and percentage of S. aureus isolates from different samples

n	Type of sample	No. of Samples	No. of +Ve	%
1	Workers hand	26	14	53.84
2	Chicken	26	14	53.84
3	Ventilator	26	12	46.15
4	Feeder	26	9	34.61
5	Water	26	9	34.61
6	Soil	26	12	46.15
7	Bedding	26	10	38.46
8	Grass	26	11	42.30
9	Chicken feed	26	10	38.46
Total		**234**	**101**	**43.16**

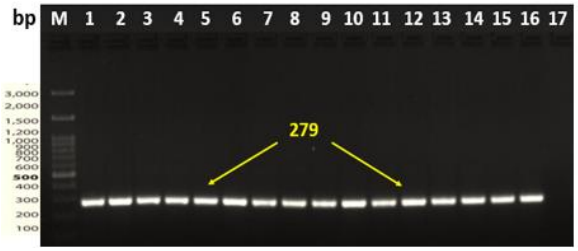

Fig. 1: PCR amplification product for S. aureus isolates for nuc gene at 279bp (M: 100-3000 bp ladder; 1-16: positive sample; 17: negative samples).

The highest prevalence rate of S. aureus was recorded among the samples, from the workers hands and chickens at 5.98%, while the lowest prevalence rate was in the feeder and water samples at 3.85%, as shown in figure (2).

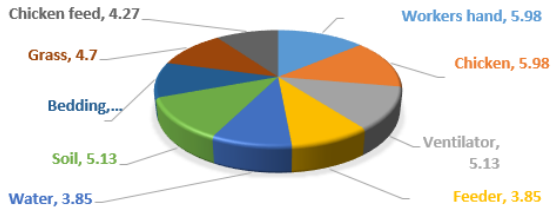

Fig. 2: Prevalence of S. aureus of different studied samples.

The results of MRSA isolation showed that 38 samples out of 234 samples were positive, i.e. 16.23% by chromogenic agar medium and molecular methods by electrophoresis of amplification products for mecA gene at 147 bp on an agarose gel. The highest percentage of MRSA isolation was recorded from Bedding and Chicken at 34.61% and 26.92% respectively, and the lowest percentage of isolation was from Grass and Chicken feed at 3.84% and 7.69% respectively (Table 4 and Figure 3 and 4).

Table (4): Number and percentage of MRSA isolates from different samples

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No.	Type of sample	No. of Samples	No. of +Ve	%
1	Workers hand	26	5	19.23
2	Chicken	26	7	26.92
3	Ventilator	26	3	11.53
4	Feeder	26	5	19.23
5	Water	26	4	15.38
6	Soil	26	2	7.69
7	Bedding	26	9	34.61
8	Grass	26	1	3.84
9	Chicken feed	26	2	7.69
Total		**234**	**38**	**16.23**

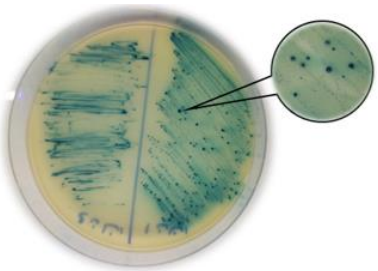

Fig. 3: Growth of methicillin-resistant S.aureus (MRSA) on MRSA chromogenic medium.

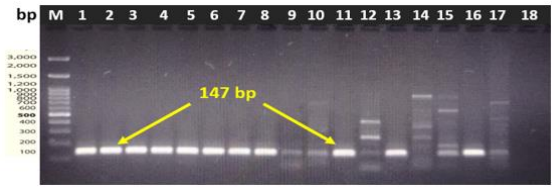

Fig. 4: PCR reaction product of MRSA isolates for the mecA gene at 147 bp (M: 100-3000 bp ladder; 1-8,11,13,16: positive sample;9,10,12,14,15,17: negative samples; 18: control negative)

The highest prevalence rate of MRSA was recorded among the samples of different fields and from different areas, from the bedding and chicken at 23.68% and 18.42% respectively, while the lowest prevalence rate was in the Grass, Soil and Chicken feed samples at 2.63%, 5.27% and 5.27% respectively, as shown in figure (figure 5).

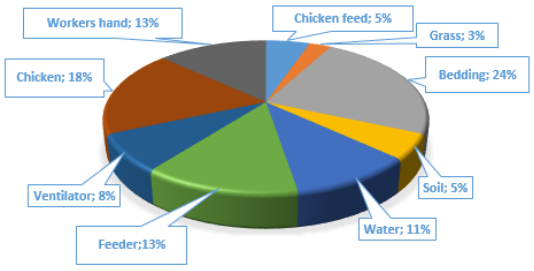

Figure (٥): Prevalence of MRSA of different studied samples

Discussion

Our study aimed to evaluate the level of S. aureus (MRSA) contamination, which is an indicator of the level of management and biosecurity measures followed in poultry farms, which have recently begun to grow and increase in Iraq in general.

In the northern regions of Iraq. Our study revealed a high prevalence of S. aureus and MRSA isolated from chickens, workers' hands, ventilators, feeders, drinking water, chicken feed, bedding, soil and grass.

Our study showed that out of a total of 234 samples, 101 samples, or 43.16%, were positive for Staphylococcus aureus, of which 38 samples, or 16.23%, were MRSA from all samples included in the study. Compared to studies conducted on workers and poultry, the study conducted by Assafi et al., (27) in Duhok province, recorded an isolation rate of 24% and 27.3%, respectively, which is higher than the rates we reached in our current study.

In comparison with studies conducted locally by (AL-Salihi et al., (34), a study conducted in Kirkuk Governorate on poultry workers recorded a rate of 16.6%, which is less than the rate we reached, which was 19.23%. The reason for the difference is likely to be due to the difference in the sample collection area, as the first was from the nose and the second was from the hands. Our results were completely similar to the results reached by (16) in Duhok Governorate, which reached 27.77% when compared to our results, which reached 26.92%. While the isolation results of MRSA from poultry meat varied between 40%, 33.3% and 14.81% in Iran, Wasit and Basra (13,35,36).

Internationally, studies have recorded varying isolation rates of MRSA from poultry including 71.5% in Germany (37). In contrast, the results of the study conducted in Egypt 36% (12), in Iran 67% (2), in addition to recording high levels of MRSA contamination in Austria, Denmark 52% (38) and 43.3% in Korea (39).

We did not observe any studies that addressed the isolation and diagnosis of MRSA from Ventilator, Feeder, Water, Soil, Bedding, Grass and Chicken feed in poultry farms, despite their importance in the occurrence of transmission and contamination (40), However, in an epidemiological study conducted by Hussein et al., (41) to investigate the presence of bacteria in the air inside poultry farms, it was revealed at a rate of 50%, which is a major cause of water and food contamination for workers, etc.

The variation in the isolation rates obtained in our study compared to other studies may be explained by many factors, including the administrative and health practices of poultry farms, breeding methods, the diagnostic methods used, geographical differences, etc., in addition to other factors like the environment in

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ISSN P: 1818-5746
E: 2313-4429
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which poultry are raised, such as water, soil and bedding, feces and waste, sick and dead birds, and other poultry products, this is what is confirmed by Khan et al., (22) and Laban et al., (23).

The possession of the mecA gene by S. aureus, which adds to the bacteria another new mechanism for resistance to antibiotics, especially methicillin, is a criterion for diagnosing MRSA molecularly S. aureus isolates, as it is considered an inevitable criterion for confirming MRSA (42).

The results of the study were based on diagnosis and characterization by molecular methods in order to obtain the best and most accurate and sensitive results. The detection and diagnosis of S. aureus and MRSA bacteria depends on traditional and molecular methods (43).

Direct contact by poultry farm workers with birds during field management operations is an important factor in the transmission of S. aureus from poultry to farm workers and vice versa (27), and therefore, S. aureus isolated from poultry is considered a risk indicator for humans living near poultry farms or dealing with them or with their production chains, as confirmed by the researcher (44,45). This may also occur due to the failure of biosecurity procedures, which include cleaning and sterilization of field components from chicken remains, or through handling birds for therapeutic purposes (41).

Therefore, reverse transmission from workers can also cause contamination and transmission of Staphylococcus aureus to birds and their environment if biosecurity procedures are not applied properly (8,9,27,41,45).

Conclusion

MRSA was isolated from broiler chickens, and its spread in the environment surrounding poultry houses is striking, as it constitutes a dangerous factor and indicator that threatens public health. The results highlight the importance of implementing biosecurity programs and adopting the Hazard Analysis and Critical Control Points (HACCP) system in poultry farms, placing restrictions and monitoring on the use of antibiotics, and applying strict health measures during poultry farming, as these measures are essential to prevent the spread of resistant bacteria and protect public health. Confirming the presence of MRSA emphasizes the need to implement continuous monitoring and surveillance programs and studies and collect data from other environments of livestock of all kinds, society and hospitals in order to develop insights into the relationship between human and animal strains, which is important for controlling and combating the microbe

Acknowledgment

The researchers would like to thank and appreciate the Deanship of the College of Veterinary Medicine / University of Mosul for its support and provision of the research environment. Thanks are also extended to those who contributed to the completion of this work by facilitating the collection of data.

Conflict of interest

The researcher declares that there is no conflict of interest regarding this work.

Funding source

This research had no specific fund; however, it was self-funded by the authors.

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Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (2) 10.29079/qjvms.2025.157137.1069

ISSN P: 1818-5746
E: 2313-4429
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2025;10(2):0-0. proof.
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