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The Relation Between Biosecurity Measures and the Level of *Staphylococcus aureus* Contamination in Poultry Farms in Northern Iraq

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ABSTRACT

Despite receiving great attention to the poultry industry in Iraq, it is still affected by various problems that have led to great economic losses. Including Staphylococcus aureus, which causes invasive diseases such as arthritis and septicemia, as well as food poisoning in humans. This study aimed to assess the levels of biosecurity measures in poultry farms by evaluating the prevalence of *Staphylococcus aureus* contamination. in broiler farms located in different regions of Duhok, Nineveh and Erbil governorates, Iraq. A total of two hundred and thirty-four samples were collected fromTwenty-six poultry farms. The samples included swabs from chickens (skin swab), workers' hands, ventilators, feeders, drinking water, chicken feed, bedding, soil and grass (26 samples each); furthermore, the biosecurity levels in the investigated broiler farms were assessed via response to a descriptive questionnaire during the period from September 2024 to December 2024. The samples were subjected to biochemical and molecular tests, and the questionnaire data were statistically analyzed. *Staphylococcus aureus* was found in 43.16% (101 out of 234) of the samples. The highest isolation rate was from workers' hands, chicken (53.85%), while the lowest was from feeder and water (34.61%). According to this study, there were notable differences in the investigated farms' biosecurity levels with an inverse relation to the occurrence of *S. aureus*. The lack of supporting evidence for the effectiveness of biosecurity measures to reduce the introduction and transmission of *S. aureus* in poultry farms in northern Iraq is a matter of concern and requires further studies on the sources of contamination and the mechanism of its spread and conducting sensitivity tests at regular intervals to determine the development of resistance to the antibiotics used.

Keywords: Biosecurity measures, Iraq, *Staphylococcus aureus*, Poultry farms.

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INTRODUCTION

Recently the poultry industry, especially commercial systems, has received great attention, despite restrictions on food and disease infections, including many pathogens that cause health and economic losses, especially in developing countries, including Iraq. Biosecurity measures are an integral part of any successful poultry production system and refer to the preventive measures taken to control and prevent the introduction and spread of pathogens into flocks, failure of which can lead to disease outbreaks and significant economic losses (**Sharma, 2010**). The key components of biosecurity measures include cleaning, disinfection, isolation, movement control, and monitoring **(Abdelal *et al.*, 2016).**

Staphylococcus aureus (S. aureus) is regarded as a commensal and opportunistic pathogen, which is found in water, soil, air and food; contamination has the ability to be the cause of food poisoning around the world (**Newell *et al.*, 2011**). *S. aureus* is considered one of the most important pathogens that threaten human life due to the high rates of mortality, despite their symbiotic presence with living organisms (**Ramana *et al.*, 2009; Pollitt *et al.*, 2018**). Water, soil, air. and food contamination can be the cause of food poisoning around the world (**Friese *et al.*, 2013; Wang *et al.*, 2018**).

S. aureus was isolated from the intestine, respiratory tract, feathers, and skin in poultry (**Casey *et al.*, 2007; Olayinka *et al.*, 2010**), which is associated with many pathological conditions such as femoral head necrosis, bumblefoot, omphalitis, tenosynovitis, dermatitis, osteomyelitis, arthritis, and synovitis (**Abd El Tawab *et al.*, 2017**).

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 (**Okonko *et al.*, 2010; Begum *et al.*, 2023**),

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 (**Hossain *et al.*, 2008; Igbinosa, 2014; Khan *et al.*, 2014; Laban *et al.*, 2014**).

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 (**Suleiman *et al.*, 2013; Sonola *et al.*, 2023**). S. aureus isolated from poultry is considered a risk indicator for humans and poultry or those who deal with them, whether in the field or through their production chains **(Wertheim *et al.*, 2005).** 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 (**Cuny *et al.*, 2010; Ferreira *et al.*, 2011**). Also, direct contact by poultry farm workers during field management operations is an important factor in the transmission of S. aureus from poultry to farm workers and vice versa (**Assafi *et al.*, 2020**).

Transmission occurs through several pathways, including inhalation of air, consumption of contaminated water and food, direct contact through hands or contact with secretions or contaminated materials and vectors (**Cuny *et al.*, 2010; Ferreira *et al.*, 2011**), this is also due to the failure of cleaning and sterilization operations for the fields and their components and their contamination with chicken remains, or through handling birds for therapeutic purposes, on the other hand, an epidemiological study revealed the presence of bacteria at a rate of 50% in air samples from inside poultry fields, which is a cause of water and food contamination for workers (**Hussein *et al.*, 2015**).

The most important sources of contamination in poultry farms with *S. aureus* are field workers, human waste, drinking water, feed, tools used in the field, rodents and hatcheries (**Begum *et al.*, 2023**), bedding (**Igbinosa, 2014**), feces and waste (**Khan *et al.*, 2014**), sick and dead birds (**Hossain *et al.*, 2008**), eggs and other poultry products (**Laban *et al.*, 2014**) and wild birds (**El-Mahallawy *et al.*, 2022**). On poultry farms, studies have revealed the presence of *S. aureus* in samples collected from humans, chickens, rodents, poultry litter, and soil surrounding the farm (**Assafi *et al.*, 2020**).

Moreover, *S. aureus* was isolated in large quantities from live, sick and dead chickens by **Sonola *et al.*, (2023) and Bakheet *et al.*, (2014),** the cross transmission from workers can also cause contamination and transmission of *S. aureus* to birds and their environment if biosecurity procedures are improper (**Hussein *et al.*, 2015; Ştefan, 2024**). The objective of this study was to assess the relationship between *S. aureus* and biosecurity measures and management in poultry farms in northern Iraq.

MATERIALS 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

The study was conducted on poultry farms in three governorates Duhok, Nineveh and Erbil which are located in northern Iraq between latitudes 34° and 39° and longitudes 37° and 46°, with a semi-tropical and moderate climate. Poultry farming system in Iraq generally depends on the controlled environment house, floor farming, artificial ventilation and lighting with a single age system (all in - all out system).

Biosecurity Survey

A biosecurity questionnaire was created to investigate the application of the pyramidal biosecurity level in poultry farms under investigation. The biosecurity framework was categorized into three main axes, which totally include 22 questions. The first axis is cleaning and disinfection, which focused on the availability of cleaning and disinfection equipment, the availability of barriers and shoe sinks, washing and disinfecting cleaning vehicles, changing clothes when entering and leaving the farm, allowing the disinfectant to come into contact with the sterile material, applying minimal movement and transition, washing and disinfecting all equipment in the farm, cleaning and disinfecting drinking systems, cleaning and disinfecting feeders and feed stores, and around the farm, disposing of dead birds in a healthy way and replacing the mattress.

The second one is isolation, which included the direction of movement from small to large herds, barriers to prevent the access of animals, insect and rodent control programs, new animals entering directly to the farm, and special field vehicles for transporting fodder. The third line is monitoring which focused on the assessment of the risks and challenges, health status of the herd assessed, feed monitored by periodic inspection, water monitored and treated on-site, rodent control programs and display of remaining fodder from previous herds **(Jennison, 2021)**. The form also included information about the area, the number of birds and their ages.

Data collection and management

The questionnaire was pre-selected to ensure that all important issues were identified and covered, and data were collected through field visits to poultry farms conducting a face-to-face interview with farm

owners. In addition, to observe and verify these procedures. The answers of the questionnaire were documented as yes or no for biosecurity procedures and then compared to the ideal biosecurity standard (**Al-Mahmood, 2023**). Data were entered into a Microsoft Excel program designed to retain, store and retrieve field survey data and results during analysis. The percentages of answers were calculated by dividing the number of yes or no / number of criteria adopted in the questionnaire multiplied by 100.

Sample and the sampling procedures

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. Two hundred and thirty-four samples were collected from broiler farms and their environment. Nine samples were collected from each field randomly; the samples included cotton swabs from chickens (skin), workers' hands, ventilators, and feeders placed in sterile tubes containing peptone (Himedia®/India); drinking water from drinkers was collected in sterile containers; and chicken feed, bedding, soil and grass were collected in sterile plastic bags (**Borkar, 2017; MacFaddin, 2000**). The samples were collected in a cooler container and transferred immediately to the Scientific Research Laboratory at the College of Veterinary Medicine / University of Mosul for bacteriological analysis.

Isolation of *S. aureus*

According to **Borkar, (2017)** the swab samples were placed in peptone water medium and incubated for 2 to 3 hours at 37°C and then transferred to Mannitol Salt Agar (MSA) (Himedia®/India) medium. Water samples were treated by taking 50 ml of each sample and placing it in a centrifuge (5000 rpm for 5 minutes), the sediment was taken and added to 2 ml of peptone water and incubated for 2 to 3 hours at 37°C and then transferred to Mannitol Salt Agar (MSA), while chicken feed, bedding, soil and grass samples were treated by placing 10 g of the sample in sterile glass beakers containing 90 ml of sterile phosphate buffered saline (PBS) solution was shaken well for one minute in an electric shaker and left for two hours to separate the bacteria attached to the samples, then the liquid was distributed in sterile test tubes and placed in a centrifuged at 5000 rpm for 5 minutes, after which 100 microliters were taken from the supernatant and transferred to MSA **(MacFaddin, 2000**)

Molecular Identification of *S. aureus*

Extraction of DNA

Accurately following microbiological testing, the DNA of *S. aureus* isolates were extracted and analyzed. The samples were first cultivated on Mannitol Salt agar and incubated at 37°C for 24 hours. 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* 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, 1 µl 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 conditions. 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 along with a 100-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: The used primers for testing of *S. aureus*

Gene	Primer	Primer sequence (5'-3')	Product size (bp)	Reference
nuc	*nuc-F*	GCGATTGATGGTGATACGGTT	279	**Rahman *et al.*, (2018)**
	nuc-R	AGCCAAGCCTTGACGAACTAAAGC		

PCR program: A: 35 times (94°C for 45s, 55°C for 60s, 72°C for 60s).

Statistical Analysis

Pearson correlation test was used to find the relationship between the percentages of the applied biosecurity measures studied and the percentage of isolation obtained from the different samples in the different study areas at $P < 0.05$ (**Thrane, 2024**).

RESULTS

The results of *S. aureus* isolation showed that out of 234 (workers hand, chicken, ventilator, feeder and water, soil, bedding, grass and chicken feed), 101 samples were positive for *S. aureus* isolation at a rate of 43.16%. The highest isolation rate was from workers' hand and chicken skin at 53.85%, while the lowest isolation rate was from feeder and water at 34.61%, as shown in **table 2 and Fig.1.**

Table 2: Prevalence *S. aureus* isolates from different sources collected from poultry farms in northern Iraq.

N	Source of sample	No. of Samples	No. of +Ve	%
1	Worker's hand	26	14	53.85
2	Chicken (skin)	26	14	53.85
3	Ventilator	26	12	46.15
4	Feeder	26	9	34.61
5	Water (drinker's)	26	9	34.61
6	Soil	26	12	46.15
7	Bedding	26	10	38.46
8	Grass	26	11	42.31
9	Chicken feed	26	10	38.46
		234	101	43.16

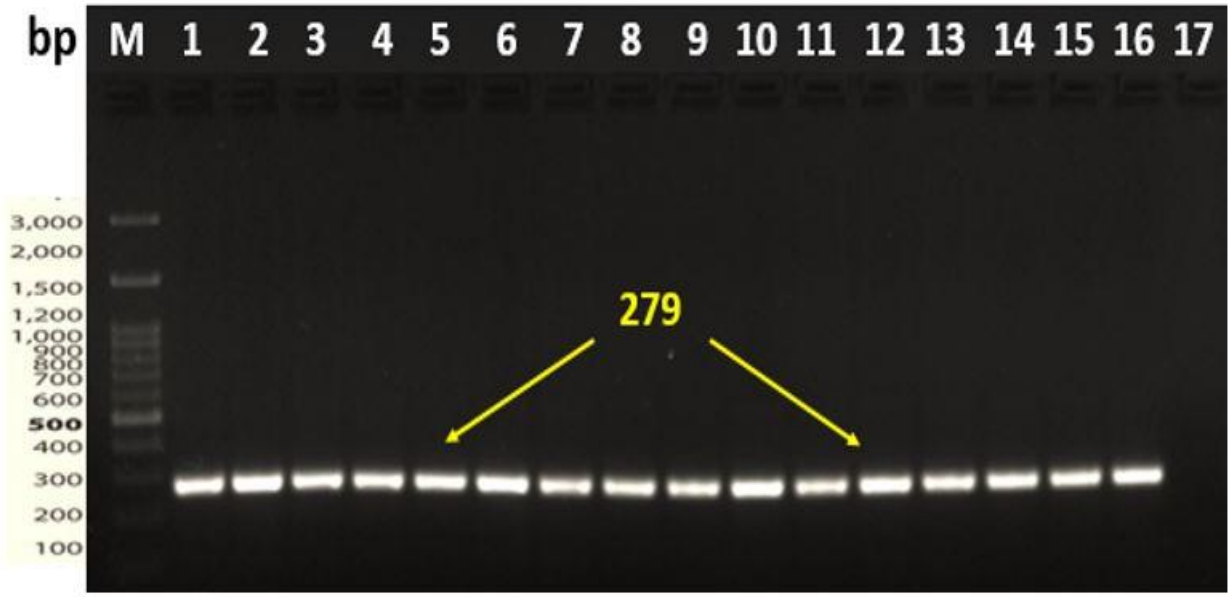

Fig 1: PCR amplification product for *S. aureus* isolates for *nuc* gene at 279bp.

The results of the questionnaire were included in order to investigate the biosecurity procedures for 22 measures for 26 fields, where the application of 19, i.e., 86.36% of the measures, was documented in field No. 6 at the age of 7 days, in which no isolation rate was recorded; on the contrary, in field No. 22, which was 40 days old, the application of 5 measures was documented at a rate of 22.73% and a 100% isolation rate was recorded in the studied samples.

The procedure of movement direction from small to large herds was the least applied biosecurity procedure, as it was applied in 3% of the studied farms which is considered a very bad biosecurity score (BS), and on the contrary, all measures were taken before introducing animals brought from markets to the fields; this procedure was applied in 100% of the studied fields which is considered a good BS, as shown in **Table 3.**

Table 3: The assumed score of biosecurity measures in the examined poultry farms.

N	Type of biosecurity measures (category)	No. of farms	Apply No. (%)	Not Apply No. (%)
1	Availability of cleaning and disinfection Equipment	26	14(53.85)	12(46.15)
2	Availability of barriers and shoe sinks	26	14(53.85)	12(46.15)
3	Wash and disinfect vehicles	26	10(38.46)	16(61.54)
4	Changing the clothes when entering and leaving farm	26	12(46.15)	14(53.85)
5	Allow the disinfectant to come into contact with the sterile material	26	7(26.92)	19(73.08)
6	Apply minimal movement and transition	26	14(53.85)	12(46.15)
7	Wash and disinfect all equipment in the farm	26	17(65.38)	9(34.62)
8	Cleaning and disinfecting drinking systems	26	17(65.38)	9(34.62)
9	Cleaning and disinfecting feeders and feed stores and around the farm	26	15(57.69)	11(42.31)
10	Dispose of dead birds in a healthy way	26	19(73.08)	7(26.92)
11	Mattress replaced	26	25(96.15)	1(3.85)
12	Direction of movement from small to large herds	26	3(11.54)	23(88.46)
13	Barriers to prevent the access of animals	26	13(50.00)	13(50.00)
14	Insect and rodent control programs	26	19(73.08)	7(26.92)
15	New animals inter directly to the farm	26	26(100.00)	0(00.00)
16	Special field vehicles for transporting fodder	26	24(92.31)	2(7.69)
17	Monitoring is based on an assessment of the risks and challenges	26	20(76.92)	6(23.08)
18	Health status of the herd assessed	26	18(69.23)	8(30.77)
19	Feed monitored by periodic inspection	26	5(19.23)	21(80.77)
20	Water monitored and treated on site	26	7(26.92)	19(73.08)
21	Rodent control programs	26	17(65.38)	9(34.62)
22	Disposed of remaining fodder from previous herds	26	25(96.15)	1(3.85)

In poultry farm No. 6, implemented 19 of 22 biosecurity requirements, accounting (86.36%) of the biosecurity scores, with a 0% isolation rate. In contrast, farm No. 22, implemented 5/22 (22.73%) of the biosecurity requirements, compared to a 100% isolation rate. The relationship was significant at ($P < 0.01$), as shown in **table 3 and Fig.3.**

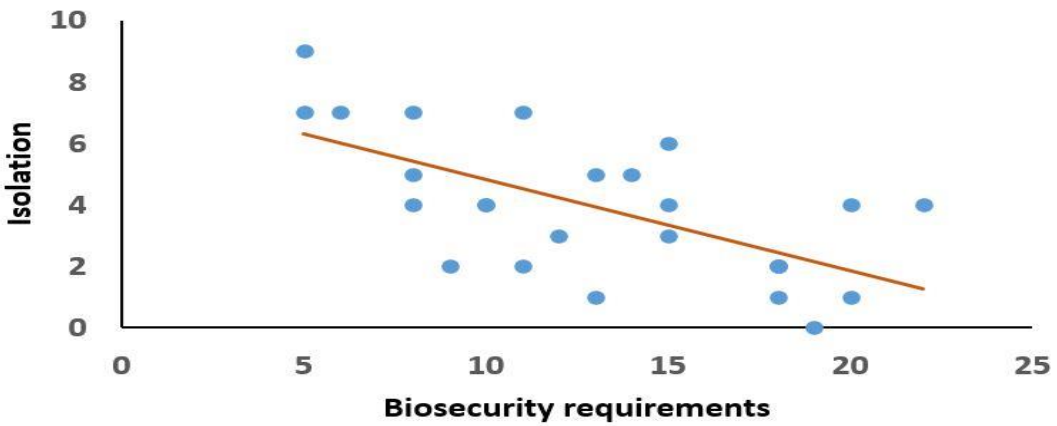

Fig 3: Correlation between applied biosecurity measures and *S. aureus* isolation among different samples in studied farms from different areas covered by the study. Pearson's Correlation (r = -0.6425, P-value < 0.01).

The impact of age of the chicken flocks on the biosecurity measures and the isolation rates was clarified in **table 4.** The older the flock, the less biosecurity measures were applied, and this was reflected in the isolation rate. Data obtained from the Pearson correlation test showed an inverse relationship between the application of biosecurity requirements and the isolation rates of S. aureus in this investigation, as the relationship revealed an increase in contamination rates with a decrease in the application of biosecurity. The same effect was found between the isolation *S. aureus* and the age of the birds at ($P < 0.01$), as shown in **table 3 and Fig.4.**

Table 4: Correlation between of biosecurity level, *S. aureus* and age of flocks in the studied poultry farms.

N	Biosecurity procedures (measures)			*S. aureus* isolates from different sources		Age (day)
	No. of category	Apply No. (%)	Not apply No. (%)	No. of sample	No. of positive (%)	
1	22	6(27.27)	16(72.73)	9	7(77.77)	36
2	22	11(50.00)	11(50.00)	9	7(77.77)	36
3	22	18(81.81)	4(18.19)	9	2(22.22)	15
4	22	18(81.81)	4(18.19)	9	2(22.22)	18
5	22	18(81.81)	4(18.19)	9	1(11.11)	11
6	22	19(86.36)	3(13.64)	9	0(00.00)	7
7	22	15(68.18)	7(31.82)	9	3(33.33)	28
8	22	22(100.00)	0(00.00)	9	1(11.11)	15
9	22	20(90.90)	2(10.10)	9	4(44.44)	32
10	22	20(90.90)	2(10.10)	9	1(11.11)	15
11	22	18(81.81)	4(18.19)	9	2(22.22)	22
12	22	14(63.64)	8(36.36)	9	5(55.55)	34
13	22	11(50.00)	11(50.00)	9	2(22.22)	22
14	22	9(40.91)	13(59.09)	9	2(22.22)	23
15	22	13(59.10)	9(40.90)	9	5(55.55)	35
16	22	15(68.18)	7(31.82)	9	4(44.44)	33
17	22	8(36.36)	14(63.64)	9	7(77.77)	35
18	22	12(54.55)	10(45.45)	9	3(33.33)	25
19	22	10(45.45)	12(54.54)	9	4(44.44)	32
20	22	10(45.45)	12(54.54)	9	4(44.44)	28
21	22	8(36.36)	14(63.64)	9	5(55.55)	34
22	22	5(22.73)	17(77.72)	9	9(100)	40
23	22	15(68.18)	7(31.82)	9	6(66.66)	36
24	22	8(36.36)	14(63.64)	9	4(44.44)	35
25	22	5(22.73)	17(77.27)	9	7(77.77)	35
26	22	13(59.10)	9(40.90)	9	1(11.11)	15

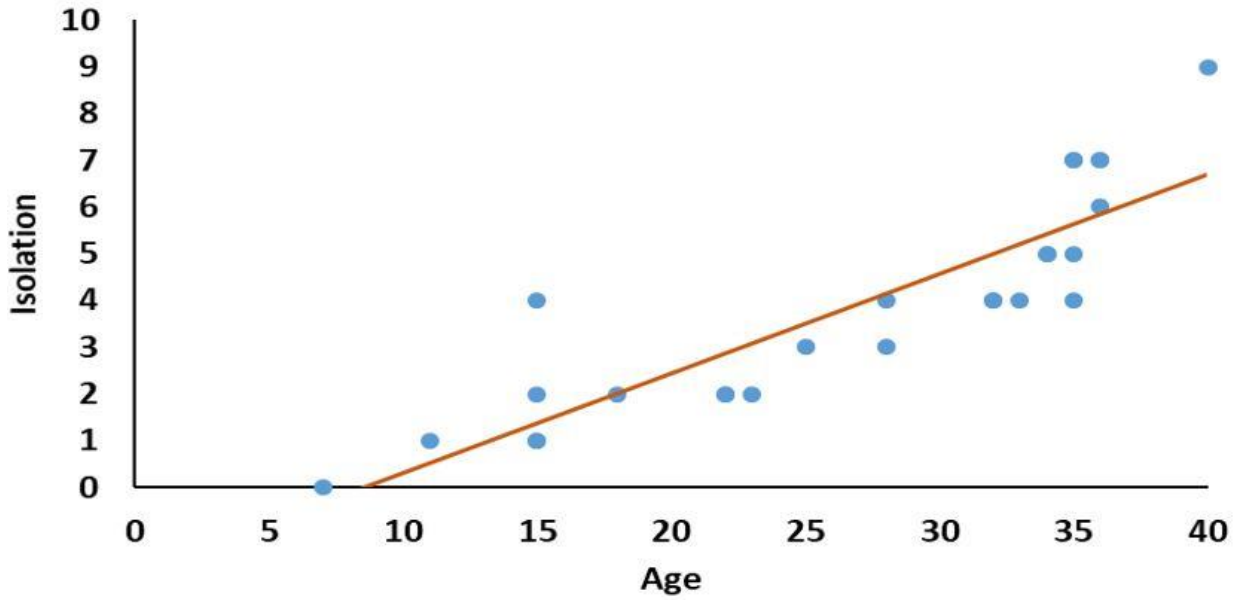

Fig 4: Correlation between *S. aureus* isolation and age in studied farms from different areas covered by the study. Pearson's Correlation (r = 0.8766, P-value < 0.01).

DISCUSSION

Staphylococcus is one of the most important pathogens in poultry pressing.chain, but few studies have addressed these pathogens in the poultry environment. Our study aimed to evaluate the level of S. aureus contamination and the level of management and biosecurity measures in poultry farms, which have recently been increasing in the northern region of Iraq. The isolation rate of S. aureus in our study was lower than the rates reported by Almousawi and Alhatami, (2020), who recorded 37.7% from chickens in Babylon, and Abdulrahman, (2020) who reported 28% in Duhok.

In another study similar to ours, conducted on six poultry farms in Ethiopia on chickens, workers, bedding and water to investigate S. aureus, the results were lower than our results, with the rates being 17%, 15%, 4.7% and 7%, respectively **(Abunna *et al.*, 2020).**

The variation in the isolation rates obtained in our study compared to other studies may be due to 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 which poultry are raised, such as Insulation level of the sheds, water, soil and the disposal method of bedding, feces and waste, sick and dead birds, and other poultry products, this is what is confirmed by **Hossain *et al.*, (2008); Igbinosa, (2014); Khan *et al.*, (2014); Laban *et al.*, (2014)**.

The study's results supported the identification and characterization by molecular methods, which are effective and sensitive, to obtain the best and most accurate results **(Taha *et al.*, 2025)**. Applying biosecurity standards and procedures to limit pathogens and avoid over-prescribing antibiotics can be the basic step in preserving livestock, reducing the spread of infectious diseases, and improving their health and productivity (**Kirtonia *et al.*, 2021**). Thus, the effectiveness of traditional antimicrobials will be maintained, as will sterilizers and bactericides (**Butucel *et al.*, 2022**). In northern Iraq, we have not observed any studies or data on linking biosecurity applications to levels of contamination with microorganisms, especially *S. aureus*, in poultry farms and their surrounding environment; therefore, we decided to study this topic because of its great importance in the poultry industry and human health.

The study revealed a difference in the application of biosecurity requirements in the poultry farms covered by the study. The reasons for this are due to the unwillingness of breeders to apply some requirements or procedures as they believe that they may add a financial burden to them under the fluctuations in the poultry markets. The other reason is also due to the lack of application or loss of application with the advancement of the birds' age. The older birds in the poultry farms, the less we find in the application of biosecurity procedures or requirements or their lack of strict application. This was clear from the results we reached. In returning to Table No. 3, we find that the isolation rate in Field No. 6 at the age of 7 days was 0%, as 19 requirements were applied out of a total of 22 requirements, i.e., 86.36% of the biosecurity requirements, which is considered good BS. On the contrary, in Field No. 22, the isolation rate was 100%, while applying 5 requirements out of a total of 22, i.e.,

22.73% of what is required, which is considered bad BS. This will lead to air pollution, which will be considered a reservoir for microscopic organisms and lead to pollution. Field components with pathogens, including S. aureus, cast their shadows on the health and productivity of poultry due to the increasing concentrations of their numbers with age; this is what **Vučemilo *et al.*, (2010)** indicated.

In a recent study conducted on poultry farms in Duhok governorate, northern Iraq, to verify contamination with *S. aureus* between workers and chickens by researchers **(Hado and Assafi 2021**) who confirmed the role played by chickens in exposing workers to *S. aureus* through direct contact, in addition to contaminating the rest of the field components. This was confirmed by the results of our study in recording the highest isolation rate from chickens and workers' hands, at a rate of 53.85%. It also played a role in the contamination of the air ventilator and the external environment of the breeding halls, including grass and soil.

The results of our study also showed that workers in most fields moved randomly between poultry breeding halls, while biosecurity rules require movement from young age halls to old ones and not vice versa, as this was applied in 3 fields out of 26 of the fields included in the study. It is believed that it is also a contributing factor to the occurrence of *S. aureus* contamination (**Abdelal *et al.*, 2016**).

All the poultry farms included in the study share the same breeding system, which is a floor breeding system, as the litter plays a role in preserving and transferring microorganisms by providing the appropriate environmental conditions (**Bolan *et al.*, 2010; Khalafalla *et al.*, 2019**). It is also worth noting that most of the places where poultry farms are located are in environments that lack infrastructure and sanitation; there is certainly pollution from sewage water and wells, in addition to the transmission of bacteria between poultry and other animals and workers through waste or when carried in the air or dust, which may be a reason for the high isolation rates in the samples under study, and this is what is confirmed by **Rahma and Jwher, (2024)**.

A study of the relationship between the application of biosecurity measures and isolation rates indicates a close connection between them. The study showed an increase in the isolation rate with a decrease in the level of biosecurity applications; this is what previous studies have supported. (**Laban *et al.*, 2014**).

CONCLUSION

The current study confirms that *S. aureus* is one of the organisms widely spread in poultry farms and their environment. Poultry and workers play a major role in the transmission and spread of bacteria, in addition to mismanagement. The study also indicates the importance of biosecurity measures to control and prevent the entry of pathogens into production chains and contribute to the evidence-based decision-making process to adopt certain standards and concepts for biosecurity. The lack of supporting evidence for the effectiveness of biosecurity measures followed in poultry farms in northern Iraq is a matter of concern and requires further studies on the sources of *staphylococcal* contamination and the mechanism of its spread and conducting sensitivity tests at regular intervals to determine the development of resistance to the antibiotics used.

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Conflict of interest

The authors declare that there is no conflict of interest

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