Original Article

Molecular Investigation of Some Virulence Factors of *Staphylococcus aureus* Isolated from Local Chickens with Bumblefoot

Ezzalden Burhan Ghaib, Sumaya Yaseen A. Al-Dabbagh
Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

Background: *Staphylococcus aureus* is considered a commensal microorganism on the skin of humans and animals. The study aimed to isolate of *S. aureus* from chickens that suffered from bumblefoot and molecular diagnosis using the *nuc* and *coa* genes. **Materials and Methods:** One hundred swabs were collected from chickens with bumblefoot across different regions of Mosul city between September 2025 and January 2026. The samples were transferred directly under cooled conditions to the microbiology lab in the College of Veterinary Medicine for microbiological examination. The swabs were streaked on MSA medium and then incubated at 37°C for 24 h. Suspected isolates were cultured on blood agar for detecting the type of haemolysis. Catalase and coagulase tests were used to confirm isolates. Polymerase chain assay was done for the detection of *S. aureus* using the thermonuclease gene (*nuc*) and the virulence gene *coa*. **Results:** The study showed that *S. aureus* formed 68/100 (68%) from chickens with bumblefoot disease, followed by *Staphylococcus hyicus* 12/100 (12%), whereas *Staphylococcus intermedius* formed 4/100 (4%). The results of molecular detection showed that all tested isolates possessed the (*nuc,* and *coa*) genes that gave amplicon products at (166 bp and 304 bp), respectively. **Conclusion:** *S. aureus* plays a significant role in the development of bumblefoot disease in chickens, as evidenced by its high isolation rate and its possession of key virulence factors, including haemolysins and coagulase.

Keywords: Bumblefoot, *coa* genes, haemolysin, *Staphylococcus aureus*

Introduction

The poultry industry is affected by many diseases, including bacterial infections that affect various body systems, including the musculoskeletal system (1). Staphylococci are the primary cause of this infection, producing a yellowish, caseous pus that turns black in advanced cases. *Staphylococcus aureus* was the most frequently isolated pathogen from bumblefoot cases (2). The infection begins after the entry of *S. aureus* into the toes and pads of the chicken feet through cuts, scratches or injuries and creates an abscess full of pus (3). Staphylococci are opportunistic microorganisms that may cause severe infections in both humans and animals under certain conditions. *S. aureus* strains are non-motile, Gram-positive cocci, facultative anaerobic and nonspore-forming bacteria that can easily spread between animals and humans (4,5). *S. aureus* has many virulence genes that aid its pathogenicity, such as the ability to produce haemolysins, coagulase, hyaluronidase and thermonuclease (6). Haemolysins are essential virulence factors that help the bacterium to evade the immune system and acquire iron for survival. Haemolysins participate in infection by killing leucocytes and epithelial cells in host tissue, facilitating bacterial spread. *S. aureus* has alpha and gamma toxins that cause pore-forming and a sphingomyelinase, also known as β-haemolysin, that causes the breakdown of host cells, enabling the bacterium to obtain nutrients and evade the immune system (7,8). *S. aureus* has the ability to cause coagulation of human or rabbit plasma due to the production of the coagulase enzyme. This enzyme is responsible for the conversion of fibrinogen to fibrin and

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Address for correspondence: Prof. Sumaya Yaseen A. Al-Dabbagh, Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.
E-mail: sumayayase18@uomosul.edu.iq

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plays a role as a defense mechanism against bacterial pathogens through immobilisation of bacteria in a clot that hides them from phagocytic cells (9,10). The thermonuclease (*nuc*) gene catalyses the hydrolysis of nucleic acids RNA and DNA at the 5' position of the phosphodiester bond, this gene is known as the *S. aureus*-specific gene. Amplification of the *nuc* gene is used to detect and quantify *S. aureus* (11,12). Due to the bacterium's significance to the chicken industry and its potential for zoonotic transmission (13). Therefore, the aims of the study were to isolate *S. aureus* from infected chickens that suffered from bumblefoot and perform molecular identification using standard genes and detection of some virulence factors.

Materials and Methods

Ethical approval

All samples were taken in accordance with permission granted by the Institutional Animal Care, University of Mosul, College of Veterinary Medicine, under authority number UM.VET.2025.093.

Specimens collection

One hundred samples were taken from the feet of chickens suffering from bumble foot from different regions of Mosul City for the period from September 2025 to January 2026. The soles of the feet were cleaned and disinfected with 70% alcohol, then a small incision was made with a sterile scalpel and a swab was taken from the affected area. The swabs were transported directly under cooled conditions to the microbiology lab in the College of Veterinary Medicine for microbiological examination.

Isolation and identification

Swabs were inoculated on nutrient broth medium and incubated at 37°C for 4 h. One loopful of fresh culture was streaked on mannitol salt agar medium and incubated at 37°C for 24 h. The cultural characteristics were studied in terms of mannitol fermentation. Gram stain was used to study morphological features, including the shape, arrangement and staining reaction of bacteria. Then, isolates were inoculated on blood agar medium and incubated at 37°C for 24 h to detect the type of haemolysis. Biochemical tests, which include catalase and coagulase, were done according to(14).

DNA extraction

Only *S. aureus* isolates were subjected to molecular conformation and detection of *nuc* and *coa* genes. DNA extraction was performed by Add Prep Genomic DNA Extraction (Korea) according to company instructions, and the DNA concentration was determined using Nanodrop (Nano Photometer® N50/Germany). The DNA stored in −80°C until used (15).

Molecular detection

A polymerase chain reaction (PCR) test was used for 2 primers, the *nuc* and *coa* of *S. aureus*. The molecular weights of (*nuc* and *coa* genes) were (166 bp, 300 bp), respectively [Table 1]. The mixture of PCR comprised this process, which was carried out in a 200 µl tube (Biozym, Germany). A total of 25 µl was used for the PCR reaction, including 12.5 µl of 2× Taq Green Mix-Master (Promega Corporation, USA) and 1 µl of primer 1. One microliter of primer 2, then 6.5 µl of DNeasy-free water and 4 µl of DNA templates [Table 2]. Gel electrophoresis on a 2% agarose gel (Peqlab, Germany) with a 100 bp ladder as a reference was used to analyse the resulting amplicons.

Results and Discussion

S. aureus is the only staphylococcal species in poultry considered to be pathogenic, bumble foot in poultry is a disease that occurs as a result of unsanitary rearing conditions and high humidity (18,19). Bacteriological examination of 100 samples collected from chickens infected with bumble foot showed according to phenotypic and cultural characteristics, including fermentation of mannitol on mannitol salt agar medium, types of haemolysis on blood agar medium, coagulase and catalase tests, *S. aureus* colonies appear as circular, golden yellow and smooth colonies on nutrient agar [Figure 1]. This result is consistent with what is described by (4).

S. aureus formed 68/100 (68%) followed by *Staphylococcus hyicus* 12/100 (12%), whereas *Staphylococcus intermedius* formed 4/100 (4%), from chickens with bumble foot disease, as shown in Table 3.

S. aureus constituted the highest isolation percentage at 68%. This percentage is closest to that of Al-Dabbagh (20) and Algammal *et al*. (21), who recorded 75% and 78%, respectively, of chickens infected with bumblefoot. While disagreeing with the results of Hanoon and Alkhafagi (22), who recorded an isolate rate of (48%) from infected chickens with bumble foot in Iraq. Many researchers (13,20,21) have indicated that *S. aureus* bacteria are the main etiological agents of this disease. *S. aureus* are naturally present on the skin, in the respiratory tract and in the intestinal tract. When wounds and scratches occur on the bottom of the foot, this bacterium enters initially leading to local inflammation in the damaged skin, and then infiltrates the deeper located tissue leading

Table 1: Primers of *Staphylococcus aureus*

Primers	Sequence (5–3)	Amplicon (bp)	References
Nuc – Reverse	CCTGAAGCAAGTGCATTTACGA	166	(16)
Nuc – Forward	CTTTAGCCAA GCCTTGACGAACT		
Coa – Reverse	ATAGAGATGCTGGTACAGG	304	(17)
Coa – Forward	GCTTCCGATTGTTCGATGC		

to severe inflammation, ulceration, difficulty walking, and eventually lameness (1,19). The study results also indicated the isolation of other coagulase-positive Staphylococci, such as *S. hyicus* and *S. intermedius*, from cases of bumblefoot disease in local chickens. In addition, some isolates were identified as coagulase-negative staphylococci, whereas a few isolates belonged to non-Staphylococcus species such as *Trueperella pyogenes* and *Proteus mirabilis*; these findings are consistent with previous studies (20,22).

S. aureus has several virulence agents that help the bacterium to avoid the immune system of the host, adhere to epithelial cell and invading the host tissue, leading to sepsis (23,24). Haemolysins are one of the most important of these factors. Current study showed that most of *Staph aureus* isolates showed beta (β-) haemolysis on blood agar, others showed alpha haemolysis, as shown in Figure 2, this finding in agreement with Divyakolu *et al*. (7) who indicated that *S. aureus* has alpha and gamma toxins that cause pore-forming and a sphingomyelinase, also known as β-haemolysin, that causes the breakdown of host cells, enabling the bacterium to obtain nutrients and evade the immune system.

The finding of molecular investigation of *S. aureus* isolates using *nuc* gene, which encodes thermonuclear, showed that all isolates subjected to PCR were given a positive (166 bp) amplicon products as shown in Figure 3. This result is in agreement with previous studies that indicated the *nuc* gene is widely used by PCR as a particular target for the observation of *S. aureus* (15,25,26). Thermonuclease gene (*nuc*) is considered the brilliant standard goal for identifying *S. aureus* rapidly using PCR because of this action in breaking down the DNA and biofilm disarrangement (27).

Furthermore, the results showed that all *S. aureus* isolates have *coa* gene, given a positive (304 bp) amplicon product as shown in Figure 4. These results are in agreement with the findings of Dallal *et al*. (28) who indicated that coagulase is considered an important determinant agent for the recognition of *S. aureus* strains. Coagulase is binds firmly to the *S.*

Table 2: Polymerase chain reaction programme used for *Staphylococcus aureus* strains

Primers	Programs	Temperature (°C)	Time	Cycle
Nuc	Initial-denaturation	94	2 min	1
	Denaturation	94	1 min	35
	Annealing	55	30 s	
	Primary extension	72	30 s	
	Last extension	72	30 s	1
Coa	Initial denaturation	94	2 min	1
	Denaturation	94	30 s	30
	Annealing	54	30 s	
	Primary extension	72	30 s	
	Last extension	72	30 s	1
	Cooling	4		

Table 3: Numbers and percentage of *Staphylococcus* spp. isolates from chickens

Types of isolates	*n* (%)
Staphylococcus aureus	68 (68)
Staphylococcus hyicus	12 (12)
Staphylococcus intermedius	4 (4)
Coagulase-negative staphylococci	6 (8)
Nonstaphylococci	10 (10)

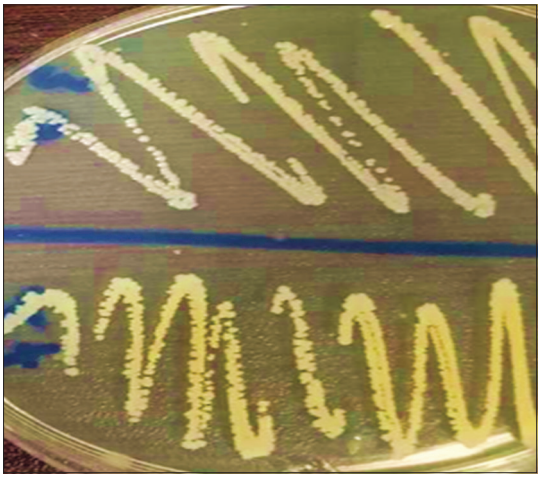

Figure 1: *Staphylococcus aureus* on nutrient agar

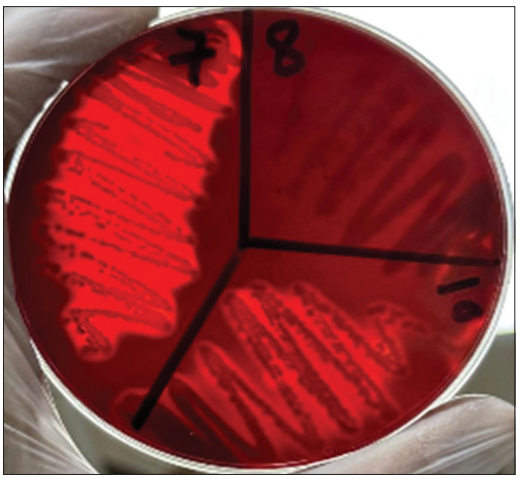

Figure 2: Types of haemolysis of blood by *Staphylococcus aureus* on blood agar

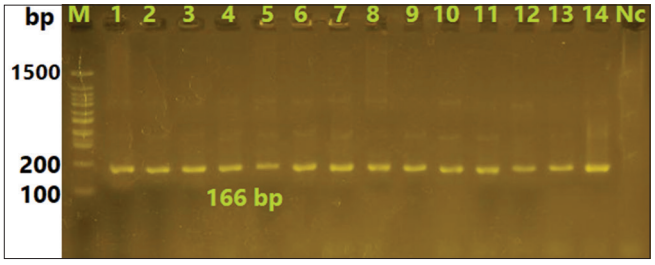

Figure 3: PCR test; amplicon products of *nuc* gene. Lane M; DNA molecular marker. Lane (1to14) positive tested isolates giving 166 bp by agarose gel electrophoresis

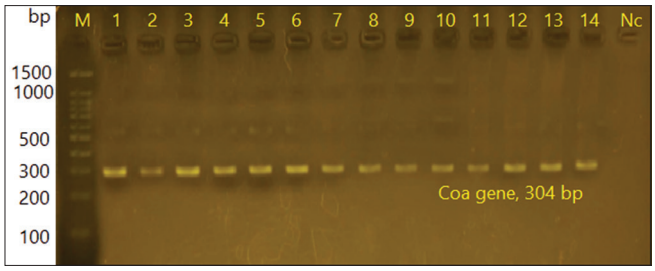

Figure 4: Polymerase chain reaction test; amplicon products of *coa* gene. Lane M; DNA molecular marker. Lane (1 to 14) positive tested isolates giving 304 bp by agarose gel electrophoresis

aureus bacterium and can constitute a coat at the surface with fibrin when in contact with blood. This clot can protect the *S. aureus* from phagocytosis and separate it from the host's defenses; therefore, the fibrin coat makes the bacterium more virulent (29,30).

Conclusion

The study concluded that *S. aureus* plays a significant role in the development of bumblefoot disease in chickens, as evidenced by its high isolation rate and its possession of key virulence factors, including haemolysins and coagulase. These findings highlight the potential negative impact of this bacterium on the poultry industry and underscore the risk of its transmission to other animals and humans.

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Conflicts of interest

There are no conflicts of interest.

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