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**ORIGINAL ARTICLE**

# *Staphylococcus aureus* Causing Subclinical Mastitis in Goats: Prevalence, Phenotypic and Genotypic Characterization

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## Abstract

The dairy goat industry is rapidly developing worldwide as a result of increasing the awareness about the high quality and nutritional properties of caprine milk. Subclinical mastitis (SCM) is one of the most important challenges facing goat industry and leading to great economic losses. *S. aureus* has been regarded for long time as one of the most leading cause of mastitis either clinical or subclinical. The present study aimed to investigate the prevalence of SCM among goats and to isolate *S. aureus* as well as study some of their phenotypic and genotypic characters. A total of 143 individual half milk samples (HMSs) were collected aseptically from 75 apparently healthy goats and examined. *S. aureus* were isolated and identified phenotypically using conventional methods in addition to using Vitek2 compact system. The selected isolates were confirmed by the detection of staphylococcal 16S rRNA gene. The prevalence of SCM based on California Mastitis Test (CMT) was 41.3 and 34.3% at goats and udder HMSs levels, respectively. The prevalence of *S. aureus* isolation in subclinically mastitic goats was investigated in 49 HMSs as 26.5%. The results of *in-vitro* antimicrobial susceptibility of *S. aureus* isolates against 12 antimicrobial agents showed high resistance against ampicillin, amoxicillin-clavulanic, cefoxitin, cefotaxime and vancomycin. Meanwhile, high susceptibilities were recorded against ciprofloxacin, levofloxacin, florophenicol, doxycycline HCl, clindamycin, gentamicin and sulfamethoxazole-trimethoprim. The hemolytic activity and biofilm formation on CRA medium were investigated in all isolates. The hemolytic activity was detected in 76.9% of isolates meanwhile 53.8% of isolates were biofilm formers, respectively. The results of genotypic detection of *mec*A, *bla*Z and *van*A resistance genes using PCR showed that they were detected in 100, 71.4 and 42.9% of the tested isolates, respectively. Meanwhile, biofilm and α-hemolysin coding genes (*ica*D and *hla*) were detected in 71.4 and 42.9% of the tested isolates, respectively. It was concluded that *S. aureus* is one of the most prevalent cause of caprine SCM and the existence of high percentages of antimicrobials resistance as well as resistance and virulence genes represent risk factors and public health hazards and possible danger of lateral transfer of resistance genes to other microorganisms in both animals and humans.

## Keywords

Biofilm, Goats, Hemolysis, *S. aureus,* Subclinical Mastitis

## 1. Introduction

Economically, SCM is considered more critical to the dairy industry than clinical mastitis (CM) not only because of the hidden symptoms but also because the milk production does not increase even after SCM full recovery leading to persistent economic loss **(El-Zamkan and Mohamed, 2021)**. SCM is more frequently occurring 15-40 times than CM and is longer-lasting and it was found to persist even after antibiotic treatment that leading to acquire the clinical form **(Cobirka et al. 2020)**. Moreover, SCM serves as a reservoir of different pathogens that can disseminate the udder infection among different animals and is considered as of public health concern **(El-Zamkan and Mohamed, 2021)**.

The intra mammary infections (IMIs) are mainly with contagious pathogens; such as *S. aureus* and *S. agalactiae*, or environmental pathogens; such as coagulase negative staphylococci (CNS), *E. coli*, *P. aeruginosa* and *S. uberis* **(Azab, 2007)**.

*S. aureus* was recorded as the most prevalent cause of SCM in goats in many studies in Egypt and worldwide **(El-Bassiony et al. 2008; Alemu and Abraha, 2017; Haggag et al., 2019; Öztürk et al., 2019)**.

Bacteriological examination and identification of the etiological agents is regarded as the gold standard diagnosis of IMI in goats **(Paterna et al., 2014)** although its high costs and time consuming for mastitis treatment **(Hussein et al., 2020)**. PCR assay is more reliable, accurate and confirmatory technique for the identification of different pathogens recovered from caprine mastitic milk samples; especially *S. aureus*, **(Abdallah et al., 2018)**. *S. aureus* isolates could be detected using the molecular analysis of the 16SrRNA gene **(Qazi et al., 2019)**.

The biofilm formation ability is a substantial staphylococcal virulence factor allowing their organization into multicellular layers clusters those embedded in an extracellular polysaccharide matrix; called slime, and allowing staphylococci to be resistant to antimicrobials and host immunity **(Abed et al., 2021b).** Biofilm formation is encoded by the *ica*A, *ica*B, *ica*C and *ica*D genes **(Nasr et al., 2012)**. A significant correlation was found between biofilm formation, multidrug resistance and virulence genes of the isolates **(El-Zamkan and Mohamed, 2021)**. **Jain and Agarwal (2009)** evaluated the sensitivity and specificity of biofilm production in *Staphylococcus* spp. on Congo red agar (CRA) medium as a gold standard. *S. aureus* can form biofilm that impairs the drug exposure participating drug resistance and in chronic infection **(Tras et al., 2019)**.

Bacterial antimicrobial resistance (AMR) can be resulted from the overuse of antimicrobial drugs in veterinary practices **(Abed et al., 2021a)**. Staphylococcal methicillin-resistance is one of AMR mechanisms that regarded for to β-lactams resistance and coded by several *mec* genes such as *mec*A or *mec*C **(Abed et al., 2018; Abed et al., 2021b)** as well as vancomycin-resistant *S. aureus* (VRSA) coded by *van*A or other van resistance genes **(Azhar et al., 2017; Cong et al., 2020)**. Animals and their environments are regarded as reservoirs of resistant bacteria as well as resistance genes those can be transmitted to human **(WHO, 2011)**.

The present study was carried out to investigate the prevalence of SCM among goats and identify *S. aureus* as an etiological bacterial agent on bacteriological and molecular bases as well as study some phenotypic and genotypic characters of *S. aureus* isolates.

## 2. Materials and Methods

### 2.1. Animals

A total of 75 apparently healthy native breed lactating goats from 3 private farms located in Alexandria desert district in the north of Egypt were subjected to the current study along the period from January to September 2018. Animals mainly were selected in middle and late lactation stages; between the 2$^{nd}$ and 4$^{th}$ seasons of lactation. All animals were examined clinically for detection of abnormalities suggestive for clinical mastitis such as swelling, hotness, asymmetry and/or any physical changes.

### 2.2. Collection of Individual Half Milk Samples (NMC, 2017)

A total of 143 individual half milk samples (HMSs); while 7 udder halves showed complete loss of function, were collected aseptically at mid-lactation through a cluster sampling method and investigated using California mastitis test (CMT) for detection of SCM according to **APHA (2004)**. All samples were transferred in an ice box; as soon as possible, to the laboratory of Animal Health Research Institute Alexandria, Egypt for the bacteriologic examination.

### 2.3. Staphylococci Isolation (Waller et al., 2011)

CMT-positive HMSs were centrifuged for 15min at 3,000 rpm with discarding the supernatant and cream layer. Then, the sediment was inoculated into tryptone soy broth; TSB, (Oxoid) and incubated at 37˚C for 18-24hrs. A loopful was taken from turbid broth and streaked onto7% sheep blood agar as well as Baird-Parker and mannitol salt agar; MSA, (Oxoid) and incubated at 37˚C for18-24hrs. All plates were examined for their bacterial growth and cultural characters according to **Collee et al., (1996)** and **Quinn et al., (2011)**.

### 2.4. Identification of S. aureus isolates

#### 2.4.1. Morphological and biochemical identification

Bacterial smears from suspected pure colonies were prepared, stained by Gram's stain technique, and examined microscopically for the morphological identification and to confirm being Staphylococci.

*Staphylococcus* isolates were identified biochemical depending on the following tests; catalase, oxidase and coagulase tests in addition to hemolytic and lecithinase activities; on sheep blood and Baird Parker agars, according to Collee et al., (1996), Quinn et al., (2011) and Waller et al., (2011).

### 2.4.2. Biochemical identification of S. aureus isolates using Vitek2 compact system: (Using ID-GP kits)

The Vitek2 compact system using ID-GP kits; used for Gram positive cocci identification, was applied on pure cultures for complete identification according to BioMérieux (2013).

## 2.5. Antimicrobial Ssusceptibility Testing of *S. aureus* Isolates

All isolates were examined for their antimicrobial susceptibility (AMS) to 12 different antimicrobials using disc diffusion method. Antimicrobial discs included ampicillin (10µg), amoxicillin-clavulanic A (30µg), cefoxitin (30µg), cefotaxime (30µg), vancomycin (30µg), clindamycin (2µg), gentamicin (10µg), doxycycline HCl (30µg), ciprofloxacin (5µg), levofloxacin (5µg), florfenicol (30µg) and sulfamethoxazole-trimethoprim (25µg) (Oxoid, Basing Stoke, UK). AMS tests were applied via disc diffusion method using Muller-Hinton agar and judged according to the guidelines of to CLSI (2018).

## 4.5. Phenotypic Detection of Biofilm Formation on Congo Red Agar Medium

Biofilm formation was phenotypically assessed for all CNS isolates by using CRA medium as described previously by El-Seedy et al., (2017). All the tested isolates were inoculated onto the medium and incubated for 24 hrs. at 37°C. After that, they were kept for 48 hrs. at room temperature. Colonies colors were detected using a four-color reference scale varies from red-black. Black colonies were regarded as positive biofilm formers while negative were indicated as pink or purple color. Indeterminate biofilm formers colonies appeared somewhat black.

## 2.6. Polymerase Chain Reaction

PCR was conducted on 7 *S. aureus* isolates those were phenotypically β-lactams and methicillin resistant, hemolytic and biofilm formers. The tested isolates were genetically confirmed by harboring specific staphylococcal 16S rRNA gene. Then they were screened for estimation of 3 AMR coding genes (*mec*A, *bla*Z, and *van*A) and 2 virulence-associated genes (*hla*; hemolysin alpha coding gene, and *ica*D; biofilm coding gene). The primers specificities and sequences in addition to the amplified products lengths and sizes (Metabion, Germany) were represented in Table (1).

**Table (1).** Primers of virulence and resistance genes used in PCR for *S. aureus* isolates.

| Tested genes | | Primer Sequence (5'-3') | Product size | References |
|---|---|---|---|---|
| *16S rRNA* | F | *CCTATAAGACTGGGATAACTTCGGG* | 791 bp | Mason et al., (2001) |
| | R | *CTTTGAGTTTCAACCTTGCGGTCG* | | |
| *mec*A | F | *GTAGAAATGACTGAACGTCCGATAA* | 310bp | McClure et al., (2006) |
| | R | *CCAATTCCACATTGTTTCGGTCTAA* | | |
| *blaZ* | F | *ACTTCAACACCTGCTGCTTTC* | 173bp | Duran et al., (2012) |
| | R | *TGACCACTTTTATCAGCAACC* | | |
| *van*A | F | *CATGACGTATCGGTAAAATC* | 885 bp | Patel et al., (1997) |
| | R | *ACCGGGCAGRGTATTGAC* | | |
| *ica*D | F | *AAACGTAAGAGAGGTGG* | 381 bp | Ciftci et al., (2009) |
| | R | *GGCAATATGATCAAGATA* | | |
| *hla* | F | *GAAGTCTGGTGAAAACCCTGA* | 704 bp | Fei et al., (2011) |
| | R | *TGAATCCTGTCGCTAATGCC* | | |

# 3. Results

## 3.1. Clinical examination of lactating goats.

The results of clinical examination of the udders of lactating ewes (*n=75*) revealed that out of 150 examined udder halves, 143 halves were apparently normal while 7 halves showed complete loss of function.

## 3.2. The prevalence of subclinical mastitis in lactating goats.

Regarding animals, results of CMT in milk samples collected from lactating goats revealed that out of 75 apparently healthy examined animals, 31 animals (41.3%) were positive CMT (subclinically mastitic), while 44 animals (58.7%) were negative. Regarding HMSs, out of 143 collected individual HMSs, 49 samples (34.3%) were positive CMT (subclinically mastitic) while 94 samples (65.7%) were negative (Table, 2).

## 3.3. Prevalence of *S. aureus* in CMT-Positive Goat HMSs.

Out of 49 subclinically mastitic goat HMSs, 13 *S. aureus* were isolated with a prevalence of 26.5%.

## 3.4. Antimicrobial Susceptibility Testing of *S. aureus* Isolates.

Results of *in-vitro* antimicrobial susceptibility of all *S. aureus* isolates (*n=13*) from subclinically mastitic goat milk samples against 12 antimicrobial agents (Table, 3) represented that *S. aureus* isolates mostly resistant to ampicillin (92.3%), followed by amoxicillin-clavulanic (76.9%), cefoxitin (61.5%), and finally both of cefotaxime sodium and vancomycin (53.8% for each). Meanwhile, they were highly sensitive to ciprofloxacin (76.9%), levofloxacin (69.2%) and florophenicol (61.5%), then, each of doxycycline HCl, clindamycin, gentamicin and sulfamethoxazole-trimethoprim (53.1% for each).

**Table (2).** CMT results of individual HMSs of the examined goats.

| Examined apparently healthy goats | | | | | Individual HMSs | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Total No. | CMT-positive | | CMT-negative | | Total No. | CMT-positive | | CMT-negative | |
| | No. | % | No. | % | | No. | % | No. | % |
| 75 | 31 | 41.3 | 44 | 58.7 | 143 | 49 | 34.3 | 94 | 65.7 |

%: were calculated according to the corresponding Total No.

**Table (3).** Results of antimicrobial susceptibility testing of *S. aureus* isolates.

| Class | Antimicrobial agent | Disc content (µg) | *S. aureus* tested isolates (*n=13*) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | R | | I | | S | |
| | | | No. | % | No. | % | No. | % |
| Penicillins | Ampicillin | 10 | 12 | 92.3 | - | - | 1 | 7.7 |
| | Amoxicillin-clavulanic A | 30 | 10 | 76.9 | 1 | 7.7 | 2 | 15.4 |
| Cephalosporins | Cefoxitin | 30 | 8 | 61.5 | 2 | 15.4 | 3 | 23.1 |
| | Cefotaxime sodium | 30 | 7 | 53.8 | 2 | 15.4 | 4 | 30.8 |
| Glycopeptides | Vancomycin | 30 | 7 | 53.8 | 1 | 7..7 | 5 | 38.5 |
| Fluoroquinolones | Levofloxacin | 5 | 3 | 23.1 | 1 | 7.7 | 9 | 69.2 |
| | Ciprofloxacin | 5 | 2 | 15.4 | 1 | 7.7 | 10 | 76.9 |
| Tetracyclines | Doxycycline HCl | 30 | 5 | 38.5 | 1 | 7.7 | 7 | 53.8 |
| Lincosamides | Clindamycin | 2 | 4 | 30.8 | 2 | 15.4 | 7 | 53.8 |
| Aminoglycosides | Gentamicin | 10 | 4 | 30.8 | 2 | 15.4 | 7 | 53.8 |
| Chloramphenicol | Florophenicol | 30 | 5 | 38.5 | 1 | 7.7 | 8 | 61.5 |
| Potentiated sulfonamides | Sulfamethoxazole-trimethoprim | 25 | 4 | 30.8 | 2 | 15.4 | 7 | 53.8 |

R=Resistant. S=Sensitive. I=intermediate. %: were calculated according to the No. of tested isolates (*n=8*).

## 3.5. Hemolytic Activity and Biofilm Formation Ability for *S. aureus* Isolates

Out of 13 *S. aureus* isolates, 10 isolates (76.9%) were β-haemolytic meanwhile 3 isolates (23.1%) were non (γ)-haemolytic.

Regarding biofilm formation on CRA medium, 7/13 (53.8%) of *S. aureus* isolates were phenotypically biofilm formers. Of them, 6 (46.2%) were strong biofilm formers while only one isolates (7.7%) was intermediate biofilm former. Meanwhile, 6 isolates (46.2%) were negative.

## 2.6. PCR of *S. aureus* Isolates

The PCR results were represented in **Table (4)** and **Figs. (1-6)** revealing that, all the tested isolates (*n=7*; 100%) were genetically confirmed as being Staphylococci by harboring staphylococcal 16S rRNA gene. Regarding the screened resistance-associated genes; *mec*A was recorded in all the tested isolates (*n=7*; 100%), while *bla*Z and *van*A genes were recorded in 5 (71.4%) and 3 isolates (42.9%), respectively. On the other hand, both *ica*D and *hla* virulence-associated genes were detected in 5 (71.4%) and 3 isolates (42.9%), respectively.

**Table (4):** Prevalence of resistance associated genes in the examined *S. aureus* isolates.

| No. of *S. aureus* tested isolates | Target genes | Positive | | Negative | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| 7 | *16S rRNA* | 7 | 100 | - | - |
| | *mec*A | 7 | 100 | - | - |
| | *blaZ* | 5 | 71.4 | 2 | 28.6 |
| | *van*A | 3 | 42.9 | 4 | 57.1 |
| | *ica*D | 5 | 71.4 | 2 | 28.6 |
| | *hla* | 3 | 42.9 | 4 | 57.1 |

% was calculated according to No. of *S. aureus* tested isolates (*n=5*).

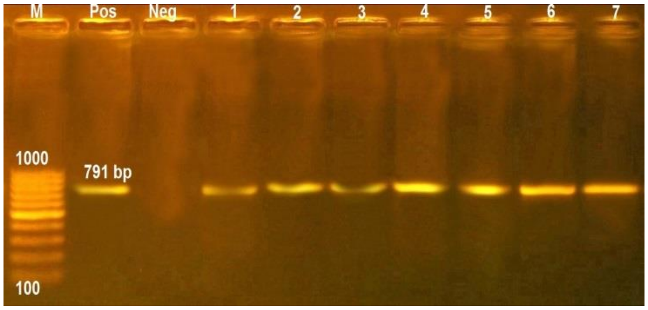


**Fig. (1).** PCR results of *16S rRNA* gene; at 791bp, for 7 *S. aureus* isolates (Lanes 1-7); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

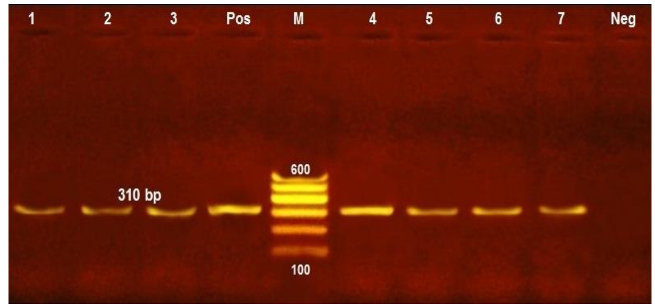


**Fig. (2).** PCR results of mecA gene; at 310bp, for 7 S. aureus isolates (Lanes 1-7); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

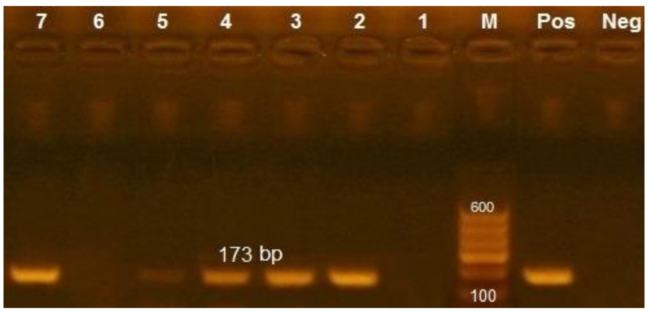


**Fig. (3).** PCR results of blaZ gene; at 173bp, for 7 S. aureus isolates (Lanes 1-7); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

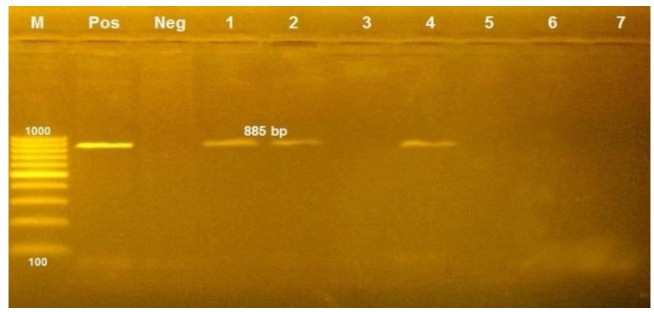


**Fig. (4).** PCR results of vanA gene; at 885bp, for 7 S. aureus isolates (Lanes 1-7); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

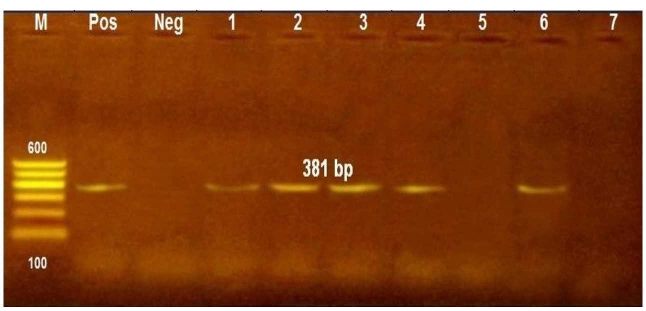


**Fig. (5).** PCR results of icaD gene; at 381bp, for 7 S. aureus isolates (Lanes 1-7); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

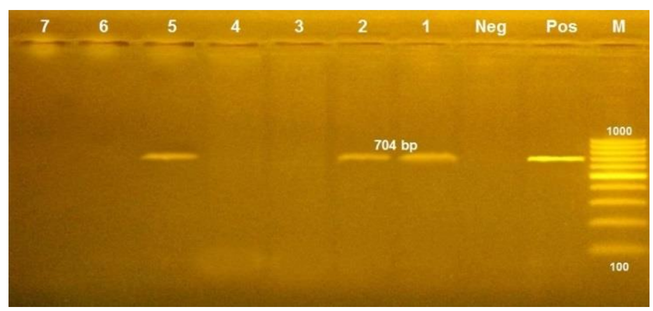


**Fig. (6.** PCR results of hla gene; at 704bp, for 7 S. aureus isolates (Lanes 1-7); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

## 4. Discussion

The dairy goat industry is rapidly developing worldwide as a result of increasing the awareness about high quality and nutritional properties of caprine milk **(Lima et al., 2018)**. There are more than half pillion goats all over the world producing annually about 4.5 million tons of milk **(Ebrahimi et al., 2007)**. In Africa, small ruminants produce about 14% of the world's milk **(Adane and Girma, 2008).** SCM is one of the most important challenges facing goat industry and leading to great economic losses **(Hussein et al., 2020).** Moreover, SCM is considered a constant risk of infection for the stock. Therefore, early diagnosis of SCM not only protects the farmer but rather the consumer. SCM is one of the most important challenges facing goat industry and leading to great economic losses **(Hussein et al., 2020).** The relevance of IMI infection in dairy goats is not only economic but also hygienic and safety issue with respect to the bacteriological quality of milk in the dairy industry **(Doğruer et al., 2016).** Therefore, early diagnosis of SCM not only protects the farmer but also the consumer.

Among 250 potential infectious pathogens causing mastitis, *Staphylococcus* members are considered the principal pathogens as a sequent of their high prevalence as well as their serious disease developed **(Hassan et al., 2016).** There are 50 staphylococci or more those have been incriminated as cause of staphylococcal mastitis **(El-jakee et al., 2013).** Among them, *S. aureus* was considered for long times as one of the major causes of mastitis due to its various virulence components such as toxins, enzymes in addition to wide ranges of AMS **(Darwish and Asfour, 2013).**

The present study investigated the prevalence of *S. aureus* SCM in goats as well as studied some phenotypic and genotypic characters *S. aureus* isolates.

In the current work, the prevalence of SCM; according to CMT, was 41.3 and 34.3% at goats and udder HMSs levels, respectively. These results coincided with those obtained by **El-Bassiony et al., (2008)** who recorded the prevalence of SCM in goats and HMSs as 44 and 34.5%, respectively. Meanwhile, **Abdallah et al., (2018)** detected the prevalence of SCM in goats and their HMSs in small private flocks in different localities at Sharkia Governorate as 29.8 and 48.1%, respectively. Additionally on the level of HMSs, nearly the same results were recorded in Egypt; **Haggag et al., (2019);** 31%, and worldwide; **McDougall et al. (2002);** in USA as 35.5%, **Hall and Rycroft (2007);** in U.K. ranged from 33-42%, **Bourabah et al., (2013);** in Algeria as 33.9%, **Alemu and Abraha (2017);** in Ethiopia as 30.8%. Moreover, such results were supported by **Omar and Mat-Kamir (2018)** who reported that SCM is the most common form of mastitis with the prevalence between 15-40 % of infected dairy goats. On the other hand, lower prevalences were recorded by **Abd El-Tawab et al., (2018);** 45.2%, **Hussein et al., (2020);** 52.6%, and **El-Zamkan and Mohamed (2021);** 52.1%. Meanwhile, much lower prevalence was recorded by **Ebrahimi et al., (2007);** in Iran ass 5.3%. It was reported that the prevalence of IMIs increased with age in goats due to higher exposure to pathogens in older animals than young in addition, increased duration length of infection and lower spontaneous recovery rate **(Al-Majali and Jawabreh, 2003).**

In the present study, *S. aureus* isolates were identified phenotypically using conventional methods including morphological, colonial and biochemical characteristics in addition to using Vitek2 compact system. All the tested isolates were genetically confirmed as being staphylococci by harboring staphylococcal 16S rRNA gene which was detected in all isolates. This was supported by **Krimmer et al., (1999)** who reported that the genotypic bacterial identification and detection depending on 16S rRNAs have several advantages where bacterial cells involve several copies of the 16S rRNA in their ribosomes. Therefore, this assay is highly sensitive enough to estimate any bacterial cell although, 16S rRNA genes are highly protected during bacterial evolution.

In the present study, the prevalence of *S. aureus* isolaion in subclinically mastitic goats was investigated in 49 HMSs as 26.5%. These results coincided with those obtained by **Hussein et al., (2020)** who recorded the prevalence of *S. aureus* SCM in goats as 24.4%. On the other hand, lower prevalences were recorded in Egypt; **El-Bassiony et al., (2008)** as 6.6% and **Haggag et al., (2019)** as 16.8%, and worldwide**; Ebrahimi et al., (2007);** in Iran as 14.3%, and **Omar and Mat-Kamir (2018);** in Malaysia as 4.9%. Meanwhile, much higher prevalences were recorded in Egypt by **Abdallah et al., (2018);** 46.4%, and worldwide; **İşnel and Kırkan (2012)** in Turkey as 69.6%, and **Alemu and Abraha (2017);** in Ethiopia as 33.3%. Also, **Öztürk et al., (2019)** in Turkey recorded *S. aureus* as 34.2% and concluded that *S. aureus* and CNS were found to be the most isolated species from goat milk. The high persistence of *S. aureus* mastitis was attributed to the high ability of *S. aureus* to produce exo-polysaccharides (‘‘slime’’) that forming a protective barrier restricting the efficiency of both the immune responses and chemotherapy **(Baselga et al., 1994).**

Antimicrobial therapy is still regarded as the base of any mastitis control measures. In Egypt, several antimicrobial drugs including β-lactams, glycopeptides, aminoglycosides, lincosamides, phenicols, tetracyclines, polymyxins, fluoroquinolones and sulfonamides have been incriminated in mastitis controlling **(Abed et al., 2021b).** However, the extensive and misuse of antimicrobials have led to the emergence of strains resistance. Therefore, identification of etiological agents and their AMS profiles prior treatment achieves the proper treatment **(Srednik et al., 2017).**

In the current work, results of *in-vitro* antimicrobial susceptibility of *S. aureus* isolates from subclinically mastitic goat against 12 antimicrobial agents showed high resistance against β-lactams antibiotics either penicillins; ampicillin and amoxicillin-clavulanic or cephalosporins; cefoxitin and cefotaxime sodium, in addition to glycopeptides; vancomycin. On the other hand, high susceptibilities were recorded against the other tested antimicrobials including ciprofloxacin, levofloxacin, florophenicol, doxycycline HCl, clindamycin, gentamicin and sulfamethoxazole-trimethoprim. Nearly similar results were previously recorded in Egypt and worldwide **(Hande et al., 2015; Awad et al., 2017; Abed et al., 2018; Ren et al., 2020).** Meanwhile, **Algammal et al., (2020)** recorded moderate cephalosporins susceptibility against *S. aureus* isolates showing stable effect in the existence of β-lactamase enzyme **(Algammal et al., 2020).**

*Staphylococci* capable of production various enzymes; enabling invasion of host tissues and spreading of inflammatory processes, in addition to hemolysins and proteolytic enzymes those facilitating the iron uptake **(El-Seedy et al., 2017).** *Staphylococci* are able to produce four types of hemolysins (α, β, γ, and δ) those are cytolytic exotoxins which can invade the host cell and destroy the red blood cell membrane assisting staphylococci for iron uptake especially hemoglobin iron **(Moraveji et al., 2014).**

In this work, hemolytic activity was investigated in all *Staphylococcus* isolates and the majority of isolates; 76.9%, were β-hemolytic while α-hemolysis was not recorded. These results ran parallel to those recorded by **Abed et al., (2021a)** who recorded the hemolytic activity in 76.6% of the examined isolates; of which 50.6% were β-hemolytic while 26% of isolates showed α-hemolysis. Meanwhile, **Moraveji et al., (2014)** reported 60% of *Staphylococcus* isolates as hemolytic.

Production of slime and the capability of surfaces' attachment to assist the formation of biofilm is an essential prosperity related to the pathogenicity of *Staphylococcus* spp. and their intra mammary survival **(El-Seedy et al., 2017).** Additionally, biofilms reduce AMS impairing antimicrobial therapy **(Tremblay et al., 2013).** CRA is running parallel with PCR for routine detection of biofilm **(Hou et al., 2012; Osman et al., 2015).**

In the current study, biofilm formation ability was phenotypically investigated in all *Staphylococcus* isolates 53.8% of isolates were found to be biofilm former on CRA medium; of which 46.2% were strong biofilm formers while 7.7% was intermediate biofilm former. These results were supported by those recorded by **Bochniarz et al., (2014)** recorded slime-production in 54% of *Staphylococcus* isolates. Somewhat lower results were recorded by **Abed et al., (2021a)** who found biofilm formation in 46.8% of *Staphylococcus* isolates; of which 33.8% were strong while 13% were intermediate. These results were similar to those reported by **El-Seedy et al., (2017).** Meanwhile, higher results were recorded **(Murugan et al., 2010; Hou et al., 2012; Osman et al., 2015).**

Phenotypic cefoxitin susceptibility was used for methicillin resistance detection **(Abed et al., 2018).** High methicillin resistance rates is very characteristic in infamous staphylococci leading to limited treatment options as well as effective antibiotic therapy **(Srednik et al., 2017).** The methicillin-resistance is encoded by a *mec*A gene **(Abed et al., 2021a, b).** Therefore, methicillin-resistant staphylococci (MRS) strains have huge public health importance due to carriage of other resistance genes on the chromosome acquiescing *mec*A gene that promoting MRS as well as resistance to other β-lactams antibiotics **(Srednik et al., 2017)**. Moreover, *bla*Z gene is encoding for β-lactamases and responsible for staphylococcal β-lactams resistance. In addition, the huge involvement of β-lactams antibiotics in mastitis therapies makes *bla*Z acquisition and dissemination among staphylococci from human and animals a great problem regarding the efficiency of mastitis therapy programs as well as the public health **(Sawant et al., 2009).** β-lactamase enzyme production is the most prevalent staphylococcal resistance mechanism **(Abed et al., 2018).**

In the current work, the *mec*A & *bla*Z resistance-genes were assessed using PCR among 7 *S. aureus* isolates and both genes were found in 100 and 71.4%of tested isolates, respectively. These results were somewhat supported by **Abed et al., (2018)** who detected *mec*A and *bla*Z genes in75 and 65%of isolates, respectively. Much lower results were recorded by **Abed et al., (2021b)** who detected both genes in 60 and 46.7%of tested MDR *S. aureus* isolates, respectively.

The existence of MDR *S. aureus* harboring *mec*A gene in dairy animals distributed worldwide **(Haran et al., 2012; Awad et al., 2017).** Interestingly, **Abed et al., (2018)** study revealed that all *mec*A posing *S. aureus* harbored also the *bla*Z gene. The MDR *S. aureus* isolates from milk and farm environment carrying *mec*A and/or *bla*Z genes have been considered a great threat for the consumers, veterinarians and farmworkers. Most of MDR *S. aureus* are often of human origin and transferred to dairy animals as a result of poor hygienic and management measures **(Haran et al., 2012; Awad et al., 2017).**

Vancomycin is considered one of the first-line of treatment for MRSA infections **(Abed et al., 2021a).** Recently, emergence of vancomycin resistance (VR) has become a great public health threats. Vancomycin resistance is encoded by a *van* gene cluster which was transmitted from VR enterococci **(Cong et al., 2020).** Although there are 11 *van* gene clusters conferring VR (*van*A, B, D, F, I, M, C, E, G, L and N phenotypes), only the *van*A gene cluster is associated with the vancomycin resistant *S. aureus* (VRSA) strains **(Werner et al., 2008).** The characterization of VR associated genes showed a strong correlation between the phenotypes and genotypes of AMR and showed also that the existence of VR in animals has great hazardous effect on human health **(Abed et al., 2021a)** through direct infection with resistant pathogens or through the lateral transmission of these genes between different staphylococci **(Werner et al., 2008).**

In the present work, *van*A gene was assessed using PCR in7 *S. aureus* isolates and was detected in 42.9% of tested isolates. Such result was nearly similar to that reported by **Abed et al., (2021a)** who recorded *van*A with a rate of 41.7%. They also recorded *van*C1 gene with higher prevalence; 83.3%.

The extracellular slime components synthesis is encoded by the genes of the *ica*RADBC locus, which is an operon of four biosynthetic genes and is regarded as the first step in biofilm formation (*ica*ADBC) **(Osman et al., 2015).** Such genes are considered virulence markers for staphylococci and their existence is indicating high pathogenic potential of the strain **(Abed et al., 2021b).** The *icaD* gene was considered as one of the most important genes encoding for biofilm production **(Osman et al., 2015).** Biofilm-producing bacteria become highly resistant to opsono-phagocytosis and antimicrobial drugs **(Srednik et al., 2017).** This bacterial resistance is considered the main cause for the chronic disease status development **(Burki et al., 2015).** In addition, biofilm production can damage the host tissues due to enhance the release of phagocytic lysosomal enzymes **(Hermeyer et al., 2011).** The biofilms play an important role in the development and dissemination of microbial resistance through the interactions occurring via the biofilm **(Morente et al., 2013).**

In the current study, the biofilm coding gene; *ica*D, was assessed using PCR in7 *S. aureus* isolates and detected in 71.4% of the tested isolates. These findings were nearly similar to the prevalences obtained by **Osman et al., (2015);** 77%, and **Abed et al., (2021a);** 77.8%. Meanwhile, **Abed et al., (2021b)** found *ica*D gene in 20%of tested MDR *S. aureus* isolates. Such findings suggested that biofilm production needs several factors of which *ica*D gene is considered the most reliable gene marker for biofilm formation **(Osman et al., 2017; Srednik et al., 2017; Abed et al., 2021b).**

The expression of hemolysins is the main factor contributing to bacterial infection and prohibiting the host's immune response **(Almeida et al., 2013).** The cytolytic toxins production is the principal mechanism of *S. aureus* for targeting host phagocytic cells **(Saleem, 2017).** *S. aureus* produces variable exotoxins those invading host cell including *Staphylococci* are able to produce four types of hemolysins (α, β, γ, and δ) those are cytolytic exotoxins which can invade the host cell and destroy the red blood cell membrane assisting staphylococci for iron uptake especially hemoglobin iron the different types of hemolysins those coded by *hla*, *hlb*, *hlg* and *hld* genes. These hemolysins have a cytolytic effect against wide range of cells including red blood cells, platelets, monocytes and neutrophils **(Moraveji et al., 2014).**

In this study, alpha hemolysin coding gene; *hla* was assessed using PCR in 7 *S. aureus* isolates and detected in 42.9% of the test isolates. A higher prevalence was recorded by **Abed et al., (2021a)** who detected *hla*, gene was in 50% of *Staphylococcus* isolates. Meanwhile, **Moraveji et al., (2014) and Schmidt et al., (2017)** found *hla* gene in all *S. aureus*. These results supported that *hla* existence in staphylococci is essential for establish of infection in humans and animals **(Moraveji et al., 2014).**

## 5. Conclusion

*S. aureus* is one of the most prevalent causes of caprine SCM. The existence of high percentages of antimicrobials resistance as well as resistance and virulence genes represent risk factors rendering the farmers and the veterinarians under pressure of choosing effective antimicrobial therapies or prophylaxes, in addition to the public health hazards as well as the danger of lateral transferring of resistance associated genes among human and animal pathogens.

## 6. Authors Contributions

All authors contributed equally to study design methodology, interpretation of results and preparing of the manuscript.

## 7. Conflict of Interest

The authors declare no conflict of interest.

## 8. References


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