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ORIGINAL ARTICLE

Coagulase Negative *Staphylococci* Causing Subclinical Mastitis in Sheep: Prevalence, Phenotypic and Genotypic Characterization

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Abstract

Subclinical mastitis (SCM) is one of the most prevalent diseases affecting dairy animals and hindering the development of animal production sector worldwide. Staphylococci are the most significant causative bacterial pathogens in both clinical and subclinical cases. The present study aimed to investigate the prevalence of SCM among sheep detecting the prevalence of coagulase negative staphylococci (CNS) and studying some of their phenotypic and genotypic characters. A total of 145 individual half milk samples (HMSs) were collected aseptically from 75 apparently healthy ewes and examined. The prevalence of SCM based on California Mastitis Test (CMT) was 29.3 and 21.4% at sheep and udder HMSs levels, respectively. The prevalence of CNS in subclinically mastitic sheep was investigated in 31 (25.8%) HMSs. Identification of CNS isolates revealed that, *S. epidermidis* was the most prevalent (37.5%) followed by *S. xylosus* (25%) and each of *S. simulans*, *S. chromogenes* and *S. haemolyticus* (12.5%). The results of *in-vitro* antimicrobial susceptibility of CNS isolates against 12 antimicrobial agents showed high resistance against ampicillin, amoxicillin-clavulanic acid, cefoxitin and cefotaxime. Meanwhile, high susceptibilities were recorded against ciprofloxacin, levofloxacin, florophenicol, vancomycin, doxycycline, clindamycin, gentamicin and sulfamethoxazole-trimethoprim. The haemolytic activity and biofilm formation on CRA medium were investigated in all isolates. The haemolytic activity was detected in 75% of isolates meanwhile 62.5% of isolates were biofilm formers. The results of genotypic detection of *mec*A and *bla*Z resistance genes and *ica*D biofilm coding gene using PCR showed that they were detected in 80, 60 and 60% of the tested isolates, respectively. It was concluded that CNS isolates were the most prevalent causes of ovine 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, β-lactams resistance, blaZ, icaD and mecA, Coagulase Negative *Staphylococci*, Sheep, Subclinical Mastitis

1. Introduction

Subclinical mastitis (SCM) is considered one of the most serious economic diseases of the ovine mammary glands worldwide **(Abdallah et al., 2018)**. The main reasons for its economic significance are its higher prevalence rates and adverse effects on animal health and production including milk yield reduction, growth retardation and higher mortalities among suckling kids **(Kumar et al., 2016)**.

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 intramammary 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)**.

Staphylococci are the main etiological agents of small ruminants' IMIs, where, *S. aureus* is the most frequently common isolated pathogen in CM while CNS are the most predominant in SCM **(Moawad and Osman, 2005)**. Moreover, many studies recorded CNS as the most prevalent cause of SCM in sheep in Egypt and worldwide **(Beheshti et al., 2010; Omar and Mat-Kamir, 2018; Haggag 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.

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).** 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 investigated the prevalence of SCM among sheep detecting the prevalence of CNS as an etiological bacterial agent as well as studying some phenotypic and genotypic characters of CNS isolates.

2. Materials and Methods

2.1. Animals:

A total of 75 apparently healthy native breed lactating sheep from 4 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 2nd and 4th seasons of lactation. All animals were examined clinically for detection of abnormalities suggestive for clinical mastitis.

2.2. Collection of individual Half Milk Samples: (NMC, 2017)

A total of 145 individual half milk samples (HMSs); while 5 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 given a serial numbers and detailed information. 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 15 min 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, 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 CNS 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 as well as sugar fermentation test for sucrose, maltose, lactose, mannitol, arabinose, mannose and xylose in addition to haemolytic 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 CNS isolates using Vitek2 Compact System

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

2.5. Antimicrobial Susceptibility Testing of CNS isolates

All isolates were examined for their antimicrobial susceptibility (AMS) to 12 different antimicrobials of the most important antimicrobials used in the field 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 **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 at 37°C for 24 hrs. After that, they were kept for 48 hrs at room temperature. Colonies were examined 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 5 CNS isolates; representing different species, those were phenotypically β-lactams and methicillin resistant, haemolytic and biofilm formers. PCR-tested isolates were screened for estimation of 2 AMR coding genes (*mec*A and *bla*Z) as well as biofilm coding gene; *ica*D. 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 CNS isolates.

Tested genes		Primer Sequence (5'-3')	Product size	References
***mec*A**	***F***	***GTAGAAATGACTGAACGTCCGATAA***	**310bp**	**McClure et al., (2006)**
	R	***CCAATTCCACATTGTTTCGGTCTAA***		
***bla*Z**	***F***	***ACTTCAACACCTGCTGCTTTC***	**173bp**	**Duran et al, (2012)**
	R	***TGACCACTTTTATCAGCAACC***		
***ica*D**	***F***	***AAACGTAAGAGAGGTGG***	**381 bp**	**Ciftci et al., (2009)**
	R	***GGCAATATGATCAAGATA***		

3. Results

3.1. Clinical Examination of Lactating Sheep

The results of clinical examination of the udders of lactating ewes (*n=75*) revealed that out of 150 examined udder halves, 145 halves were apparently normal while 5 halves showed complete loss of function.

3.2. Prevalence of Subclinical Mastitis in Lactating Sheep

Regarding animals, results of CMT in milk samples collected from lactating sheep revealed that out of 75 apparently healthy examined animals, 22 animals (29.3%) were positive CMT (subclinically mastitic), while 53 animals (70.7%) were negative. Regarding HMSs, out of 145 collected individual HMSs, 31 samples (21.4%) were positive CMT (subclinically mastitic) while 114 samples (78.6%) were negative **(Table, 2)**.

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

CMT	Apparently healthy Sheep		Individual HMSs	
	No.	%	No.	%
Positive	**22**	**29.3**	**31**	**21.4**
Negative	**53**	**70.7**	**114**	**78.6**
Total No.	**75**	**100**	**145**	**100**

%: were calculated according to the corresponding Total No.

3.3. Prevalence and identification of CNS spp. in CMT-positive sheep HMSs.

Out of 31 subclinically mastitic sheep HMSs, 8 CNS were isolates with a prevalence of 25.8%.

Identification of CNS isolates (*n=8*) revealed that, *S. epidermidis* was the most prevalent (3isolates; 37.5%) followed by *S. xylosus* (2 isolates; 25%) and each of *S. simulans*, *S. chromogenes* and *S. haemolyticus* (1 isolate; 12.5% for each) **(Table, 3)**.

3.4. Antimicrobial Susceptibility Testing of CNS isolates.

Results of *in-vitro* antimicrobial susceptibility of all CNS isolates (*n=8*) from subclinically mastitic sheep milk samples against 12 antimicrobial agents **(Table, 4).** Results indicated that CNS isolates were mostly resistant to ampicillin (87.5%), followed by amoxicillin-clavulanic (75%), cefoxitin (67.5%), and finally cefotaxime sodium (50%). Meanwhile, they were highly sensitive to ciprofloxacin (87.5%), both of levofloxacin and florophenicol (75% for each), both of gentamicin and sulfamethoxazole-trimethoprim (62.5% for each) and finally each of vancomycin, doxycycline and clindamycin (50% for each).

Table (3).CNS spp. in CMT-positive sheep HMSs

S. epidermidis		*S. xylosus*		*S. simulans*		*S. chromogenes*		*S. haemolyticus*		Total No.
No.	%	No.	%	No.	%	No.	%	No.	%	
3	37.5	2	25	1	12.5	1	12.5	1	12.5	8

%: percentage was calculated according to total No. of isolates (*n=8*).

Table (4): Results of antimicrobial susceptibility testing of CNS isolates.

Class	Antimicrobial agent	Disc content (µg)	CNS tested isolates (*n=8*)					
			R		I		S	
			No.	%	No.	%	No.	%
Penicillins	Ampicillin	10	7	87.5	1	12.5	-	-
	Amoxicillin-clavulanic A	30	6	75	1	12.5	1	12.5
Cephalosporins	Cefoxitin	30	5	67.5	2	25	1	12.5
	Cefotaxime sodium	30	4	50	2	25	2	25
Glycopeptides	Vancomycin	30	3	37.5	1	12.5	4	50
Fluoroquinolones	Levofloxacin	5	2	25	-	-	6	75
	Ciprofloxacin	5	1	12.5	-	-	7	87.5
Tetracyclines	Doxycycline HCl	30	3	37.5	1	12.5	4	50
Lincosamides	Clindamycin	2	2	25	2	25	4	50
Aminoglycosides	Gentamicin	10	2	25	1	12.5	5	62.5
Chloramphenicol	Florophenicol	30	2	25	-	-	6	75
Potentiated sulfonamides	Sulfamethoxazole-trimethoprim	25	2	25	1	12.5	5	62.5

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

3.5. Haemolytic Activity and Biofilm Formation Ability for CNS isolates

Out of 8 CNS isolates, 6 isolates (75%) were haemolytic; among which 4 isolates (50%) showed β-haemolysis while 2 isolates (25%) were α-haemolytic. Meanwhile, 2 isolates (25%) were non-(γ)-haemolytic (25%).

Regarding biofilm formation on CRA medium, 5/8 (62.5%) of CNS isolates were phenotypically biofilm formers. Of them, 4 (50%) were strong biofilm formers while only one isolates (12.5%) was intermediate. Meanwhile, 3 isolates (37.5%) were negative.

2.6. PCR of CNS isolates

The PCR results were represented in **Table (5)** and **Figs. (1-3)** revealing that, out of 5 examined CNS isolates; *mec*A gene was detected in 4 isolates (80%), while both *bla*Z and *ica*D genes were detected in 3 isolates (60% for each).

Table (5). Prevalence of different genes in the examined CNS isolates

No. of CNS tested isolates	Target genes	Positive		Negative	
		No.	%	No.	%
5	*mec*A	4	80	1	20
	blaZ	3	60	2	40
	*ica*D	3	60	2	40

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

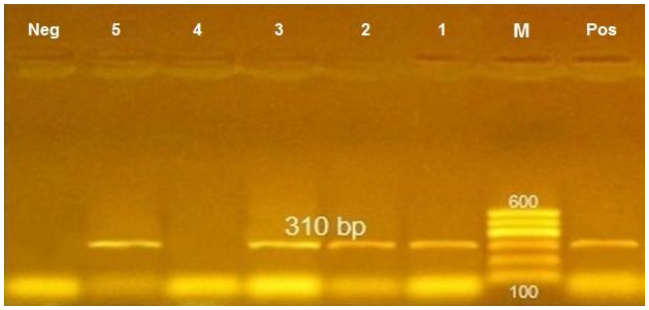

Fig. (1). PCR results of *mec*A gene; at 310bp, for 5 CNS isolates (Lanes 1-5); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

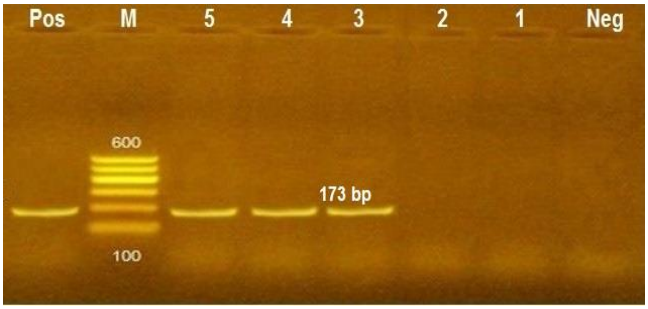

Fig. (2). PCR results of blaZ gene; at 173bp, for 5 CNS isolates (Lanes 1-5); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls.

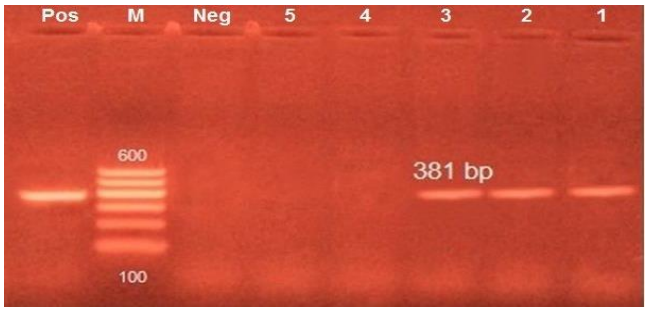

Fig. (3). PCR results of icaD gene; at 381bp, for 5 CNS isolates (Lanes 1-5); DNA size marker (M); Lanes (Pos and Neg): Positive and Negative controls

4. Discussion

Small ruminants play an important role in the people nutrition and income all over the world. In Africa, small ruminants produce about 14% of the world's milk **(Ebrahimi et al., 2007; Adane and Girma, 2008)**.

Mastitis; especially SCM, is ranking as the most prevalent diseases between dairy animals that hindering the development of animal production sector worldwide **(Haggag et al., 2019)**. 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. 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 rather the consumer.

Among 250 potential infectious pathogens causing mastitis, Staphylococci 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, CNS spp. are of the major pathogens causing mastitis throughout the world even they were described as emerging mastitis pathogens **(Taponen et al., 2006)**. CNS spp. have reported as the causative agents of mastitis either subclinical or clinical **(Soares et al., 2012)**. Moreover, CNS infections interfere with some dairy products manufacturing as a result of using antimicrobial agents as prophylaxis and therapy **(Srednik et al., 2017)**. Therefore, CNS pathogens have been considered to be the major cause of SCM **(Azab, 2007)**.

The present study investigated the prevalence of coagulase negative staphylococcus SCM in sheep and studied some phenotypic and genotypic characters *S. aureus* isolates.
According to CMT, the prevalence of SCM was 29.3 and 21.4% at sheep and udder HMSs levels, respectively. These results were supported by those recorded previously in Egypt by **Azab (2007)** who recorded the prevalence of SCM in Kafr El-Sheikh Province in sheep and their HMSs as 30.7 and 24.1% in gland and 30.7% in dairy ewes. While, **Moawad and Osman (2005)** detected them in Fayoum Province as 29.5 and 31.6%, respectively. Meanwhile, **Abdallah et al. (2018)** detected the prevalence of SCM in sheep in small private flocks in different localities at Sharkia Province as 40.7% in ewes and 27.3% in HMSs. Much lower results were detected by **El-Bassiony et al., (2008);** in Assiut Province, who recorded the prevalence of SCM in ewes and HMSs as 14 and 9.6%, respectively. Additionally on the level of HMSs, nearly the same results were recorded worldwide; **McDougall et al., (2002);** in USA as 19%, and **Alemu and Abraha (2017);** in Ethiopia as 25%. On the other hand, much higher prevalences were recorded by **Abd El-Tawab et al., (2018);** 43.8%, **Haggag et al., (2019);** 33%, and **El-Zamkan and Mohamed (2021);** 32.3%. Meanwhile, much lower prevalence was recorded by **Ebrahimi et al., (2007);** in Iran as 4.8%. It was reported that the prevalence of IMIs increased with age in sheep due to higher exposure to pathogens in older

animals than young in addition, long duration of infection and lower spontaneous recovery rate **(Al-Majali and Jawabreh, 2003).**

In the present study, the prevalence of CNS isolation in sheep SCM was investigated in 31 HMSs as 25.8%. Identification of CNS isolates revealed that, *S. epidermidis* was the most prevalent (37.5%) followed by *S. xylosus* (25%) and each of *S. simulans*, *S. chromogenes* and *S. haemolyticus* (12.5%). Regarding the CNS prevalence, such results coincided with those obtained by **Abdallah et al., (2018)** in Egypt who recorded CNS as 26.0%, and **Alemu and Abraha (2017)** in Ethiopia who recorded CNS as 25%. On the other hand, lower prevalences were recorded by **El-Bassiony et al., (2008);** 4.6%, and **Haggag et al., (2019);** 14.8%. Meanwhile, much higher prevalences were recorded in Egypt by **Moawad and Osman (2005);** 50%, and **Azab (2007);** 67.3%, and worldwide; **Ebrahimi et al., (2007);** 36.8%, and **Beheshti et al., (2010);** in Iran as 69.2%. Regarding CNS isolates identification; the obtained results were supported by those recorded by **Azab (2007)** who recorded *S. epidermidis* as the most prevalent, followed by *S. haemolyticus*, *S. xylosus*, *S. simulans*, and *S. caprae*. Also, such results were supported by those reported by **Contreras et al., (2007)** where *S. epidermidis*, *S. simulans*, *S.chromogenes*,*S. xylosus*, and *S.haemolyticus* were the most commonly CNS isolates in persistent sheep SCM. **Ergün et al., (2009)** in Turkey recorded higher prevalence of CNS isolates as 76.5% and identified them with reporting *S. epidermidis* as the most prevalent species as 35.7%, followed by *S. xylosus* (10.2%), *S. saprophyticus* (10.2%), *S. warneri* (9.2%), and *S. intermedius* (7.1%). Streptococci with a rate of 12.2% were the second most isolated bacterial group among the flocks. Meanwhile, **Doğruer et al., (2016)** in Turkey identified CNS isolates as *S. capitis* (14.3%), *S. haemolyticus* (9.5%), *S. simulans*, *S. xylosus* and *S. caprae* (7.9% for each), both *S. epidermidis* and *S. warneii* (6.4% for each), *S. scuiri* (4.8%), *S. auricularis* and *S. hominis* (3.2% for each).

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 CNS isolates from subclinically mastitic sheep against 12 antimicrobial agents showed high resistance against β-lactams antibiotics either penicillins; ampicillin and amoxicillin-clavulanic or cephalosporins; cefoxitin and cefotaxime sodium. On the other hand, high susceptibilities were recorded against the other tested antimicrobials including ciprofloxacin, levofloxacin, florophenicol, vancomycin doxycycline HCl, clindamycin, gentamicin and sulfamethoxazole-trimethoprim. Nearly similar results were previously recorded in Egypt and worldwide **(Waller et al. 2011; Alekish et al., 2013; Hande et al., 2015; Abed et al., 2018).**

Staphylococci capable of production various enzymes; enabling invasion of host tissues and spreading of inflammatory processes, in addition to haemolysins and proteolytic enzymes those facilitating the iron uptake **(El-Seedy et al., 2017).** *Staphylococci* are able to produce four types of haemolysins (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 haemoglobin iron **(Moraveji et al., 2014).**

In this work, haemolytic activity was investigated in all *Staphylococcus* isolates and the majority of isolates; 75%, were haemolytic. Of them, 50% showed β-haemolysis while 25% showed α-haemolysis. These results ran parallel to those recorded by **Abed et al., (2021a)** who recorded the haemolytic activity in 76.6% of the examined isolates; of which 50.6% were β-haemolytic while 26% of isolates showed α-haemolysis. Meanwhile, **Moraveji et al., (2014)** reported 60% of *Staphylococcus* isolates as haemolytic.

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 intramammary 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 them 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 reported 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 5 CNS isolates and both genes were found in 80 and 60%of the tested isolates, respectively. These results ran parallel to those recorded by **Abed et al. (2018)** who found *mec*A and *bla*Z were in 70% and 55% of isolates, respectively. Lower prevalences were reported by **Abed et al. (2021b)** who found both genes in 26.7 and 53.3% of MDR CNS, respectively. The recorded results regarding *mecA* gene was greater than those previously recorded **(Soares etal., 2012; Frey et al., 2013; Klimiene et al., 2016)** meanwhile lower than that reported by **Abed et al., (2021a);** 93.1%. Regarding *blaZ* gene in CNS isolates recovered from SCM, higher findings were reported by **Krewer et al., (2015);** 93.1%, meanwhile lower results were also reported 65.7% by **Hosseinzadeh and Saei (2014);** and 20.4% by **Srednik et al., (2015).**

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 this work, the biofilm coding gene; *ica*D,was assessed using PCR in5 CNS isolates and detected in 60% 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 6.7%of MDR 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., et al., 2017; Srednik et al., 2017; Abed et al., 2021b).**

5. Conclusion

CNS are one the most prevalent causes of ovine SCM in several countries. 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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