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MOLECULAR DETECTION OF MULTIDRUG-RESISTANT STAPHYLOCOCCUS AUREUS IN COWS AFFECTED WITH SUBCLINICAL MASTITIS IN SOHAG GOVERNORATE

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is the most important bacterial cause of subclinical mastitis (SCM), which is resistant to treatment. This study was conducted on cows with SCM to estimate hematobiochemical changes. It also aimed to isolate and identify multidrug-resistant *S.aureus* and its antibiotic susceptibility pattern to detect antibiotic-resistant genes. One hundred cattle from different farmholders were examined by the California mastitis test, which revealed that 32 (32%) SCM cases were positive. Blood and milk samples were collected from subclinical cases and 10 control healthy cows. This study showed a significant increase in AST, ALT, ALP, total leucocytic count, neutrophil, basophil percentage, and CRP, while a significant decrease in the erythrocytic count, total protein and albumin in subclinical cases, compared to the control group. The antibiograms of 32 *S. aureus* isolates showed high resistance toward penicillin (65.6%), ampicillin (56%), imipenem, and amoxicillin (44%) each. These were followed by tetracycline (40.6%), vancomycin (37.5%), amoxicillin-clavulanic acid, ciprofloxacin (34%) each, and chloramphenicol (31%). All the isolates resistant to the antibiotics encode *blaZ, tetK, vanA, optrA, norA, mphC,* and *mecA* genes. The haemato-biochemical parameters associated with SCM can be used as vital indicators of SCM. *S. aureus* is an important cause of SCM, which is highly resistant to antibiotic treatment. Antibiotic susceptibility tests improve the selection of appropriate antimicrobial drugs and avoid antibiotic resistance. Molecular surveillance is essential for the detection of *S. aureus* resistance genes.

Keywords: Subclinical mastitis, *S. aureus,* PCR, Resistance genes.

INTRODUCTION

Mastitis is a common disease in dairy cows that causes inflammation of the mammary gland with a high economic cost for treatment, low milk production, and sometimes ends with animal deaths (Romero, Benavides *et al.,* 2018). The prevalence of subclinical mastitis (SCM) is higher than clinical mastitis and is more common in older lactating animals (Mbindyo *et al.,* 2020). It is a multi-etiological disease. *Staphylococcus aureus (S. aureus)* is the main bacterial cause of SCM, which leads to deep chronic mammary tissue infection resistant to

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treatment (Jaradat *et al.,* 2014). A high incidence of *S. aureus* is commonly associated with improper farm hygiene and management systems, especially a lack of post-milking teat dipping and sanitization of the milking system (El-Diasty *et al.,* 2021), (Mohammed *et al.,* 2019).

Intramammary antibiotic treatment of SCM cases is generally unsuccessful in eliminating existing *S. aureus,* resulting in the culling of the diseased animals (Ghobrial *et al.,* 2018). Recently, methicillin-resistant *S. aureus* isolates have become widely spread, with the risk of resistance to all beta-lactams and other classes of antibiotics used in the animal field. So, the therapeutic choices for antibiotics in treating infected animals are very limited (Diederen *et al.,* 2005).

S. aureus isolates show pathogenic characteristics, such as clonal evolution, mutation, horizontal gene transfer, and antibiotic resistance. The *mecA* gene encodes PBP2a-mediated resistance to methicillin. *tetK* gene encodes for drug efflux pump-mediated resistance to tetracycline (Ochoa *et al.* 2008). Many genetic determinants encoding antimicrobial resistance, such as *blaZ* (penicillin resistance), *mecA*, *tetK*, *erm* type genes (macrolides resistance) help *S. aureus* to survive for a long period inside the herd environment or the host and avoid the efficacy of antimicrobial therapy (Kumar *et al.,* 2011).

The hematological parameters including total RBC count, total WBC count, and differential leukocytic count are considered important diagnostic tools for SCM. Liver enzymes are sensitive to udder infection (El-Demerdash *et al.,* 2023). Acute phase protein is mainly formed in the liver hepatocytes, and its concentration differs in case of trauma, infection, or inflammation. It includes ferritin and C-reactive protein CPR (Chen *et al.,* 2006).

This study was conducted to estimate hematobiochemical changes in SCM cattle. It also aimed to isolate and identify multidrug-resistant *S. aureus*, detect their antibiotic susceptibility pattern, and study resistance genes in these isolates.

MATERIALS AND METHODS

1. Ethical approval:

This work complied with all relevant national regulations and institutional policies for the care and use of animals. It was approved by the Medical Research Ethics Committee, Faculty of Medicine, Sohag University (OHRP #: IRB00013006) under registration number: Soh- Med-23-10-11PD, on 9th October 2023.

2. Study sample:

This study included one hundred lactating cattle from dairy farms aged 3 - 6 years. It was conducted from December 2022 to February 2023 at Sohag governorate, Egypt.

3. Clinical examination of cattle:

This was done according to Constable *et al.* (2016) to detect the signs of inflammation. Then, the California Mastitis Test (CMT) was carried out as a screening tool to identify SCM-infected cows (Schalm and Noorlander 1957). Ten healthy cows without clinical signs, showed a negative result for CMT, were used as control animals.

4. California Mastitis Test (CMT):

An equal volume of milk samples and the CMT reagent (2ml of each quarter) were mixed thoroughly in a cup of black plastic paddle. Then the mixture was gently rotated for 10 seconds, and the results were recorded (Moroni *et al.,* 2006).

5. Haemato-biochemical examinations:

Five ml of blood were collected and divided into two parts, part in a plain tube for biochemical analysis, and the other part in an EDTA test tube for haematological analysis. The blood in the plain tube was allowed to stand for clotting for 20 minutes at 37˚C. Centrifugation of samples at 2000-3000 rpm for 10-15 minutes was done. Serum

was aspirated using Pasteur pipette into Eppendorf tubes at -20°c until testing (Coles 1967). Care measures were considered to keep these samples suitable for biochemical analysis. These measures included constant freezing, protection from light, and thawing when needed (Norbert 1986). This serum was used for the determination of total protein and albumin (g/dl) after the method described by Tietz (1995). Serum globulin (g/dl) was determined mathematically by subtraction of the serum albumin level from the serum total protein level. ALT, AST, and ALP (u/l) were measured after the method described by Bauer *et al.* (1999), Young (1997), and Zwata *et al.* (1994) respectively. All parameters were measured using a test kit supplied by Spectrum, an Egyptian company of biotechnology, using the (RoBonic) Prietest ECO biochemistry analyzer. Serum was also used for CRP detection using immunoassay-based fluorescence kits (QAYEE-BIO, Life Science, Co., Ltd., Daegu, South Korea), according to the manufacturer's instructions.

About 2.5 ml whole blood was collected in an EDTA test tube and used for estimation of RBC count, Hemoglobin concentration (Hb), Packed cell volume (PCV), White blood cell count (WBCs), and differential leukocytic count by using (Mindray, BC-1800) hematology analyzer.

6. Milk samples collection:

Milk samples were collected for bacteriological examination after cleaning and disinfecting the animal teat end and hands of milkers with soap and water. Alcohol (70%) was used for disinfection of teats and teat orifices before the collection of milk samples for examination (Kerro and Tareke 2003). The first jets of milk from each quarter of the cow udder were discarded. Then 5-10 ml was taken in sterile vials. They were labeled with the identification number of affected cows and the date of collection, then the samples were brought to the Animal Health Research Institute laboratory, Sohag Lab., Egypt for bacteriological examination.

7. *S. aureus* isolation:

A volume of 25 ml of collected milk sample was kept in a sterile glass flask with 225 ml of tryptose soya broth containing 7% NaCl (HI-MEDIA®, Mumbai, India) and incubated for 24 hrs at 37 °C. A loopful of the culture was added onto Baird-Parker agar (HI-MEDIA®, Mumbai, India) plates, and Mannitol Salt Agar (HI-MEDIA®, Mumbai, India) incubated at 37 °C for 24 hrs. Identification of the suspected colonies according to morphology and Gram staining was done, then catalase tests and coagulase tests were performed (Cruickshank *et al.,* 2000).

8. Antibiotic sensitivity testing of *S. aureus*:

The 32 isolates of *S. aureus* were tested for their antibiogram using the disc diffusion method against 10 antibiotics (Oxoid) (Breuer 1996). The antibiotic discs used in the current study were penicillin (10 µg), ampicillin (10 µg), vancomycin (30 µg), chloramphenicol (30 µg), amoxicillin and clavulanic acid (30 µg), imipenem (10 µg), azithromycin (15 µg), amoxicillin (25 µg), tetracycline (50 µg), and ciprofloxacin (5 µg). The interpretation of results was applied according to CLSI (He *et al.,* 2024).

9. DNA extraction:

DNA extraction of S. aureus isolates was performed using the QIAamp DNA Mini kit (Qiagen, Germany). Briefly, 200 µl, 20 µl and 200 µl of overnight culture suspension, proteinase K (20mg/ml), and lysis buffer, respectively, were mixed and incubated at 56 °C for 10 min. Then, 200 µl of 100% ethanol was added to the lysate. Washing and centrifugation of samples was done following the manufacturer's recommendations, and nucleic acid was eluted with 100 µl of elution buffer of the kit. Primers used in this study were supplied by Metabion (Germany), as shown in Table (1).

10. Molecular identification of the suspected *S. aureus* isolates and detection of antibiotic resistance genes:

The 32 recovered *S. aureus* isolates were confirmed by PCR amplification of

Staphylococcal *16S rRNA* (Mason *et al.,* 2001) then screening for antibiotic resistance genes: *blaZ* for detection of β-lactam resistance (Penicillinase) (Bagcigil *et al.,* 2012), *tetK* encoding tetracycline resistance (Duran *et al.,* 2012), *optrA* mediating phenicols resistance (chloramphenicol) (Wang *et al.,* 2015), *vanA* encoding vancomycin (glycopeptides) resistance (Patel *et al.,* 1997), *mecA* for detection of methicillin resistance (McClure *et al.,* 2006), *mphC* for examination of macrolide resistance (Schlegelova *et al.,* 2008), and *norA* for the detection of quinolone resistance (Pourmand *et al.,* 2014) by using primers (supplied by Metabion (Germany)) listed in Table (1). PCR amplification was performed in an Applied Biosystem 2720 thermal cycler.

11. Analysis of the PCR Products:

At room temperature, the products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer using gradients of 5V/cm. For gel analysis a gene ruler 100 bp ladder (Fermentas, thermo, Germany), gelpilot 100 bp and 100 bp plus ladders (Qiagen, Gmbh, Germany), and Genedirex 50 bp DNA ladder RTU, Cat. No. DM012-R500 were used to determine the fragment sizes. The gel was photographed (Alpha Innotech, Biometra) and analyzed using computer software.

12. Statistical analysis

Data of serum biochemistry and hematology were analyzed for the mean and standard deviations. The significance of the results was evaluated using an independent sample T-test (SPSS program), after the methods described by Snedecor and Cochran (1980). The results were considered significant at $p < 0.05$.

RESULTS

The examination of 100 lactating cows for cases of mastitis revealed that 32 cows (32%) had SCM by CMT. The current study revealed an increase in levels of AST, ALT, ALP, total protein, albumin, and CRP in SCM cows, compared to controls (Table 2).

Hematological analysis in SCM cases when compared to controls at the level of P value < 0.05 revealed a significant decrease in RBCs count, Hb, and PCV, while a significant increase in the levels of total leukocytic count, neutrophils, and monocytes percentage, and there was no significant change in the levels of globulin, monocytes, lymphocytes and eosinophils percentage (Tables 2 and 3).

S. aureus was confirmed in 32 milk samples by bacteriological isolation and identification.

The phenotypic antimicrobial sensitivity pattern of 32 *S. aureus* isolates from SCM cases proved that all isolates were susceptible to gentamycin, erythromycin, trimethoprim and sulfamethoxazole. The isolates were resistant to penicillin (65.6%), followed by ampicillin (56%), imipenem and amoxicillin (44%) each, tetracycline (40.6%), vancomycin (37.5%), amoxicillin and clavulanic acid and ciprofloxacin (34%) each and chloramphenicol (31%) (Table 4).

Molecular identification by PCR proved that all 32 isolates were positive for the 16S rRNA gene. All beta-lactam-resistant isolates carried the *blaz* gene. All methicillin-resistant isolates possess the *mecA* gene. All tetracycline-resistant isolates had the *tetK* gene. All vancomycin-resistant isolates possess the *vanA* gene. All quinolones resistant isolates encode *norA* gene. Although phenotypic antimicrobial resistance profiles showed 10 samples resistant to phenicols (chloramphenicol), genotypic resistance proved that the *oprtA* gene was detected in 7 samples while absent in three samples. Also, phenotypic antimicrobial resistance showed 9 samples resistant to macrolides, and the genotypic resistance detected the *mphC* gene in 7 samples, while it was absent in two samples (Figure 1).

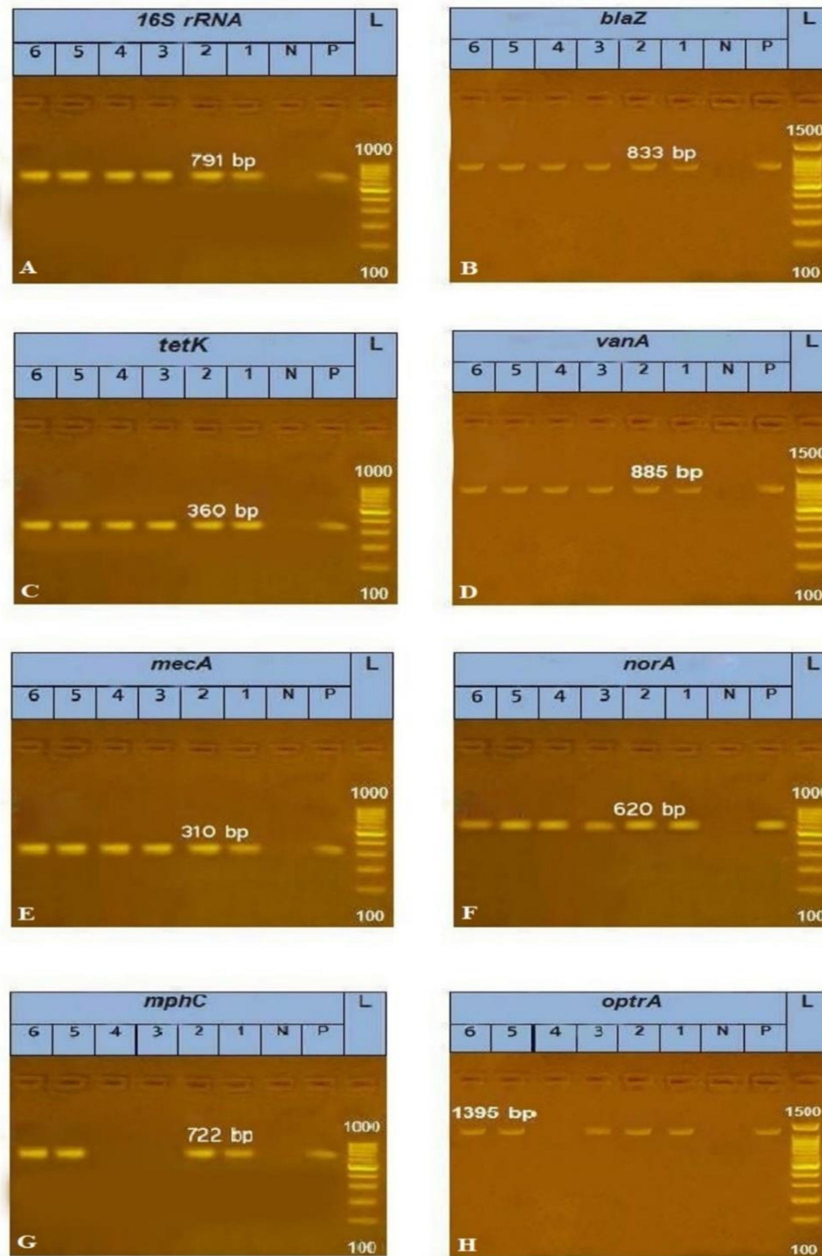

Figure (1): Gel electrophoresis and PCR amplification for S. aureus. Lane (L): DNA marker, lane (P): positive control, lane (N): negative control, (bp) base pair.
(A): 1, 2, 3,4,5,6 lanes are positive for the 16S rRNA gene (791 bp).
(B): 1, 2, 3,4,5,6 lanes are positive for the blaZ gene (833 bp).
(C): 1, 2, 3,4,5,6 lanes are positive for tetK gene (360 bp).
(D): 1, 2, 3,4,5,6 lanes are positive for vanA gene (885 bp).
(E): 1, 2, 3,4,5,6 lanes are positive for mecA gene (310 bp).
(F): 1, 2, 3,4,5,6 lanes are positive for norA gene (620 bp).
(G): 1, 2,5,6 lanes are positive, while 3, and 4 lanes are negative for the mphC gene (722 bp).
(H): 1, 2, 3,5,6 lanes are positive, while lane٤ is negative for the optrA gene (1395 bp).

Table 1: Oligonucleotide primers sequences for detection of the suspected *S. aureus* isolates and detection of antibiotic resistance genes

Name of genes	Primers (5'-3')		Condition of amplification					Reference
16S rRNA	CCTATAAGACTGGGATAACTTCGGG CTTTGAGTTTCAACCTTGCGGTCG	791 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	(Mason, Blevins *et al.,* 2001)
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	(McClure, Conly *et al.,* 2006)
mphC	GAGACTACCAAGAAGACCTGACG CATACGCCGATTCTCCTGAT	722 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	(Schlegelova, Vlkova *et al.,* 2008)
tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	(Duran, Ozer *et al.,* 2012)
norA	TTCACCAAGCCATCAAAAAG CTTGCCTTTCTCCAGCAATA	620 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	(Pourmand, Yousefi *et al.,* 2014)
blaZ	TACAACTGTAATATCGGAGGG CATTACACTCTTGGCGGTTTC	833 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	(Bagcigil, Taponen *et al.* 2012)
vanA	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	(Patel, Uhl *et al.,* 1997)
optrA	AGGTGGTCAGCGAACTAA ATCAACTGTTCCCATTCA	1395 bp	94˚C 5 min.	94˚C 30 sec.	53˚C 1 min.	72˚C 1.2 min.	72˚C 10 min.	(Wang, Lv *et al.,* 2015)

Table 2: Changes associated with SCM on some biochemical parameters (mean± SD)

Parameters	Control	SCM
ALT (u/l)	37±5	46±3.5**
AST (u/l)	24.5±4	28.5±5.5*
ALP (u/l)	52.5±0.9	46±0.83**
Total protein (g/dl)	7.2±0.5	5±0.32*
Albumin (g/dl)	4.5±0.37	2.4±0.2*
Globulin (g/dl)	2.7±0.4	2.6±0.19
CRP (pg/ml)	6.8±3.5	32.5±12.5**

*Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table 3: Changes associated with SCM on some hematological parameters (mean±SD).

Parameters	Control	SCM
Total erythrocytic count (10^6/ml)	7.7± 1	4.6±0.3*
Hb (gm%)	9.5±1.2	6.8±0.87*
PCV %	34.3±1.9	28±1.3**
Total leukocytic count (10^3/ml)	8.4±0.7	11.6±0.5**
Neutrophil %	45±0.4	48.5±0.58*
Lymphocyte %	42.5±0.5	45.02±0.8*
Basophil %	3.5±0.32	2.8±0.12
Eosinophil %	4.2±0.4	3.5±0.1
Monocytes %	3.9±0.12	3±0.3

*Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table 4: Antimicrobial resistance profiles of *S. aureus* isolated from SCM.

Antibiotic	Sensitive (n-%)	Intermediate (n-%)	Resistant (n-%)
Gentamycin (0.4)	21 (65.6%)	11 (34%)	-----
Erythromycin (ER 15)	19 (59.3%)	13 (40.6%)	-----
Trimethoprim and sulfamethoxazole (SMT25)	18 (56%)	14 (44%)	-----
Penicillin (P10)	7 (21.7%)	4 (12.5%)	21 (65.6%)
Tetracycline (TE 50)	9 (28%)	10 (31%)	13 (40.6%)
Chloramphenicol (C30)	15 (47%)	7 (21.7%)	10 (31%)
Amoxicillin and clavulanic acid (AMC 30)	18 (56%)	3 (9%)	11 (34%)
Vancomycin (VA 30)	13 (40.6%)	7(21.7%)	12(37.5%)
Imipenem (IMP 10)	12 (37.5%)	6 (18.5%)	14(44%)
Amoxicillin (AX 25)	14 (44%)	4 (12%)	14 (44%)
Azithromycin (AZM 15)	18 (56%)	5 (15.6%)	9 (28%)
Ciprofloxacin (CIP 5)	14 (44%)	7 (21.7%)	11(34%)
Ampicillin (AS 10/10)	12 (37.5%)	2 (6%)	18 (56%)

DISCUSSION

Mastitis is a devastating disease caused by interactions between the host animal, environment, and infectious agents. It leads to large economic losses, as it reduces the quantity and quality of milk, besides the high veterinary costs due to the antibiotic price and the withdrawal time post-treatment (Dufour *et al.,* 2019).

This study revealed that out of one hundred examined cows, 32 cows (32%) had SCM due to *S. aureus* after examination of milk samples by CMT and bacteriological isolation. On one hand, this percentage is nearly similar to that reported by EL-Damaty (2013) and Algammal *et al.* (2020), who proved that the prevalence of *S. aureus* recovered from examined cases of SCM cows was 31.9% and 36.3%; respectively. On the other hand, this ratio is greater than that reported by Talaat *et al.* (2023) and Ayano *et al.* (2013), who observed that the incidence of *S. aureus* in SCM cows was 15.7% and 13.8%; respectively.

A high incidence of *S. aureus* in milk is mainly due to improper hygiene during handling, poor management during milking, lack of teat dipping, poor housing and milking equipment, absence of dry therapy, and sometimes related to bad hygiene bedding material (Daka *et al.,* 2012). Ranjan *et al.* (2011) reported that the main sources of *S. aureus* in SCM are milk of the infected gland and the skin of the udder, leading to the spread of infection, penetration of the tissues, and production of deep foci. The variations between the results of different reports may be due to the sample size, geographical distribution, management practice, and sanitary measures (Barua *et al.,* 2014).

This study proved a significant increase in the concentration of AST, ALT, and ALP. Hussein and Ahmed (2019) reported a significant increase in liver enzyme levels in SCM animals, compared to healthy controls. This may be attributed to damage to hepatic tissues in cases of SCM (Abubaker *et al.,* 2023).

The current study showed a significant decrease in total protein and albumin in SCM cows, compared to control ones. These observations are consistent with Darwish *et al.* (2003) and Allam *et al.* (2014), who proved a decrease in levels of total protein and albumin in SCM cows. This decrease in albumin level can be explained by infiltration

of albumin from blood to milk, as a result of increased permeability of blood vessels caused by inflammation of the udder parenchyma (Honkanen and Seuna 1995, Singh and Pachauri 2002). Godden *et al.* (2002) concluded that bacterial infection and its toxins can induce damage to liver parenchyma, leading to failure of albumin production.

This work demonstrated a significant increase in CRP values in SCM animals. This agrees with Ali *et al.* (2023), who found that the concentration of CRP was 29.7 pg/ml in the serum of SCM cows. That was more than in control animals, which was 5.4 pg/ml. The acute phase protein is an important biomarker for inflammation, so its concentration increased following SCM and udder inflammation. It is produced by the liver and has an important function in phagocytosis (Black and Kushner 2004).

Hematological analysis of the blood of SCM cows revealed a significant decrease in total erythrocytic count, Hb, and PCV, while there was an elevation in the levels of the total leukocytic count, neutrophils, and lymphocytes percentage. There was a non-significant change in the basophils, eosinophils, and monocytes percentages in SCM cases, compared to controls. Similar findings were detected by Allam *et al.* (2014) and Alhussien *et al.* (2015).

The decrease in total erythrocytic count and Hb content may be attributed to the effect of bacterial toxins and enzymes of *S. aureus* that cause damage to the mammary tissues. The increase in total leukocytic count is due to the body's defense mechanism against this bacterial infection (Workineh *et al.,* 2002), (Abed *et al.,* 2021).

Antimicrobials possess an important role in SCM control programs in dairy cows (Awandkar *et al.,* 2013). Thus, the investigation of antibiotic susceptibility is essential to ensure the ideal results of antibiotic use against bacterial infection. This can be achieved through correct selection based on antibiogram studies (Awandkar *et al.,* 2009).

The phenotyping results of the current study revealed high resistance rates towards penicillin (65.6%), ampicillin (56%), imipenem (44%), amoxicillin (44%), tetracycline (40.6%), vancomycin (37.5%), amoxicillin-clavulanic acid (34%), ciprofloxacin (34%) and chloramphenicol (31%). These data are following Kenar *et al.* (2017), who found that *S. aureus* isolated from SCM cows were resistant to penicillin (63%), ampicillin (61.5%), erythromycin (48.2%), tetracycline (34.9%), chloramphenicol (18.6%) and vancomycin (4.9%). Talaat *et al.* (2023) reported high resistance of *S. aureus* isolates to ampicillin (93.3%) , tetracycline (73.3%), chloramphenicol (36.7%), and ciprofloxacin (30%). Also, our findings agreed with Daka *et al.* (2012), who concluded that *S. aureus* was highly resistant to ampicillin, followed by amoxicillin-clavulanic acid. Similarly, Abubaker *et al.* (2023) reported high resistance of *S. aureus* to penicillin (48%), amoxicillin and clavulanic acid (3%), tetracycline (67%), ciprofloxacin (8%) and chloramphenicol (60%). The rate of penicillin resistance of *S. aureus* in this research is similar to that of Abdul Qayyum *et al.* (2016), who reported high resistance of *S. aureus* to penicillin (65%) isolated from SCM.

Failure to respond to antibiotic therapy is multifactorial in lactating animals. It may be attributed to the presence of micro-abscesses within the udder and the inaccessibility of the drug to the causative agent of infection, and avoiding the effect of antibiotics by residing inside macrophages. Some strains of *S. aureus* can exist as latent bacteria covered by a capsule and then reactivate when conditions become suitable (Du Preez 2000). Other factors are related to the veterinarians, such as inappropriate therapy, suboptimal drug dose, or shortening the treatment protocol time. Prescription of antibiotics without applying antibiotic sensitivity tests increases

microorganism-resistant strains (Hendriksen *et al.,* 2008). Bacterial antibiotic sensitivity differs from one area to another. These differences in sensitivity and resistance to antibiotics may be attributed to random use of antibiotics in different herds (Abdul Qayyum *et al.,* 2016).

Molecular diagnosis is the most suitable technique for the identification of different strains of bacteria that are difficult to be identified by conventional methods (Qian *et al.,* 2014). In this study, the conventional PCR detected the *16S rRNA* gene in all isolates. All antibiotic-resistant isolates were analyzed for the presence of *blaZ, tetK, vanA, optrA, norA, mphC,* and *mecA* genes. The presence of *mecA* and *blaZ* genes was nearly similar to the findings of Jamali *et al.* (2015), who reported that all *S. aureus* isolates were positive for the *mecA* gene and *blaZ* gene was present in 97.4% of the *S. aureus*. Also, Talaat *et al.* (2023) isolated *blaZ* gene from all *S. aureus* isolates.

The *mecA* gene was found in all methicillin-resistant *S. aureus*. Similarly, Abubaker *et al.* (2023) reported the presence of the *mecA* gene in all *S. aureus* isolates, and concluded that the *mecA* gene was considered an important marker gene for detecting methicillin-resistant *S. aureus* isolates. This gene is considered an inducible 76-kDa penicillin-binding protein, that is carried on a mobile genetic element (Staphylococcal Cassette Chromosome mec (SCCmec)) (Abed *et al.,* 2021).

The *blaZ* gene was detected in all isolates resistant to beta-lactam. This agrees with Feng *et al.* (2016) and Awad *et al.* (2017), who demonstrated the prevalence of the *blaZ* gene at 95.5% and 95.7%, respectively. In this study, the *mecA* gene was detected in all isolates resistant to imipenem. On the contrary, Huber *et al.* (2010) found that the *mecA*-resistant gene percentage occurred at a low rate of 1.42%.

The resistance to beta-lactams may be attributed to the β-lactamase encoded *blaZ* production, which causes hydrolysis to penicillin (Olsen *et al.,* 2006). Sawant *et al.* (2009) reported that methicillin/oxacillin resistance is a β-lactam resistance mechanism, which occurs due to the penicillin-binding protein production low affinity encoded by the *mecA* gene.

In this work, the *tetK* gene was detected in all isolates by genotyping. This is similar to Ho *et al.*(Ho *et al.,* 2012), who concluded that the prevalence of tetracycline-resistant isolates carried the *tetK* gene was (97%).

Antimicrobial drug resistance can be performed phenotypically by antibiotic sensitivity tests or genotypically by molecular tests. In the present study, there was an agreement between phenotypic and genotypic evaluation, except in phenicols (chloramphenicol) and macrolides. Phenotypic antimicrobial resistance profiles showed 10 samples resistant to phenicols (chloramphenicol), while genotypic resistance proved that the *oprtA* gene was detected in 7 samples and absent in three samples. Also, phenotypic antimicrobial resistance showed 9 samples resistant to macrolides, while the genotypic resistance detected the presence of the *mphC* gene in 7 samples and was absent in two samples.

The differences between phenotyping and genotyping agreed with Feng *et al.* (2016), who found variation between patterns of phenotypic and genotypic tetracycline resistance. These results suggested that genes are not the only factor responsible for antibiotic resistance.

In this study, the genes vanA and norA were detected in all isolates resistant to vancomycin and quinolones, respectively. This is consistent with the study of Hoque *et al.* (2018), who reported that the *vanA* and *norA* genes were detected in 37.5% and 28%, respectively.

CONCLUSION

Subclinical mastitis is associated with multiple changes in the haemato-biochemical parameters that can be used as vital indicators of this illness in dairy farms. *S. aureus* is an important cause of SCM, which is highly resistant to antibiotic treatment. Antibiotic susceptibility tests should be performed in SCM to improve the selection of the appropriate antimicrobial drugs and to avoid antibiotic resistance. Molecular surveillance is essential for the detection of *S. aureus* resistance genes.

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الفحص الجزيئى لميكروب المكور العنقودي الذهبي "الاستافيلوكوكس أوريس" المقاوم للعديد من المضادات الحيوية في الأبقار المصابة بالتهاب الضرع تحت الإكلينيكى في محافظة سوهاج

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المكورات العنقودية الذهبية (S. aureus) هي السبب البكتيري الأكثر أهمية لالتهاب الضرع تحت الاكلينيكي (SCM) المقاوم للعلاج. أجريت هذه الدراسة على الأبقار المصابة بالتهاب الضرع تحت الاكلينيكي (SCM) لتقدير التغيرات الكيميائية الحيوية الدموية، وعزل وتحديد S.aureus المقاومة للمضادات الحيوية المتعددة وتحديد مدى حساسيتها للمضادات الحيوية للكشف عن الجينات المقاومة للمضادات الحيوية. تم فحص ١٠٠ بقرة حلابة من المزارع المختلفة بواسطة اختبار كاليفورنيا الذي كشف وجود عن ٣٢ (٣٢ ٪) حالة مصابة بالتهاب الضرع تحت الاكلينيكي (SCM). تم جمع عينات الدم واللبن من الحالات المصابة و من ١٠ عينات من حيوانات سليمة كمجموعة ضابطة.

أظهرت هذه الدراسة زيادة كبيرة في AST، ALT، ALP، إجمالي عدد كرات الدم البيضاء، و CRP بينما لوحظ انخفاض كبير في مستويات عدد كريات الدم الحمراء، إجمالي البروتين والألبومين في الحالات المصابة عند مقارنتها بالمجموعة الضابطة.

كانت المضادات الحيوية لعزلات ٣٢ S.aureus شديدة المقاومة للبنسلين (٦٥,٦ ٪) أمبيسلين (٥٦ ٪)، إيميبينيم وأموكسيسيلين (٤٤ ٪) لكل منهما. تبعها التتراسيكلين (٤٠,٦ ٪)، الفانكوميسين (٣٧,٥ ٪)، الأموكسيسيلين.

كشف التحديد الجزيئي بواسطة تفاعل البوليميراز المتسلسل أن جميع العزلات الـ ٣٢ كانت إيجابية لجين 16S rRNA وحملت جينات مقاومة المضادات الحيوية blaz، tetK، optrA، vanA، mecA، mphC.

وتستخلص الدراسة امكانية استخدام التغييرات البيوكيميائية الدموية المرتبطة بالتهاب الضرع تحت الاكلينيكي (SCM) كمؤشرات حيوية للإصابة بالمرض. وأن S. aureus هو سبب مهم لـ SCM وهو مقاوم للغاية للعلاج بالمضادات الحيوية. وأن تفاعل البوليميراز المتسلسل ضروري للكشف عن جينات مقاومة S. aureus. وتوصي الدراسة بعمل اختبارات الحساسية للمضادات الحيوية لاختيار الأدوية المضادة للميكروبات المناسبة وتجنب مقاومة المضادات الحيوية.