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# Antimicrobial Resistance of *Staphylococcus aureus* and Coagulase-negative *Staphylococci* from Bovine Mastitis Milk with Detection of Interleukins in Milk and Serum of Infected Cows

Aya Shehab*, Rasha Elkenany, Gamal Younis

Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Mansoura University, 35516 Mansoura, Egypt

***Correspondence**
Corresponding author: Aya Shehab
E-mail address: ayhm00127@gmail.com

## Abstract

*Staphylococci* are the most typical bacteria found in cattle with mastitis, either *Staphylococcus aureus* or coagulase-negative *Staphylococci* (CNS). The study's goal was to determine the prevalence of *Staphylococci* in bovine mastitis, the antimicrobial profile, and evaluate the concentration of pro-inflammatory interleukins (IL-4, IL-6, IL-10) related to the inflammatory response in clinical mastitis by ELISA. *S. aureus* (54%) and CNS (19%) were detected in 400 samples of milk from both clinical and subclinical bovine mastitis. The *S. aureus* isolates revealed higher resistance to ampicillin (100%), followed by amoxicillin-clavulanic acid (98.1%), cefotaxime (88.9%), erythromycin (63.2%), cefuroxime (63%), and tetracycline (61.1%). Furthermore, the CNS isolates showed high resistance against amoxicillin-clavulanic acid (100%), followed by ampicillin (94.7%), cefotaxime (89.5%), erythromycin (79.6%), cefuroxime (89.5%), and tetracycline (73.7%). However, the high susceptibility of *S. aureus* and CNS was observed to imipenem and ciprofloxacin. Furthermore, Cows with clinical mastitis reported high levels of IL-6 in both their serum and their milk. While they have much lower levels of IL-4 and IL-10 than normal ones ($P < 0.001$). In conclusion, it is recommended that laboratory results be carefully interpreted to avoid antimicrobial therapy for *Staphylococci* that is not clinically relevant and to ensure the advisable use of antimicrobials. Also, further study on the application of interleukins as therapeutic agents against bovine mastitis should be considered.

KEYWORDS
*Staphylococcus aureus*, Coagulase-negative *Staphylococci*, Bovine mastitis, Antimicrobial resistance, Interleukins.

## INTRODUCTION

Bovine mastitis, which is classified into clinical and subclinical forms, ranks as one of the most widespread and expensive infectious diseases affecting the dairy sector (Pol and Ruegg, 2007; Côté-Gravel and Malouin, 2019). *Staphylococcus* species are one of the most common pathogens isolated in mastitis cases (Pyörälä and Taponen, 2009). *Staphylococcus* species are categorized into coagulase-positive *Staphylococci* (CPS), which include *S. aureus*, and coagulase-negative *Staphylococci* (CNS) (Taponen and Pyörälä, 2009) Contagious bovine mastitis is primarily caused by *Staphylococcus aureus*, whereas CNS were previously thought to be minor pathogens. Currently, CNS has become the most frequently detected pathogens from bovine intramammary infections in several countries (Botrel *et al*., 2010; Kalmus *et al*., 2011; Persson *et al*., 2011; Bochniarz *et al*., 2013). *S. chromogenes*, *S. simulans, S. haemolyticus, S. xylosus*, and *S. epidermidis* are examples of CNS species that may persist in the udder and cause a mild to moderate rise in SCC as well as a potential slight decrease in milk output (Vanderhaeghen *et al*., 2014).

Antimicrobial therapy is frequently used to treat bacterial diseases like bovine mastitis. Antibiotics such as ampicillin, tetracycline, penicillin, gentamicin, and others are utilized to inhibit the progression of mastitis and can be administered intra-mammary, intramuscular, or intravenous. As a result, antimicrobial resistance (AMR) has emerged as a major issue impeding the treatment of mastitis pathogen infections. It has been reported that *Staphylococci* are resistant to various antibiotics utilized for treating mastitis in dairy cattle (Tesfaye *et al*. 2019). This decreases the effectiveness of antimicrobial therapy for bovine mastitis. Many human disease-causing bacteria species have developed resistance to the most widely used antibiotics in the past ten years, raising some serious red flags in various countries (Garrison *et al*., 2009; Kenar *et al*., 2012). Because *Staphylococcus aureus* is less antibiotic-resistant than CNS, coagulase-negative *Staphylococci* (CNS) outbreaks must be closely monitored (Taponen and Pyörälä, 2009). CNS infections are currently treated with B-lactam antimicrobials and macrolides. The CNS resistance to B-lactam antibiotics, especially penicillin, and ampicillin, has been observed to be rising in several studies, which is a global trend (Botrel *et al*., 2010; Persson *et al*., 2011). Antibiotic resistance is associated with resistant genes, such as the *Staphylococcus aureus* that is resistant to methicillin (MRSA) has the mecA gene. Furthermore, it is even more challenging to treat an infection when *S. aureus* is present because of its capacity to form biofilm and adapt to the surroundings of the host (Rainard *et al*., 2018).

Important elements in determining the outcome of a bacterial infection include the type and potency of the host immune


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responses (Riollet *et al*., 2000). Lymphocyte subpopulations determine immune responses in mammary glands that are infected with *S. aureus*, as well as Leucocytes that have been recruited elsewhere and the cytokines they secrete (Yokomizo *et al*., 1994; Ferens *et al*., 1998; Persson Waller and Golditz, 1999). Interleukins, which are immuno-regulatory mediators, are crucial in regulating the immunological response to many infections. Dairy cows' immune systems are supported by cytokines, which control several physiological processes within cells. Furthermore, during the inflammatory and immune response processes, they function as cell communication transmitters. More and more cytokines are being used in mastitis immunotherapy, diagnosis, and prognosis. As we gain a better understanding of the cytokine network in the mammary gland of cattle and create more reliable diagnostic methods (Gonzalez *et al*. 2013). There are several types of interleukins, but the most common in bovine mastitis were interleukin-4 (IL-4), interleukin-6 (IL-6), and interleukin-10 (IL-10). IL-6 is a type of glycoprotein that is produced and secreted as a multifunctional cytokine by activated macrophages, lymphocytes, and epithelial cells (Huang *et al*., 2021). IL-6 is the strongest stimulator of acute-phase production and secretion (Song and Kellum, 2005). IL-6 has effects on cells other than hepatocytes and lymphocytes; it is stimulated by cytokines such as IL-4, which are commonly found in chronic inflammatory diseases (Scheller *et al*., 2011; Ezzat Alnakip *et al*., 2014). It stimulates keratinocyte proliferation and collagen production in dermal fibroblasts, which may explain alters in the skin of patients with systemic sclerosis (Tanaka *et al*.,, 2014a; 2014b).

Also, IL-10 has been recognized as a key inhibitor of inflammatory and immunological responses. IL-10 has a significant function. in reducing inflammation by inhibiting cytokine production by activated CD 4+ Th1 helper cells, as well as monocytes and macrophages' cytotoxic actions, in addition to the creation of APP and pro-inflammatory cytokines. In the early stages of an inflammatory response, Interleukins like IL-6 and IL-10 are highly effective, whereas cytokines produced by epithelial cells help the mammary gland's Toll-like receptors detect invasive infections (Mansour and Zeitoun, 2019; Al-Taiy *et al*., 2021). In humans, an IL-6/IL-10 imbalance has been linked to the development of multiple organ dysfunction (Moore *et al*., 2001). *S. aureus* superantigens increase interleukin-10 (IL-10) and interleukin-4 (IL-4) concentrations in the body (Ferens *et al*., 1998). In the course of *S. aureus* infection, excessive IL-10 and IL-4 production helped to promote immunosuppression by temporarily inhibiting CD4+ cells and recruiting a large number of CD8+ T suppressor cells (Ferens *et al*., 1998). Furthermore, in dairy cows, Interferon is inhibited by IL-4, which also controls innate immunity (IFN) (Ezzat Alnakip *et al*., 2014). Mastitis, whether experimentally induced or naturally occurring, increases concentrations of cytokines (interleukins (IL) such as IL-4, IL-6, and IL-10) in milk (Bannerman, 2009). Thus, as a result, the current research was focused on identifying the prevalence of *Staphylococcus* species strains in bovine mastitis milk as well as the antibiotic resistance profiles of such strains. The enzyme-linked immunosorbent assay was also used to detect interleukins in cows 'milk and serum suffering from clinical mastitis caused by *S. aureus* (ELISA).

## MATERIALS AND METHODS

*Sample collection*

From lactating cows, 400 milk samples were taken (150) from cows with subclinical mastitis that appeared to be in good health, and 250 from clinical mastitis-affected cows on six different farms in the Governorates of Damietta and Daquahlia, Egypt from March to September 2022. Inflammatory symptoms such as udder swelling, mammary gland edema, fever, and rapid heart rate were observed in cows with clinical mastitis. The presence of obvious abnormalities and mastitis milk was found. The teats were then swabbed with a clean cloth that had been dipped in 70% ethyl alcohol after the udder had been carefully cleaned and dried. Then dumped the first several milk squirts, and then 10 ml of milk samples were gathered from every animal at the appearance of clinical symptoms and kept for bacteriological examination. Also, twenty blood samples of cows with clinical mastitis and five healthy cows' blood samples as Jugular vein samples were used as the control by using a fresh and clean blood-letting needle and restraining the animal in a head-catch or squeeze chute.

*Isolation and identification of Staphylococcus species*

Following a 30-minute centrifugation of samples of milk at 3000 RPM, the sediment was cultured on nutrient agar, Baird Parker agar, mannitol salt agar, and trypticase soya agar (Oxoid LTD, England) and 24 hours of incubation at 37°C. The suspected colonies were identified as *Staphylococcus* depending on colony morphology, Gram staining, hemolysis on blood agar, and biochemical tests (catalase, DNAase, oxidase, coagulase, and urease tests).

*Molecular identification of S. aureus*

By amplifying the nuc gene using oligonucleotide primers, the uniplex PCR assay specific for *S. aureus* was carried out. (Metabion, Germany) nuc-F (5GCGATTGATGGTGATACGGTT3) and nuc-R (5AGCCAAGCCTTGACGAACTAAG3) (Oliveira *et al*., 2016) in accordance with Brakstad *et al*. (2009). PCR amplifications were carried out with a thermocycler (Biometra, Germany). The reaction mixture (25 L) contains 3 L of genomic DNA, 12.5 L of 2X PCR Master Mix (Takara code no RR310A), 1 µL of each of the two primers, and the remaining volume of nuclease-free water was added to bring the final volume to 25 L. The cycling conditions were as follows: an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and final elongation at 72°C for 7 minutes. The amplified PCR products were separated by electrophoresis in 1.5% agarose gel at 100 V for 30 minutes, stained with ethidium bromide, and then seen and identified with a UV trans-illuminator (Biometra, Germany).

*Antibiotic Sensitivity testing*

The antibiotic sensitivity testing for all isolates was done according to CLSI 2018 against the most used antibiotic discs on Muller-Hinton agar (Oxoid, Basingstoke, UK). We utilized the following antibiotic disks (Oxoid, Basingstoke, UK): β-lactams (ampicillin 15µg, amoxicillin-clavulanic acid 30µg), cephalosporins (cefotaxime 30µg, cefuroxime 30µg), glycopeptide (vancomycin 30µg), quinolones (ciprofloxacin 5µg), aminoglycosides (gentamicin 10µg), tetracyclines (tetracycline 30µg), macrolides (erythromycin 15µg), and carbapenems (imipenem 10µg). The isolates were classified as intermediate, susceptible, or resistant using the inhibition zone measurement and the (CLSI 2018) recommendations. A novobiocin susceptibility test was done to differentiate the coagulase-negative *Staphylococci* and to identify *Staphylococcus* saprophyticus, which is usually novobiocin resistant. Novobiocin (NV, 5g) resistance was defined by the existence of an

inhibition halo of 12 mm or less, and susceptibility was characterized by the presence of an inhibition halo of >16 mm (Konemann *et al*., 2001).

*Measurements of interleukins in serum and milk samples*

Pro-inflammatory cytokine concentrations were determined by a sandwich ELISA for IL-4 and a competitive ELISA for IL-6 and IL-10 in milk (n= 20) and blood serum (n=20) samples collected from cows with clinical mastitis using commercially available ELISA kits for IL-4, IL-6, and IL-10 (CUSABIO, Catalog Numbers, CSB-E12898B, CSB-E12899B, CSB-E12917B, respectively), as reported by the manufacturer. Every process was carried out in accordance with the manufacturer's recommendations and the techniques described in the literature. (Hagiwara *et al*., 2001). In brief To ELISA plates that had been pre-coated with a particular antibody for IL-4 or goat-anti-rabbit antibodies for IL-6 and IL-10, 100 µl l of standard and sample per well were added. The plates were sealed with the adhesive strip and kept at 37°C for two hours. Next, without washing, the liquid was taken out of each well. The addition of 100 µl l of Biotin-antibody (1x) to each well was followed by the covering of the plates, and an hour of incubation at 37°C. The plates were cleaned with a wash buffer three times. (200 µl) and then 100µl/well of HRP-avidin (1x) was added. The plates were covered and kept at 37°C for an hour of incubation. Five times the washing process was carried out. Each well-received 90µ l of TMB Substrate and the plates were then incubated at 37°C for 15–30 minutes. Finally, each well received 50 µl of Stop Solution. Each well's optical density was calculated in 5 minutes. Absorbance measurements were taken at 450 nm using an automatic microtiter plate reader (ELx800, Winooski, Biotek Instruments, VT) with 630 nm as a reference. According to the manufacturer, the detection ranges of IL-4, IL-6, and IL-10 for cows were 12.5-800pg/ml, 5 to 1000pg/ml, and 5 to 1,000 pg/mL, with the correction wavelength for IL4 at (540nm or 570 nm), and for IL6 and IL10 at (600 nm-630nm), respectively as reported by the manufacturer. All of the studied cytokines had inter- and intra-assay coefficients of variation (15%). It should be highlighted that all samples were contrasted with healthy samples; milk (n=5) and serum (n=5); taken from cows.

*Statistical analysis of the data*

The IBM SPSS software program version (20.0) was used to analyze the data after it was input into the computer (Armonk, NY: IBM Corp) (Kirkpatrick and Feeney, 2013). The qualitative data were described using percentages and numbers. The normality of the distribution was verified by the Kolmogorov-Smirnov test. Quantitative data were described using range (minimum and maximum), mean, and standard deviation. The acquired results' significance was assessed at a 5% level. The tests were carried out (Chi-square test, Student t-test, and Mann-Whitney test).

## RESULTS

*Prevalence of Staphylococcus species*

Microbiological analysis of 400 samples of milk obtained from symptomatic and asymptomatic mastitis cows revealed that 73% (292/400) of the isolates were *Staphylococcus* species: 187/292 (64%) came from cows with clinical mastitis and 105/292 (36%) from subclinical mastitis cases (Table 1). *Staphylococci* isolates showed circular smooth colonies with dissimilar colors (golden yellow, orange, pale yellow, creamy, pale yellow, and white ) on nutrient agar, black colonies surrounded by clear zones on Baird Parker agar, yellow colonies on mannitol salt agar as well as golden yellow colonies on tryptose soya agar. *Staphylococci* microscopically appeared as gram-positive small round cocci and arranged in clusters like grapes. Based on the coagulase test, Coagulase-positive *Staphylococci* (CPS) (216/400, 54%) and coagulase-negative *Staphylococci* (CNS) (76/400, 19%) were identified in staphylococcal isolates. *S. aureus* was completely identified in all CPS isolates (216, 54%) using a nuc gene PCR assay with an amplicon size of 270 bp (Figure 1).

Table 1. Prevalence of Staphylococcal species in milk samples

| Source of milk Samples | CPS (*S. aureus*) | CNS | Total |
|---|---|---|---|
| Clinical mastitis (n=250) | 145(58 %) | 42 (16.8%) | 187 (64%) |
| Subclinical mastitis (n=150) | 71 (47.3%) | 34 (22.7%) | 105 (36%) |
| Total (n=400) | 216 (54%) | 76 (19%) | 292 (73%) |

CPS; coagulase-positive *Staphylococci*, CNS; coagulase-negative *Staphylococci*

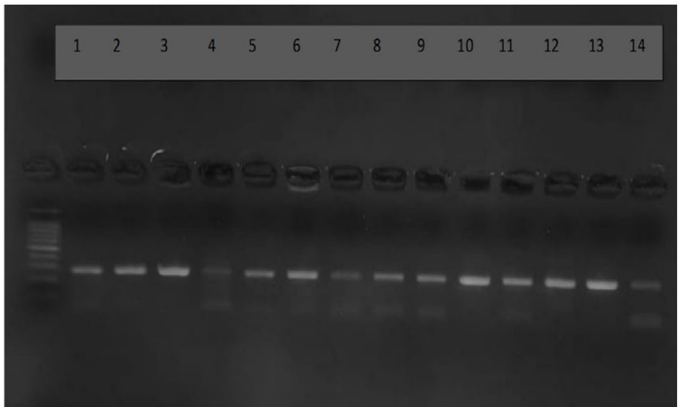


Fig. 1. Agarose gel electrophoresis showing amplification of *Staphylococcus aureus* nuc gene (270 bp). Lane M: 100 bp ladder, lanes 1-3,5,6, 8-13: positive samples; lane 14: control negative.

*Antibiotic resistance phenotypic profile*

The susceptibility of each isolate to several antimicrobial drugs from different classes was examined (Table 2 and Fig. 2). The CPS (*S. aureus*) isolates exhibited complete ampicillin resistance (100%), followed by amoxicillin- clavulanic acid (98.1%), cefotaxime (88.9%), erythromycin (79.6%), cefuroxime (63%), and tetracycline (61.11%). The CPS isolates showed high susceptibility to imipenem (94.4%) and ciprofloxacin (72.2%). Furthermore, the CNS isolates showed high resistance against amoxicillin-clavulanic acid (100%), followed by ampicillin (94.7%), cefotaxime (89.5 %), erythromycin (63.2%), cefuroxime (89.5 %), and tetracycline (73.7%). The susceptibility of CNS was observed to be highest for imipenem (94.7 %) and ciprofloxacin (63.1). According to novobiocin susceptibility tests, All CPS (*S. aureus*) isolates exhibited novobiocin resistance. While Novobiocin sensitivity was present in 57.9% of CNS isolates. And regarded as staph epidermidis while 42.1% of CNS isolates were novobiocin resistant and considered as S. saprophyticus.

*Determination of interleukins in serum and milk*

The concentrations of interleukins (IL-4, IL-6, and IL-10) found in blood serum and milk of cows with clinical mastitis caused by *S. aureus* and normal cows are shown in Table 3 and Figure 3. The current study found that IL-6 levels in clinical mastitis cows were higher in serum and milk (249.87 and 378.51 pg/mL, respectively) than in healthy cows (13.34 and 166.38 pg/mL, respectively; P< 0.001). IL-4 concentrations, on the other hand, were considerably

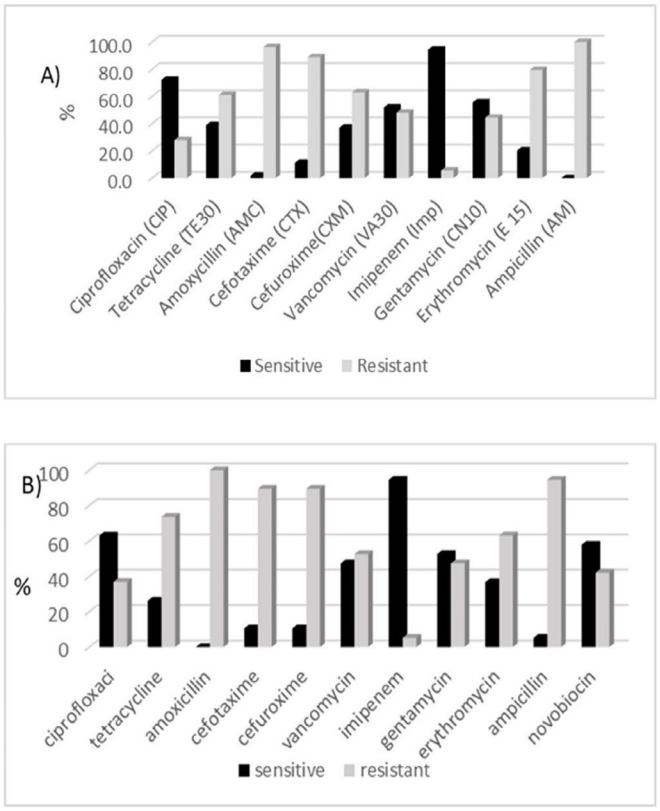


Fig. 2. The antibiotic resistance distribution of A) Coagulase-positive *Staphylococci* (n=216) and B) Coagulase-negative *Staphylococci* (n =76).

less in both serum and milk of cows with staphylococcal mastitis (118.43 and 112.73 pg/mL, respectively) compared to healthy cows (678.34 and 400.62 pg/mL, respectively; P < 0.001). In addition, staphylococcal mastitis-affected cows' serum and milk levels of IL-10 were considerably lower (20.67 and 171.76 pg/mL; P < 0.001) than those of unaffected cows (37.50 and 216.64 pg/m; P <0.001).

## DISCUSSION

*Staphylococci* are the most frequent cause of clinical or subclinical bovine mastitis, which significantly damages the Egyptian dairy industry by reducing milk production, contaminating milk, disposing of milk, and treatment costs. In the current research, a high prevalence (73%) of *Staphylococcus* species strains was obtained from cows with clinical signs of mastitis (64%) or subclinical mastitis (36%). Previous studies reported the high staphylococcal prevalence in samples of milk from cattle with either clinical or subclinical mastitis in Egypt (Asfour and Darwish, 2011; Abd Al-Azeem *et al*., 2013; Kamal *et al*., 2013). The authors detected *Staphylococci* in percentages of 68.57% and 62.5% from clinical and subclinical bovine mastitis in Egypt, respectively (Dorgham *et al*., 2013), 17.7% & 82.3% in Iran (Hosseinzadeh and Dastmalchi Saei, 2014), and 3.2% & 27.23% in Ethiopia (Dabele *et al*., 2021) from clinical and subclinical bovine mastitis, respectively.

Furthermore, 54% of isolates were determined as CPS (*S. aureus*), which was matched to other research on the milk of cows with mastitis in Egypt which revealed about 42% of *S. aureus* positive samples (Awad *et al*., 2017). Also Kemal *et al*. (2017) estimated the prevalence of *S. aureus* in 44.62% of cases of clinical and

Table 2. Antibiotic resistance distribution of Coagulase-Positive *Staphylococci* (*S. aureus*) (n= 216) and Coagulase-Negative *Staphylococci* (n= 76).

| Antimicroial class | Antibiotic disc | CPS (*S. aureus*) | | | | CNS | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | S | | R | | S | | R | |
| | | No. | % | No. | % | No. | % | No. | % |
| B-lactam | Amoxicillin-clavulanic acid | 4 | 1.9 | 212 | 98.1 | 0 | 0 | 76 | 100 |
| | Ampicillin | 0 | 0 | 216 | 100 | 4 | 5.3 | 72 | 94.7 |
| Cephalosporines | Cefotaxime | 24 | 11.1 | 192 | 88.9 | 8 | 10.5 | 68 | 89.5 |
| | Cefuroxime | 80 | 37 | 136 | 63 | 8 | 10.5 | 68 | 89.5 |
| Glycopeptides | Vancomycin | 112 | 51.9 | 104 | 48.1 | 36 | 47.4 | 40 | 52.6 |
| Carbapenems | Imipenem | 204 | 94.4 | 12 | 5.55 | 72 | 94.7 | 4 | 5.3 |
| Aminoglycosides | Gentamycin | 120 | 55.6 | 96 | 44.4 | 40 | 52.6 | 36 | 47.4 |
| Macrolides | Erythromycin | 44 | 20.4 | 172 | 79.6 | 28 | 36.8 | 48 | 63.2 |
| Tetracycline | Tetracycline | 84 | 38.9 | 132 | 61.1 | 20 | 26.3 | 56 | 73.7 |
| Quinolones | Ciprofloxacin | 156 | 72.2 | 60 | 27.8 | 48 | 63.1 | 28 | 36.9 |
| Aminocoumarin | Novobiocin | 0 | 0 | 216 | 100 | 44 | 57.9 | 32 | 42.1 |

Table 3. Comparison between study and control sample as regards interleukin (pg/ml) in milk and serum.

| | Milk | | Serum | | U | P value |
|---|---|---|---|---|---|---|
| | Study Group (n = 20) | Control Group (n = 5) | Study Group (n = 20) | Control Group (n = 5) | | |
| IL-4 | | | | | | |
| Min.-Max. | 79.7-88.5 | 380.8-420.3 | 106.3-126.7 | 650.4-700.0 | 0 | <0.001* |
| Mean± S.D | 112.73±3.134 | 400.62±15.575 | 118.43±5.744 | 678.34±19.191 | | |
| IL-6 | | | | | | |
| Min.-Max. | 374.2-382.7 | 160.1-169.9 | 238.9-260.4 | 10.8-15.0 | 0 | <0.001* |
| Mean± S.D | 378.51±2.730 | 166.38±3.975 | 249.87±6.892 | 13.34±1.677 | | |
| IL-10 | | | | | | |
| Min.-Max. | 165.1-176.4 | 210.1-224.7 | 18.4-23.7 | 35.1-40.0 | 0 | <0.001* |
| Mean± S.D | 171.76±3.216 | 216.64±5.966 | 20.67±1.749 | 37.50±1.857 | | |

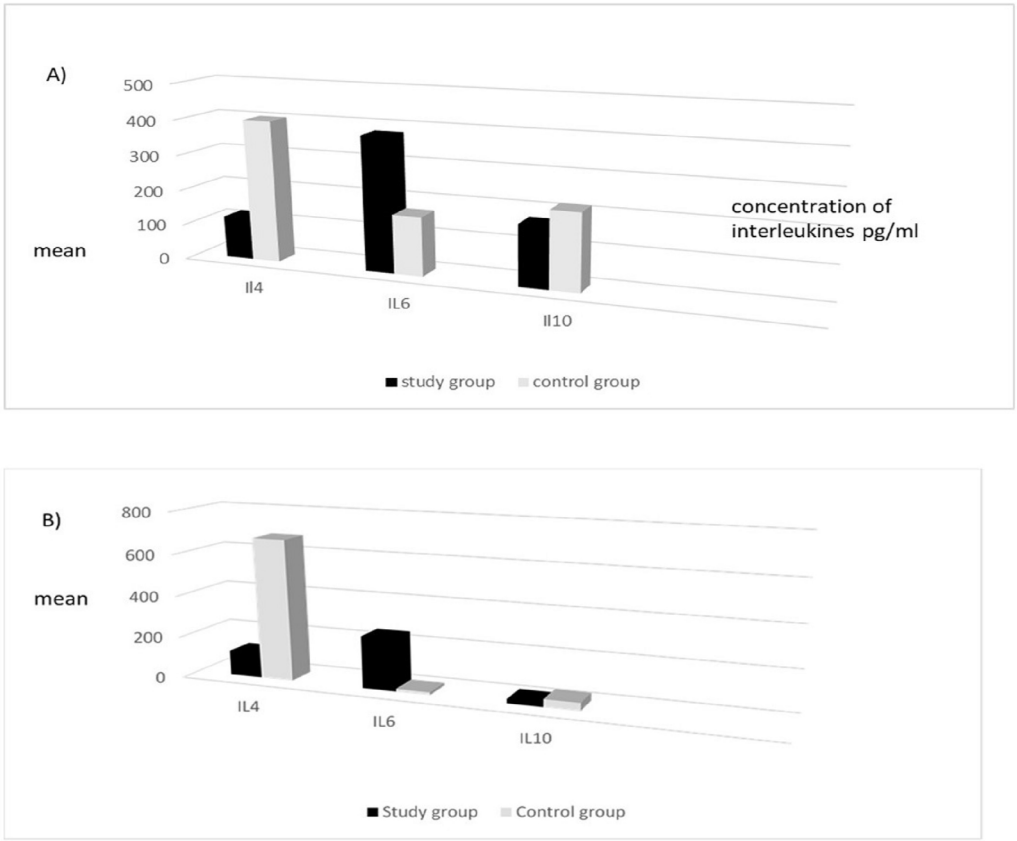


Fig. 3. Concentration (mean) of interleukins (IL-4, IL-6, and IL-10) in A) milk and B) blood serum of cows with clinical mastitis caused by *Staphylococcus aureus* in comparison to control ones.

subclinical bovine mastitis in Ethiopia. On the other hand, a low prevalence of *S. aureus* was reported in other studies on mastitis milk samples in Egypt and China showing about 14.9%, 16%, and 10.06% *S. aureus* positive samples, respectively (Amin *et al*., 2011; Ahmed *et al*., 2020; Zhang *et al*, 2022). The present work agreed with other previous studies that the commonality of *S. aureus* isolates was associated with bovine mastitis in Egypt and other countries (Elsayed *et al*., 2015). It is possible that variances in farm management practices, the breeds of targeted cows, levels of production, and study methodologies and materials were to blame for the variations in *S. aureus* prevalence rates in mastitic cows identified in different studies. The observations made during sample collection suggest that inadequate farm management methods and poor cleanliness are related to the high prevalence rates of *S. aureus* in milk. *S. aureus* is a contagious bacterium that can be spread from one animal to another or from a person to a cow during unhygienic milking techniques (Kemal *et al*., 2017).

Moreover, in this study, mastitis cow samples had a CNS prevalence of 19%. This was consistent with (El-Ashker *et al*., 2015) who isolated 12.50% CNS from cattle and buffaloes with mastitis. Also, a previous study isolated 12 % CNS from Bovine subclinical mastitis (Thorberg *et al*., 2009). In contrast, (Sharma *et al*., 2012) isolated 29.33% mastitis in a cow with subclinical CNS.

Bovine mastitis can be effectively controlled with antimicrobial therapy, as The most popular antimicrobials in Egypt for treating mastitis include B-lactams, aminoglycosides, glycopeptides, tetracyclines, fluoroquinolones, lincosamides, and sulfonamides. However, Antimicrobials are utilized widely, and this is known as the major source of antimicrobial resistance (AMR) (Abed *et al*., 2021). In vitro, there are differences in antibacterial activity of the various *Staphylococcus* species (McDougall *et al*., 2014; Nyman *et al*., 2018). Our study as regards the distribution of antibiotic resistance of CPS (*S. aureus*) showed absolute resistance to ampicillin (100%) followed by amoxicillin-clavulanic acid (98.2%), cefotaxime (88.9%), erythromycin (79.6%) and tetracycline (62.11%). They were most susceptible to imipenem (94.4%) and ciprofloxacin (72.2%). The well-known antibiotics ampicillin and other -lactams are frequently used in therapeutic settings, leading to the general resistance of *S. aureus* either in Egypt or another region (Awad *et al*., 2017; Ameen *et al*., 2019; Zhang *et al*., 2022). Resistance to other clinically important antibiotics, including cephalosporins, macrolides, and tetracyclines also occurred (Osman *et al*., 2017). Interestingly, in our research, it was discovered that CNS had higher resistance to amoxicillin-clavulanic acid (100%), followed by ampicillin (94.7%), cefotaxime (89.5 %), erythromycin (79.6%), cefuroxime (89.5 %), and tetracycline (73.7%). This result supports (Kim *et al*., 2019) findings that the CNS is more likely to develop antimicrobial agent resistance. Systemic administration of cephalosporins, ampicillin, and macrolides is particularly associated with increased CNS resistance (Nobrega *et al*., 2018). Antibiotic-resistant infections have steadily increased in developing countries, particularly Egypt, as an outcome of the overuse of antibiotics in recent years, both in therapeutic procedures and as growth promoters in food animals (Widianingrum *et al*., 2016). Due to the possibility that microorganisms in the environment of a dairy farm could produce resistant genes for both humans and animals. Consequently, from the standpoint of public health, it is crucial to monitor the antimicrobial resistance of *Staphylococcus* species in animals in addition to being important for field treatment decisions (Schmidt *et al*., 2015). Also, Bovine mastitis treatment choices should be based on a variety of cow-related parameters as well as information on antibiotic sensitivity and manifestation.

Finding the cytokine marker(s) that could be utilized as a forecasting device for the early identification of *S. aureus* infection may be helpful to monitor cytokines implicated in the control of immune responses during the infection. IL-6, a cytokine that promotes inflammation, is principally in charge of the acute phase reaction (fever) caused by both coliforms and *Staphylococci* (Elnagar *et al*., 2021). It promotes the differentiation of B lymphocytes and aids in T lymphocytes' detection of antigens (Bochniarz *et al*., 2017). In our study, upon ELISA, IL-6 levels were significantly greater in the milk and blood samples from cows with clinical mastitis caused by *S. aureus* than they were in the samples from healthy cows which confirmed the relation of IL-6 production in

milk and serum with *S. aureus* infection. The production and release of cytokines are mostly associated with *S. aureus* enterotoxins (Bjork *et al*., 1992). Correspondingly, an increased level of milk and serum IL-6 was reported by many authors in mastitis cows (Hagiwara *et al*., 2001; Griesbeck-Zilch *et al*., 2008; Osman *et al*., 2010; Vitenberga-Verza *et al*., 2022). There was a higher concentration of IL-6 in milk samples than in serum samples from cows with acute mastitis in comparison to normal cows (Hagiwara *et al*., 2001). Consequently, in clinical bovine mastitis, this high level of IL-6 is a sign of an early but generalized inflammatory condition. In contrast, similar to other studies findings, compared to healthy cows, cows with *S. aureus* mastitis had considerably lower levels of IL-4 and IL-10 in both serum and milk (Bochniarz *et al*., 2017; Šerstņova *et al*., 2022).

## CONCLUSION

The results of the current investigation showed that mastitis-affected cows' milk included a high prevalence of *Staphylococcus* species, particularly CPS (*S. aureus*). The antibiotic sensitivity testing of isolates established high resistance to β-lactam, macrolides, and tetracyclines. Concerning the prudent use of antibiotics, Staphylococcal mastitis should not be treated with antibiotics unless there is a clinical need for it, and more research is being conducted to investigate methods for controlling staphylococcal mastitis. Moreover, the immune reactions to *S. aureus* infection in the bovine mammary gland may be significantly influenced by IL-6, IL-4, and IL-10. Its considerable difference in milk and serum may be a marker of their potential in the pathobiology of *S. aureus* mastitis. Thus, further study on the elucidation of the mechanisms of interleukin production in mastitis of *S. aureus* is needed.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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