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Overview on the mastitis syndrome in cattle in Al-Sharkia governorate

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ABSTRACT

This study was conducted to determine bovine mastitis resistance genes of common etiological agents in Al-Sharkia province. In addition to monitor the changes in antioxidants profile, oxidative stress status and some of biochemical markers in dairy cows suffered from clinical mastitis. It was carried out on forty dairy cattle (40) aged from 4 – 6 years (20 healthy and 20 mastitic) from dairy farms and individual cases in Al-Sharkia Governorate, dependent on the findings of the clinical examination, the cattle were divided into 3 groups. Gp1 healthy dairy cattle (control), Gp2 mastitic dairy cattle before treatment, Gp3 mastitic dairy cattle after treatment. The clinical symptoms of mastitis including milk and udder were reported in Gp2. Within the bacterial isolates (20) it was noticed that the highest spread was of *Staphylococcus aureus* (10) followed by *E.coli* (6) *and Klebsiella Spp.* (4). Antibiotic sensitivity test displayed that gentamycin, streptomycin were the more sensitive antibiotics for *S. aureus*, *E.coli* and *Klepsiella*. While amoxicillin and clavulanic acid, tetracycline, florfenicol and cefotaxime were the more resistant antibiotics. Enrofloxacin was moderate sensitive in *s. aureus* and *E.coli* while more resist in *Klebsiella.* Real time PCR assay was used for detection of antimicrobial resistance genes. Eleven isolates representing, 5 *S. aureus*, 3 Klebsiella and 3 *E.coli* were selected for detection of multi drug resistant (MDR) genes. The *blaTEM* gene was detected in all isolates of *S. aureu*, *E. coli* and 2 isolates of *Klebsiella.* While, the *tetA* gene was detected in 4 out of 5 isolates of *S.aureus* and all isolates of *E.coli and Klebsiella*. Moreover, *floR* gene was detected in 3 isolates of *S.aureus*, 2 iso-

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late of *E.coli* and all isolates of *Klebsiella.* Biochemically, there were significantly increases in serum AST, ALT, ALP, urea, creatinine, Na, Mg, NO, PH, beta 1, total beta, total globulin and milk MDA associated with non-significant increases in t.protien, alpha 1, total alpha, gamma 1, gamma 2 and total gamma. Meanwhile, there were a significant decrease in serum Cl, Ca, K, TAC, beta 2, A:G ratio and milk SOD, CAT, GSH, associated with non-significant decrease in alpha 2. On the other hand, there were significant increases in serum albumin, immune-globulin and other proteins in whey milk, meanwhile, there were a significant decrease in α–lactoglobulin and ß- lactoglobulin in whey milk in mastitic group when compared with normal healthy cows. The results of the current investigation suggested that the body antioxidant defense system was compromised in dairy cows with clinical mastitis generating a state of oxidative stress.

INTRODUCTION

The economic value of dairy cows is especially de-termined via the milk yield and longevity. The main important factor affecting the quantity and quality of the produced milk is the manifestation of produc-tion diseases, mainly mastitis **(Zajac et al. 2012)**. Bovine mastitis still one of the most serious conditions of dairy cows globally (**FAO 2014; Abebe et al. 2016)**. Aside from the significant economic losses related to the disease, mastitis has dangerous zoonotic potential and has been linked to the increasing development and the rapid emergence of multidrug resistance strains globally **(Oliver et al. 2011; Beyene et al. 2017)**. Such hazards can mainly occur through the ingestion of contaminated milk, milk derivatives or direct contact with infected animals **(Maity and Ambatipudi 2021).**

Mastitis, which is the inflammation of the udder and teats, prevails in two primary forms: clinical as well as subclinical mastitis (reduction in milk production without clinical anomalies in udder or milk) **(Ndahetuye et al. 2019)**. Clinical mastitis, which is less predominant, is distinguished by systemic indications in the cow and notable abnormalities in the milk and udder **(Jamali et al. 2018)**, involving fever, depression, anorexia, lethargy, additionally a reduction in grooming could also transpire **(Medrano Galarza et al. 2012; Dittrich et al. 2019)**.

Mastitis is more predominant when the mammary gland's immunological and antioxidant defense mechanisms are compromised. Dairy cows are subjected to numerous physiological, genetic, and environmental factors related to both the host and pathogens, which can suppress the host's immunity, hence, mastitis incidence increase **(Andrei et al. 2016)**. Wide ranges of microbes have been registered as causative agents of mastitis worldwide **(Jamali et al. 2018)**. These involve both environmental and contagious bacteria, as well as algae, fungi and viruses. Evidence-depended on research studies have revealed marked variance in the distribution of mastitis-causing pathogens and mastitis between countries, regions, and farms **(Gao et al. 2017)**. These variances are affected by farm management practices with regional environmental factors **(Amer et al. 2018)**.

Bacteria are the primary source of mastitis, and more than 140 different pathogenic species have been mentioned **(Motaung et al. 2017)**.

Staphylococcus aureus the most widespread contagious agent linked with mastitis, because this bacterium is persistent inside the udder **(Rainard et al. 2018; Lamari et al. 2021)**.

Escherichia coli is the most prevalent gram-negative coliform responsible for inducing environmental clinical mastitis **(Campos et al. 2022)**, essentially because of high genotypic variation **(Bag et al. 2021)**. *Klebsiella pneumoniae* is the second most prevalent cause of mastitis and, is assumed the most detrimental

in dropping milk production and quality, as well as in economic terms. Even so it is categorized as an environmental pathogen, it has additionally been found to be transferred from infected to healthy animals **(Cheng et al. 2021; Fu et al. 2022**). Immediate identification as well as understanding diversity of pathogens related to mastitis is crucial for successful prevention and control **(Bi et al. 2016)**. Antibiotics are being used indiscriminately as a result of improperly identifying the cause of mastitis, resulting in higher development rates and the quick generation of multidrug resistance strains **(Sharma et al. 2018)**. Crucially, the indiscriminate applied of antibiotics to treat bovine mastitis has led to the generation of antibiotic-resistant bacteria in milk and dairy products, which is a critical concern **(Singh et al. 2018)**. Therefore, this study aims to identify the bacteriological mastitis causative agent and antibiotic resistance genes, additionally to determinate the biochemical alteration, antioxidant enzymes and oxiditive stress role associated with clinical mastitis in the Sharkia governorate.

MATERIAL AND METHODS

Ethical consent:

The experimental protocol was accepted by AHRI in conformity with the ARC and IACUC committee (ARC, AHRI, IACUC, **17**/24).

AHRI approved the experimental protocol in accordance with the ARC and IACUC committee guidelines.

MATERIALSs:

Eosin Methylene Blue (EMB) Agar, Brain Heart Infusion (BHI) Agar, Mac-Conkey Agar, Manitol salt agar, Blood Agar, Baird Parker Agar (BPA) incorporated with egg-yolk tellurite emulsion and Muller-Hinton agar (MHA) (Hi Media, Mumbai) were purchased from Arena BioScien Company, Egypt. Antibiotics disks gentamicin (CN, 10 µg), tetracycline (TE, 30 µg), Amoxicillin + clavulanic acid (Amc,30 µg), Cefotaxim (CTX,30 µg), Enrofloxacin (ENR,5 µg), florfenicol(FFC,30) and Streptomycin (S,10 µg) were obtained from Oxid Ltd. UK for microbiology media culture and diagnostic products. Different isolates that displayed phenotypic resistance were selected for the existence of anti-microbial resistant genes (5 *S. aureus*, 3 *E.coli* and 3 *Klebsiella*). For that purpose, Real Time PCR was applied to estimate the existence of genes encoding resistance to B-lactams (blaTEM), tetracycline (tetA) and florfenicol (floR), Oligonucleotide primers used were supplied from Metabion, Germany (table 1). Extraction of DNA from different isolates was applied using QIAamp DNA Mini kit (Qiagen, Germany, GmbH) Cat. No. 51304. Real Time PCR was performed using PanGreen™ Universal SYBR® Green Master Mix (purchased from Bio Helix) (table 2), the reaction was performed in a StepOne real time PCR system (table 3).

Table 1. Primers sequences, target genes and Ref.

Target gene	Primers sequences 5′- 3′	Reference
blaTEM	ATCAGCAATAAACCAGC0	**Colom et al. 2003**
	CCCCGAAGAACGTTTTC	
floR	GAACACGACGCCCGCTAT	**Wang et al. 2013**
	TTCCGCTTGGCCTATGAG	
tetA	GGTTCACTCGAACGACGTCA	**Ng et al. 2001**
	CTGTCCGACAAGTTGGGTAA	

Table 2. Reaction mix used for qPCR

Component	Volume
PanGreen™ Universal SYBR ® Green Master Mix (2x)	10 µl
Forward primer	1 µl
Reverse primer	1 µl
DNA template	5 µl
Nuclease-Free water	Up to 20 µl

Table 3. Thermal profile used in qPCR

Stage	No of cycles	Program
primary denaturation	1	95°C for 5 min
Denaturation	40	95°C for 15 sec
Annealing		60°C for 60 sec
Extension		72°C for 10 sec

Treatment trails:

Mastitic cows were treated by the most sensitive antimicrobials in addition to non-steroidal anti-inflammatory drug.

BANAMINE® (flunixin meglumine, Merck Animal Health CO.) 1ml/45 kg b.wt., slow intravenous once daily for up to 3 days.

Gentamycin: (Gentamycin 10%, El Nasr pharmaceutical Chemicals Co.) each ml contains gentamycin 100mg.the recommended dose is 4ml/ 100 kg b. wt., i.m. twice daily for 3 successive days injection.

Streptomycin: (Pen and Strep, Norbrook Company) each ml contains: procaine penicillin 200mg, dihydrostreptomycin sulphate 250mg, was. The recommended dose rate is 4 ml / 100 kg.b.wt., i.m. for Three consecutive day.

Experimental design:

Forty cattle aged 4 – 6 years (20 healthy and 20 mastitic) from dairy farms and individual cases in Sharkia Governorate were divided into 3 groups. Gp1 healthy cattle (control), Gp2 mastitic cattle before treatment, Gp3 mastitic cattle after treatment (72 hours after last doese). The clinical symptoms of mastitis were reported in Gp2.

Clinical assessment

All cattle were thoroughly checked according to **Radostits et al. (2000)**, then the clinical findings were reported. Udder examination involved inspection with palpation of the udder to ascertain the size and consistency of different quarters additionally if any possible anomalies were present. The supra mammary lymph nodes were manually palpated to assess their size and look for any noticeable indurations.

Samples collection:

Collection of blood samples:

Blood samples (about 7 ml), for serum obtaining, were collected from jugular vein from each group. Serum samples were obtained by centrifuging the blood samples at 3000 rpm for 7 minutes. Sera were separated and stored at -20°C until biochemical analysis.

Collection of milk samples:

Milk samples were taken from the affected quarters, while in healthy cow's milk sampling was taken from any quarter of the mammary gland. Teats were prepared aseptically before sample collection, according to the **National Mastitis Council (1999)**. After cleaning and drying the mammary teats, we sprayed 70% ethanol and discarded some streams of milk. 50 ml of milk samples were collected in sterile

screw-capped bottles and were targeted for the subsequent examinations and immediately transferred to the laboratory of bacteriological examination for isolation of the causative microorganisms, and also for biochemical analysis.

Microbiological examinations:

Milk samples delivered to the laboratory were mixed accurately and one loop full of the milk sample was inoculated on EMB Agar, BHI Agar, MacConkey Agar, Manitol salt agar, BPA incorporated with egg-yolk tellurite emulsion and Blood Agar. Then, these inoculated plates were incubated at 37˚C for 16-24 hours. The isolated bacterial colonies were exposed to gram's staining for the identification of gram-positive and negative and were exposed to distinct biochemical tests (oxidase, catalase, indole, Voges Proskauer's, methyl red, triple sugar iron, citrate, etc) for assurance. The bacteriological culture and biochemical estimation for isolates were done according to **National Mastitis Council (1999)**.

Antimicrobial susceptibility examinations:

Susceptibility was examined via Kirby–Bauer disk diffusion method on MHA. Pure cultures of *S. aureus*, *Klebsiella spp* and *E coli* were grown in brain heart infusion (BHI) broth, and then incubated at 37 °C for 18 hours. BHI broth cultures were additionally equally spread on MHA plates. After that, inoculated antimicrobial disks were left at room temperature for 30 min then incubated at 37 °C for 24 hours for inhibition zone diameter measurement. Strains were divided as resistant, susceptible, or intermediate based on the size of the inhibition zone (in millimeters) as well as minimum inhibitory concentrations (MICs) used for interpretation the diameters of inhibition zones were as depending on **Clinical and Laboratory Standards Institute (2014)**.

Physical and biochemical examination of milk samples:

Whole milk pH

Fresh milk was used for pH analysis, pH reading was reported by digital pH meter **(JENWAY,3510 manufactured in EU.)**

Skimmed milk:

Milk samples were centrifuged at 3000 rpm for 15 min. The fat layer in each sample was removed, and the defatted milk was immediately stored at -20°C until analysis of some antioxidant and oxidative parameters **(Colakoglu et al. 2017)**.

Whey milk:

Whey milk was prepared from a 40-ml of skimmed milk sample by adding 0.6 ml of a 1:50 dilution of rennin and 0.4 ml of saturated calcium chloride, keeping at 37 °C for 30 min., then centrifuging for 5 min at 3000 rpm. Then whey was filtered and frozen till used **(Kumar and Mikolajcik 1972)**.

Biochemical Analysis:

Activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined according to **(Reitman and Frankel 1957)**, alkaline phosphatase (ALP) was estimated according to **EL-Aaser and EL-Merzabani (1975)**. Level of urea and creatinine assayed be method described with **Wybenga et al. 1971**; **Henry 1974** respectively. Serum sodium (Na), chloride (Cl) and potassium (K) concentration was estimated with electrolyte analyzer model number PL1000 A. Calcium (Ca) and magnesium (Mg) was estimated using a kit reagent test (Biodiagnostic, Giza, Egypt) **Gindler and King 1972; Chauhan and Sarkar (1969)** respectively. The concentration of nitric oxide (NO) and total antioxidant capacity (TAC) in serum were assyed by **Montgomery and Dymock 1961**; **Koracevic et al. 2001** respectively. l-malonaldehyde (L-MDA), reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in skimmed milk were estimated according to **Okhawa et al. (1979), Beutler et al. (1963), Aebi (1974)** and **Nishikimi et al. (1972)** respectively. Serum and whey milk of total protein and their electrophoretic pattern were determined according to **Sonnenwirth et al. 1980** and **Davis 1964**, respectively and calculated based on SynGene S.No. 17292*14518 sme*mpcs.

Statistical Analysis:

The obtained data were statistically ana-

lyzed by One-Way ANOVA test followed by Duncan Multiple Range Test. The results were given as mean ± SE using SPSS 14 **(2006).** Results at P<0.05 was considered significant.

RESULTS

The cows of the mastitic group displayed rise body temperature, dullness, anorexia, congested mucus membranes, dropped milk production, the affected quarters of the udder were swelled, redness, hot, and firm in consistency. The supra mammary lymph nodes were enlarged. The milk secreted from the mastitic cattle appeared physical alteration likewise discoloration, alterations in consistency and flakes, beside there was an increase in pH of mastitic milk (average 7.5) in comparison to normal milk (average 6.7). Meanwhile, the control cows showed absence of systemic reaction and udder inflammatory signs with normal excreted milk.

Bacteriological examination of milk samples illustrated that, out of 20 tested samples, 10 isolates were identified as *S. aureus*, 6 isolate as *E.coli* and 4 isolates were identified as *Klebsiella sp*. The results of in-vitro antimicrobial susceptibility tests in table (4) revealed that, *staphylococcus aureus* showed the highest sensitivity to gentamycin and streptomycin followed by enrofloxacin and resistance to amoxycillin & clavulanic acid, cefotaxime and tetracycline and moderate resistance to florfenicol. Moreover, *E.coli* was more sensitive to gentamycin followed by streptomycin and enrofloxacin and resistant to amoxycillin & clavulanic and tetracycline followed by florfenicol and cefotaxime. Also, *Klebsiella* was sensitive to gentamycin, streptomycin and resistant to amoxycillin & clavulanic acid, tetracycline, cefotaxime, enrofloxacin and florfenicol.

Results concerning detection of antimicrobial resistance genes using Real time PCR assay showed that, the *blaTEM* gene was detected in all 5 selected isolates of *S. aureu* and 3 isolates of *E. coli* while it was detected in 2 out of 3 isolates of *Klebsiella.* The *tetA* gene was detected in 4 out of 5 isolates of *S.aureus*, all isolates of *E.coli* and *Klebsiella.* Moreover, *floR* gene was detected in 3 isolates of *S.aureus*, 2 isolates of *E.coli* and all isolates of *Klebsiella* **(**Fig.1-3).

The results of our study in Table (5) showed that mastitic group indicated a significant elevation in serum AST, ALT, ALP, urea, and creatinine when compared with normal healthy cows. All these parameters return to normal levels after treatment.

The results of our study in Table (6) showed that, the mastitic group indicated a significant elevation in serum Na, Mg and NO associated with a significant decline in serum Cl, Ca, K and TAC when compared with normal healthy cows. All these parameters return to normal levels after treatment.

pH and oxidative stress markers in milk illustrated in Table (7), there were a significant (P<0.05) increase pH and MDA, which associated with a significant (P<0.05) decrease in SOD, CAT and GSH in mastitic cows when compared with healthy cows, and all these parameters return to normal after treatment.

Serum biochemical analysis of serum T. protein and its main fractions showed in Table (8a), revealed a non-significant increase in t. protein, total alpha, total gamma associated with a significant increase in total beta and total globulin, meanwhile, there were a significant decrease in albumin and A:G ratio in mastitic group compared with normal control group, and all these biochemical parameters improved after treatment by the tested drugs.

The present results presented in Table (8b), revealed a non-significant increase in alpha 1, gamma 1 and gamma 2 associated with a significant increase in beta 1, meanwhile, there were a non-significant decrease in alpha 2 associated with a significant decrease in beta 2 in serum mastitic group compared with normal healthy cows. All these biochemical parameters improved after treatment by the tested drugs.

The present data which recorded in table (9) showed a notable increase in serum albumin, Immune-globulin and other proteins associated with non-significant increase in t. pro-

tein, meanwhile, there were significant decreases in α–lactoglobulin and ß- lactoglobulin in whey milk in mastitic group when compared with normal control groups, and all these parameters improved after treatments.

Table 4. Antibiotic sensitivity results of isolated bacteria

Antimicrobial agent	Code and potency	*Staph. Aureus* *No. 10*		*E coli* *NO. 6*		*Klebsiella spp.* *No. 4*	
		Sens.	resist	Sens.	resist	Sens.	resist
Enrofloxacin	ENR_{10}	7	3	4	2	-	4
Gentamycin	CN_{10}	8	2	6	-	3	1
Streptomycin	S_{10}	8	2	4	2	3	1
Amoxycillin+Clavulenic acid	AMC_{30}	-	10	-	6	1	3
Florfenicol	FFC_{30}	5	5	2	4	-	4
Cefotaxime	CTX_{30}	3	7	2	4	-	4
Tetracycline	TE_{30}	2	8	-	6	-	4

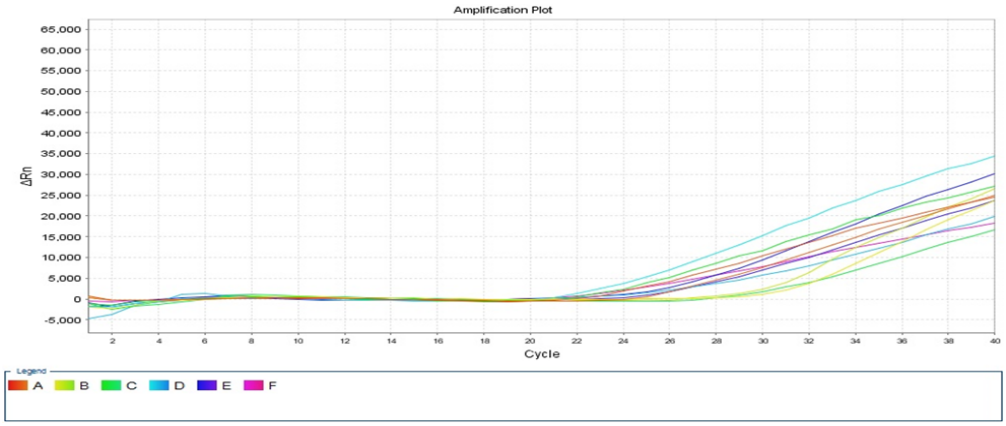

Fig. 1 Amplification Plot OF blaTEM gene.

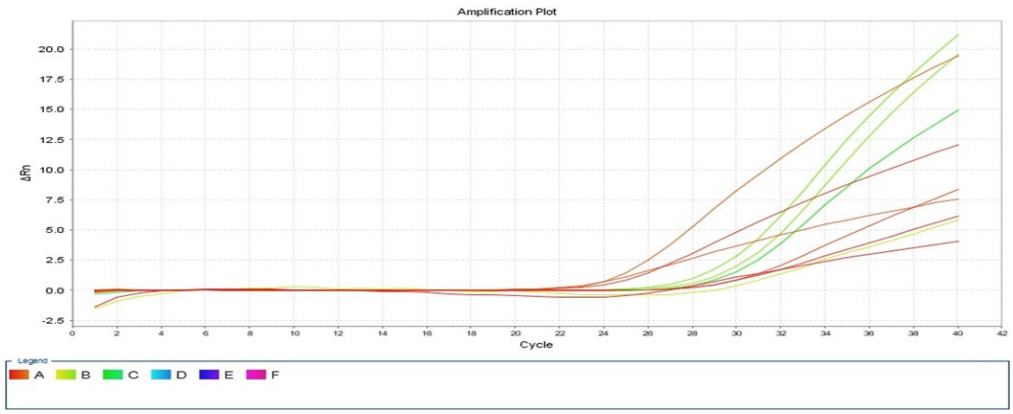

Fig. 2 Amplification Plot of tetA gene.

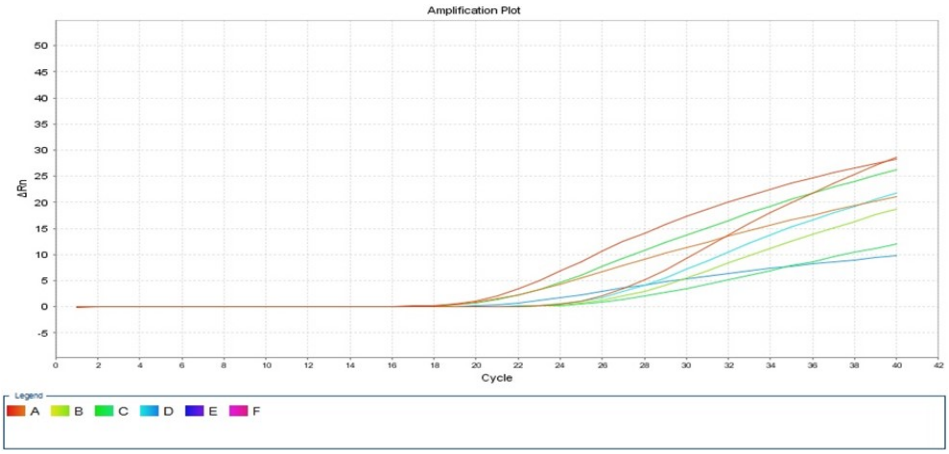

Fig. 3 Amplification Plot of floR gene.

Table 5. liver and kidney function in serum cattle suffering from mastitis.

Groups Parameters	Gp1: normal control	Gp2: mastitis gp before treatment	Gp3: mastitis gp after treatment
AST (U/L)	43.5 ± 0.87b	52.5 ± 2.02a	44.5 ± 2.29b
ALT (U/L)	26.00 ± 0.29b	28.5 ± 0.29a	26.00 ± 0.58b
ALP (IU/L)	77.33 ± 1.76b	93.00 ± 1.73a	73.67 ± 2.03b
Urea (mg/dl)	29.57 ± 1.66b	52.00 ± 1.96a	32.13 ± 1.59b
Creatinine (mg/dl)	0.75 ± 0.03b	0.96 ± 0.07a	0.75 ± 0.02b

Results are represented as (Mean±SE); Mean value in the same raw with different letter is significantly different at ($P < 0.05$)

Table 6. Electrolytes, nitric oxide and total antioxidant capacity in serum cattle suffering from mastitis.

Groups Parameters	Gp1: normal control	Gp2: mastitis gp before treatment	Gp3: mastitis gp after treatment
Na (mEq/L)	140.00 ± 1.15b	154.00 ± 1.15a	144.00 ± 2.58b
Cl (mEq/L)	116.67 ± 2.91a	96.00 ± 4.04b	115.67 ± 1.86a
Ca (mg/dl)	10.00 ± 0.12a	8.73 ± 0.15b	9.70 ± 0.17a
Mg (mg/dl)	2.73 ± 0.03c	3.13 ± 0.03a	2.93 ± 0.03b
K (mEq/L)	5.50 ± 0.06a	4.40 ± 0.21c	4.93 ± 0.15b
NO (µmol/L)	13.57 ± 0.78b	59.61 ± 0.85a	10.86 ± 0.39c
TAC (Mm/l)	0.97 ± 0.01a	0.86 ± 0.01b	0.94 ± 0.005a

Results are represented as (Mean±SE); Mean value in the same raw with different letter is significantly

Table 7. PH and oxidative stress marker in milk in cattle suffering from mastitis.

Groups Parameters	Gp1: normal control	Gp2: mastitis gp before treatment	Gp3: mastitis gp after treatment
pH	6.67 ± 0.07b	7.50 ± 0.06a	6.73 ± 0.09b
MDA (nmol/ml)	5.83 ± 0.05b	7.15 ± 0.35a	6.20 ± 0.34ab
SOD (u/ml)	5.18 ± 0.55a	3.63 ± 0.15b	5.38 ± 0.18a
CAT (u/ml)	2.53 ± 0.15a	2.00 ± 0.10b	2.45 ± 0.05a
GSH (u/ml)	2.88 ± 0.17a	2.24 ± 0.11b	3.09 ± 0.07a

Results are represented as (Mean±SE); Mean value in the same raw with different letter is significantly different at ($P < 0.05$)

Table 8a. Serum T. protein and its fractions (g/dl) in cattle suffering from mastitis.

Groups parameters	Gp1: normal control	Gp2: mastitis gp before treatment	Gp3: mastitis gp after treatment
T.protein	7.96 ± 0.09a	8.24 ± 0.11a	8.14 ± 0.23a
Albumin	2.49 ± 0.09a	2.10 ± 0.02b	2.39 ± 0.11ab
Total alpha	1.17 ± 0.05a	1.33 ± 0.05a	1.28 ± 0.14a
Total beta	1.27 ± 0.03b	1.43 ± 0.01a	1.33 ± 0.02b
Total gamma	3.03 ± 0.17a	3.38 ± 0.07a	3.14 ± 0.11a
Total globulin	5.47 ± 0.18b	6.14 ± 0.11a	5.76 ± 0.23ab
A:G ratio	0.46 ± 0.03a	0.34 ± 0.01b	0.42 ± 0.03ab

Results are represented as (Mean±SE); Mean value in the same raw with different letter is significantly different at ($P < 0.05$)

Table 8b. Serum protein sub-fraction (g/dl) in cattle suffering from mastitis.

Groups parameters	Gp1: normal control	Gp2: mastitis gp before treatment	Gp3: mastitis gp after treatment
Alpha 1	0.74 ± 0.03a	0.97 ± 0.01a	0.82 ± 0.13a
Alpha 2	0.43 ± 0.03a	0.36 ± 0.04a	0.46 ± 0.03a
Beta 1	0.89 ± 0.02b	1.13 ± 0.01a	0.91 ± 0.03b
Beta 2	0.38 ± 0.01a	0.30 ± 0.02b	0.42 ± 0.03a
Gamma 1	2.63 ± 0.18a	2.96 ± 0.07a	2.56 ± 0.11a
Gamma 2	0.39 ± 0.02a	0.43 ± 0.03a	0.59 ± 0.11a

Results are represented as (Mean ± SE); Mean value in the same raw with different letter is significantly different at ($P < 0.05$)

Table 9. T. protein and its fractions (g/dl) in Whey milk.

Groups parameters	Gp1: normal control	Gp2: mastitis gp before treatment	Gp3: mastitis gp after treatment
T. protein	1.37 ± 0.05a	1.44 ± 0.04a	1.37 ± 0.01a
ß- lactoglobulin	0.52 ± 0.04a	0.37 ± 0.01b	0.44 ± 0.02ab
α - lactoglobulin	0.25 ± 0.01a	0.21 ± 0.005b	0.23 ± 0.01ab
Serum albumin	0.14 ± 0.01b	0.19 ± 0.01a	0.16 ± 0.01ab
Immune-globulin	0.36 ± 0.02b	0.50 ± 0.01a	0.40 ± 0.04b
Other proteins	0.08 ± 0.01c	0.16 ± 0.01a	0.12 ± 0.004b

Results are represented as (Mean ± SE); Mean value in the same raw with different letter is significantly different at ($P < 0.05$)

DISCUSSION

Mastitis might be identified as inflammation of the parenchyma of the udder tissue despite the cause which causes economic losses in dairy industry **(Radostits et al. 2006 and De Vliegher et al. 2012)**. When a pathogenic bacterium penetrates the mammary gland, it usually occurs because physical barriers as the teat canal are disrupted **(Goldammer et al. 2004)**. Whenever the barrier is compromised, a rapid and influential defense response is needed to inhibit the prevalence of pathogenic organisms with additional harm to mammary gland tissue **(Aitken et al. 2011)**. It cuased physical as well as chemical changes in the secreted milk, resulting in pathological alters in the mammary gland tissue **(Babaei et al. 2007)**. In the present study, mastitic group cattle appeared increased in body temperature, loss of appetite and dullness, additionally inflammatory udder signs involving pain reaction, hotness, redness, and swelling. Furthermore, the secreted milk displayed physical al-

terations such as discoloration, existence of flakes, consistency changs with sometimes contained purulent materials. Meanwhile, neither of these signs were noted in the normal control cattle which showed normal excreted milk and absence of udder inflammatory signs. Such clinical findings were in agreement with **Radostits et al. (2006)** and **Abdel-Hamied and Mahmoud (2020)**.

In the current study, we examined the existence of *S. aureus, Klebsiella* and *E.coli* related to cattle mastitis with general study of antibiotic resistance patterns in the isolates. *S. aureus* is one of the most usually recorded bacteria in cattle mastitis cases. it is the most usuall etiological pathogen of bovine mastitis possessive several virulent pathogens and multidrug resistant make the disease difficult to treat increasing global problem which has become a major concern for dairy industry worldwide. So detect the antimicrobial susceptibility profiles is needed not only for effective therapy but also for monitoring the diffusion of resistant strains in defined ecological niches **(Coelho et al. 2009)**. The major frequency of *S. aureus* in current study may be because of absence of regular post milking teat dip, routine hand milking practice and lack of information about dry cattle therapy between the dairy owners. Spread of *staphylococci* from contagious to healthy udder quarters usually occurs among animals during the milking process via the milker's hands **(Constable et al. 2017; Sharma et al. 2007)**. The selected MDR Bacteria as *S.aureus, Klebsiella* and *E.coli* were applied for detection of resistant genes by PCR to find out the mechanism of resistant. our results are indicated the resistant mechanism of *S.aureus, klebsialla sp* and *E.coli* to tetracyclines may be due to the presence of Tet, resistance to floramphenicol due to the presence of flor and gene responsible for antibiotic resistance against β-lactam antibiotics due to presence of bla TEM. blaTEM was the most usually detected gene in gram-negative and gram-positive bacteria isolated from cattle sampled. This confirms the apparent expression of resistance of isolates to beta-lactam antibiotics. An increasing amount of beta-lactam variants have been revealed that differs in amino acid sequences as well as their catalytic activity against β-lactam antibiotics **(Bush and Jacoby 2010)**. The usage of tetracyclines as animal growth promoters is a greater contentious matter. The usage of tetracyclines in food production is numerous and contributes to the worldwide exposure of bacteria to tetracyclines. There is no suspicion that this practice results in the selection of resistant organisms and that in some cases these may be transmitted to humans. Moreover, since tet genes may be present in integrons. Eliminating the usage of tetracyclines and other antibiotics at subtherapeutic levels in animal feeds in other countries should also be a priority for feed producers and policy makers, in the hope that these alters will help to limited the incidence of resistant bacteria in the environment **(Sousa et al. 2018)**. Additionally, there are reports on other phenicol resistance systems, like the inactivation via mutations of the target site, phosphotransferases, efflux systems and permeability barriers **(Schwarz et al. 2004)**, of the latter mechanism, floR and chloramphenicol resistance (cmlA) are the most usually known genes in gram-negative bacteria **(Briggs and Fratamico 1999)**.

The present study displayed a significant elevated in ALP, ALT and AST activities in mastitic group. These results were in agreement with those recoded by **(Saleem et al. 2021)**, Mastitis caused highly elevation in ALT, AST and ALP **(Murphy 2005)**, and these increases may be because of hepatic tissues damage by bacterial toxins **(Ismail and Hatem 1998)**.

Urea and creatinine in current study showed a significant elevated in mastitic cows compared with control group. Our results were agreed with those stated by **Mosallam et al. (2014)**, this increase may be due to decrease renal perfusion (reduced glomerular filtration) which can cause azotemia, in which the tubular flow rate is lower and urea reabsorption is greater, this alteration in kidney may be due to the toxins that produced from the pathogenic bacteria **(Atroshi et al. 1996)**.

In current study, there were an increase in sodium levels in milk samples from mastitic animals, meanwhile other minerals involving

potassium and calcium were decreased. Similar observation have previously been recorded for cattle **(Rashed et al. 2002; Ahmad et al. 2007)**. The alter in milk pH in present study is thus linked with the elevated sodium levels in milk. Additionally, pH of mastitic milk was significantly higher due to the presence of clinical infections which can be used as a test for diagnosis of mastitis in animals which might be becouse of higher salts concentrations liberated due to increased permeability of cell membrane associated with inflammatory process and this might be responsible for elevate of pH in milk samples **(Hussain et al. 2012)**. On the other hand, the result show significant decrease in Ca, K, and Cl these results were agreed with **Hanan and Israa (2019); Hussain et al. (2012)**, they suggested that the declined mineral level might be linked to the mineral losses from blood into the mastitic udder **(El-Zubeir et al. 2005)**. Mastitis induces reduction in serum mineral due to anorexic condition and decreased intestinal absorption of mineral **(Naresh et al. 2001)**. Anorexia in mastitic animals may be responsible for reduction in serum mineral **(Abd Elazem et al. 2015)**.

Free radicals are produced naturally becouse of the intensive metabolism that exists in the cells of all living organism, mainly dairy cows. Oxidative stress commences when imbalance occurs in the homeostasis which caused via free radicals liberation which may ultimately lead to dairy cows being susceptible to mastitis. The discontinuity in the blood-milk barrier and the reduced secretory activity of the mammary gland's epithelial cells cause changes in the levels of most blood components during mastitis **(Krishnappa et al. 2016)**. Greater liberation of free radicals with decreased TAC through clinical and subclinical mastitis in dairy cattle was mentioned **(Atakisi et al. 2010),** with resultant occurrence of oxidative stress (**Lykkesfeldt and Svendsen 2007)**. Nitric oxide is one of the most critical reactive nitrogen radicals; which operate in different tissues like epithelial cells and macrophage of mammary gland, producing a notable amount of NO that mediates inflammation during mastitis **(Bouchard et al. 1999)**. In this study, there was a highly increase in serum NO level in dairy cows with acute clinical mastitis in comparing to control group, similar to that observed by **Atakisi et al. (2010)**. In livestock suffering mastitis, neutrophils produce large amount of NO and the myeloperoxidase enzyme, i.e. substances that together can cause nitrotyrosine formation, which has the ability to crumble proteins and have a destructive influnce on tissues **(Jóźwik et al. 2012)**. Also, the elevated in nitric oxide in present study might be due to the increased permeability of microcirculatory vessels and oxidative stress due to free radical injury **(Qayyum et al. 2016)**.

The current study revealed a significant increase in skimmed milk L-MDA, meanwhile, a significant decreases in GSH levels in mastatic group comparing with normal control, illustrating the contribution of udder allied oxidative stress with potential oxidative damage, these results are in agreement with previous results recorded by **Ranjan et al. (2005) and Kizil et al. (2007)**. L-Malondialdehyde the intermediate product of lipid peroxidation is being considered as a reliable index of oxidative stress **(Esterbauer et al. 1991)**. The increase in L-MDA concentration may be becouse of extreme free radical liberation from neutrophils in the infected mammary gland which in turn lead to peroxidative injury to mammary cells **(Boulanger et al. 2002)**, enhancing the incidence of oxidative stress in the udder tissue, which may caused increased permeability of microcirculatory arteries owing to free radical injury **(Huma et al. 2020 and Carvalho-Sombra et al. 2021)**. Oxidative stress might be one of the primary reasons in drooping in quantity and quality of milk in dairy livestock **(Kumar et al. 2022)**.

Antioxidants prevent oxidative deteriorate caused by free radicals through either directly scavenging them or via inhibiting enzymes oxidizing action **(Jóźwik et al. 2012)**. It has been noticed that milk has antioxidant effects because of existence of enzymatic such as (SOD and CAT) and non-enzymatic (glutathione and protein) defense mechanism **(Silanikove et al. 2014)**. Oxidative stress occurs because of elevated generation of ROS and increased lipid peroxidation products (MDA) concentration accompanied with reduced antioxidant protection defense mechanism, caused imbalance be-

tween ROS liberation and anti-oxidant defense system. Moreover, there is a failure of replacement and/or repair systems as well as increase levels of oxidative stress biomarkers responsible for mastatic disease **(Kumar et al. 2022; Ali et al. 2022)**.

The our data displayed that acute clinical mastitis is accompanied with a declined antioxidant defense. This might be observed through the notable increase concentrations of MDA along with the significant reduce in TAC, GSH, SOD and CAT activities. Such findings might indirectly indicate increased free radical action that reflect oxidative stress satate that occurs in such cases **(Celi 2011)**.

Glutathione is a primary intracellular reducing factor for maintenance of thiol groups on intracellular proteins or for antioxidant molecules. GSH serves considerable functions involving detoxification of electrophiles and scavenging of free radicals, also it include in phase II conjugation and other reactions **(Ribas et al. 2011)**. GSH concentration, in the current study, was highly decrease in whey milk of mastitis cows when comparing with healthy cows suggestive of oxidative stress. Similarly, **Jhambh et al. (2013)** recorded that notable reduce in GSH level in mastitic cows becouse of conversion of reduced form to oxidized form (GSSH) via ROS overproduction from damage mammary gland.

Superoxide dismutase antioxidant enzyme is localized in cytoplasm and caused dismutation of superoxide to hydrogen peroxideswhich in turn is scavenged via CAT and glutathione peroxidases (GPx) **(Kohen and Nyska 2002)**. CAT activity was highly reduced ($P<0.05$) in mastitic group. Similar observes were previously mentioned **(Jhambh et al. 2013 and Sadat et al. 2023**). Moreover, such decrease in CAT might be attributed to its increased consumption to counteract ROS generated from inflamed mammary glands, indicating an impairment in antioxidant defense of the body **(Jhambh et al. 2013)**. Also, **Sharma et al. (2010)** recorded that CAT has exerted protective influences in cattle neutrophil induced model of mammary cellular injury in staph aureus infected mastitis. Lipid peroxidation is the indicator applied to determined cellular membrane injury caused via oxidative stress **(Ranjan et al. 2005)**.

The biochemical investigation of serum samples in this study revealed, a significant decreased in serum albumin, Beta 2 and A:G ratio levels in the mastitic group. Similar observation were recorded by **Ali et al. (2017a); Abdel-Hamied and Mahmoud (2020)**. This might be attributed to the decreased albumin concentrations caused through the immune response linked with udder infection **(Singh, 2000)**. The hypoalbuminaemia could be attributed to the stress condition that happens during mastitis which induce the protein catabolism. Albumin is a negative acute-phase systemic protein that migrates to inflamed tissues by increased vascular permeability and performs a diversity of physiological activities, involving antioxidant, and is regarded as an immune-inflammatory biomarker **(Singh 2000)**. Because of vascular permeability is enhanced as a result of inflammation, this decline may be related to leakage of immunoglobulins, albumin and other serum proteins into milk **(Krishnappa et al. 2016)**, or/and because of damage of hepatic tissues by bacterial toxins **(Coles 1986)**.

Also, the present study revealed a significant increase in serum total globulin in mastitis group comparing with control one. Likewise, previous records reported high globulin concentrations in mastitic cattle **(Ali, et al. 2017b and Singh et al. 2014)**. Elevated serum globulin concentrations might be linked to antibodies development in the form of gammaglobulin, which is responsible for neutralizing the invading microorganism's influence **(Chaplin, 2010)**.

Moreover, T. protein and its fractions in whey milk showed a significant increase in serum albumin, immune-globulin and other proteins, meanwhile, there were significant decreases in main whey protein (α–lactoglobulin and ß- lactoglobulin) in whey milk comparing with healthy cows. These observations are in agreement with the previous

data recorded in milk from cows with clinical mastitis by **Rashed et al. 2002; Le Maréchal et al. 2011)**. These findings may be attributed to both inflamatory injury of the mamary secretory tissues and devastation of blood-milk permeability barriers which limits in transfer of protein from interstitial fluid in to milk **(Olumee-Shabon et al. 2013)**. Also, elevated total protein may be due to leakage of blood proteins such as immunoglobulins and serum albumin into milk as a response to immunological reactions during mastitis **(Gráff and Miko 2015)**.

CONCLUSION

There was an unexpectedly high prevalence of resistance to different antimicrobials. The most surprising fact was the high incidence of high spread of resistant genes towards tetracyclines, Florphenicol and Beta lactam. The researchers hypothesized that factors other than antimicrobial medication, as feed, environment, farm type, and/or management practices, may play a role in the development and diffusion of AMR in *E. coli, S. aureus* and *Klebsiella sp*. Regular monitoring would enable timely identification of both emerging and current forms of resistance and AMR genes in bacteria originating from food-producing animals, involving those on dairy cow farms. Additionally, our results concluded that there are alterations in some constituent of serum and milk in normal and mastitic cattle. Moreover, oxidative stress play a serious role in mastitis, may be used as a potential biomarker for diagnosing and monitoring of mastitis.

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