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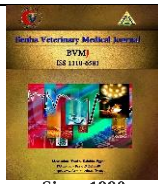

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Original Paper

Assessment of potential treatments for clinical mastitis caused by *Escherichia coli* in cattle using clinical and biochemical markers

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ABSTRACT

Bovine mastitis (BM) is dairy cattle's most significant and prevalent disease, causing significant financial losses worldwide. Among environmental causes of BM, *Escherichia coli* (*E. coli*) is considered a leading cause of subclinical, clinical mastitis (CM), developing systemic reactions, abnormal milk changes, and udder inflammation. Therefore, the purpose of this study was to determine the prevalence rate of *E. coli* in dairy cows among cases of CM, assess the effectiveness of antibiotics using antimicrobial sensitivity testing, and measure changes in biochemical markers within cows with CM as opposed to those that were treated. Out of the 64 milk samples used in the current investigation, eleven (17.1%) tested positive for *E. coli*. Antimicrobial sensitivity test results showed that *E. coli* strains are completely resistant to penicillin, tetracyclines, and beta-lactam antibiotics while being highly susceptible to the fluoroquinolone antibiotic class. The study revealed that the milk from cows with mastitis had significantly lower levels of total protein (TP), triglycerides (TG), calcium, and total antioxidant capacity (TAC) and significantly higher amounts of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), sodium chloride, and malondialdehyde (MDA) than the milk from non-mastitis and treated cows. From this study, the authors concluded that the systemic administration of fluoroquinolones is very effective with NSAIDs in the treatment of CM cases that were infected with the *E. coli* pathogen. In addition, biochemical analysis of milk samples can be achieved to diagnose and confirm the potential role of the treatment protocol in animal mastitis.

1. INTRODUCTION

Bovine mastitis (BM) is the most prevalent disease in cows, defined commonly as inflammation of the mammary gland. Three forms of BM were identified based on the level of inflammation: subclinical mastitis (SCM), clinical mastitis (CM), and chronic mastitis (Zaatout, 2022). CM is indicated by outward symptoms like redness and swelling of the affected quarter, changes in milk color, flakes, and clots. Severe CM cases can be defined using different criteria, but the usual common denominator is that the cow is systemically affected, which can be recognized by clinical signs such as lethargy, anorexia, hyper- or hypothermia, or recumbence (Wenz et al., 2006).

BM is caused by a variety of bacterial species, including Streptococci spp., Coliforms, and *Staphylococcus aureus* (S. aureus) (Ruegg, 2021). Based on the causative agent, mastitis can be classified as environmental or contagious. While contagious bacteria are mainly transmitted directly between animals or via milking equipment, environmental pathogens such as coliforms arise from bedding materials, stables, flies, and feces of cows (Klaas and Zadoks, 2018). Among coliforms, *E. coli*, which is a gram-negative bacillus within the Enterobacteriaceae family, mostly colonizes the udder during the dry season and has been connected to 80% of cases of coliform mastitis (Suojala et al., 2013). However, this colonization also develops in the early stages of lactation and calving; still, during lactation, reinfections may develop at any time (Roussel et al., 2015). *E. coli* can cause mild to severe inflammations depending on different factors such as the cow's age, the lactation stage, and parturition (Vangroenweghe et al., 2020).

Mastitis-induced changes in mammary tissue alter the quantity and quality of milk produced. In addition to raising sodium and whey proteins while decreasing fat, protein, casein, and calcium content, it also negatively affects the function of some milk enzymes (Poławska et al., 2012). Mastitis causes significant financial losses for dairy farmers, primarily due to its impact on the overall quality and amount of milk produced. In addition, it induces financial losses from discarded milk, culling of diseased animals, increased treatment expenses, and increased labor costs (Kotb et al., 2021).

Antibiotics remain the mainstay of treatment for BM (Hossain, 2017). Because the efficacy of treating cows with CM cases depends on the use of appropriate antimicrobials to eliminate the pathogen from udder tissue (Mainau et al., 2022). The present study was designed to evaluate the clinical investigation and medical treatment efficacy of CM cases due to *E. coli* after using an antibiotic sensitivity test. This aids veterinarians in minimizing expenses related to chronic management in the cattle sector, which arise from the high cost of using antibiotics and their continuous increase. It also helps to prevent more damage and

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production loss, as well as preserving animal health. Additionally, we will determine the changes in biochemical markers associated with CM-infected milk cows and the differences in their milk after therapy compared to non-mastitis cows.

2. MATERIALS AND METHODS

2.1. *Animals*

In this investigation, a total of sixty-four lactating cows suffered from CM, changes in milk and udder, from two veterinary clinics. The number of cows and scores of CM diseases according to Roberson et al. (2003) were illustrated in Table 1.
One clinic is in Minya al-Qamh, Sharkia governorate (n = 25 cows). The most significant economic activities of this city are agriculture and raising animals for milking and fattening, particularly in small breeding herds. Another veterinary clinic was located in Nūbāriyah, West Beheira Governorate (n = 39 cows). The two locations are thought of as rural villages where animals' breeders lack experience in preventing and controlling veterinary infectious diseases that occasionally or periodically may infect their animals.
Before performing CM's field treatment approach, a visual inspection of the udder to record the degree of inflammation and systemic reaction in affected animals, a visual inspection of milk samples using the strip cup test, and an inquiry about the frequency of milking seasons. Milking procedure (manual or automated) Pre- and post-milking teat dips, udder washing and drying, the duration and control of the dry period, as well as pen bedding (sort of and frequency of bedding changes) are examples of management techniques used during milking, according to Constable et al. (2017).

Table1.Assessment of CM cases during clinical examination of the cow's udder and milk and from owners' questions

List of crucial components	n=	Total proportion	P-value
Clinical signs in milk and udders according to Roberson et al. (2003)			
Mild (only abnormality of the milk as slight clots or flakes, with little evidence of udder inflammation and no systemic signs)	9	14.1%	<0.0001***
Moderate (visual changes in the udder and milk with a small systemic changes)	10	15.6%	
Sever (systemic reaction, induration, yellowish, offensive pussy discharge, or combined with acute metritis)	45	70.3%	
General health condition			
Not- affected	35	54.7%	>0.999NS
Affected	29	45.3%	
Mastitis history			
Yes	56	87.5%	<0.0001***
No	8	12.5%	
Number of lactation seasons			
1-2 season	24	37.5%	<0.0001***
3 or more	40	62.5%	

NS Non-Significant *** Highly significant

2.2. *Ethics approval*

The research protocol was reviewed and approved by the ethics committee of the Faculty of Veterinary Medicine, Zagazig University, Egypt (approval number: ZU-IACUC/2/F/161/2024).

2.3. *Sampling and laboratory examination*

Ten ml of milk was collected into a sterile test tube after the first three milking streams were removed and the teat ends were cleaned with 70% ethyl alcohol. In summary, the brain heart infusion broth was used to grow and incubate the milk samples aerobically at 37°C for a whole day, as reported by Markey et al. (2013). The materials were then streaked across MacConkey agar media (Oxoid), and pink colonies on MLA were sub-cultured on Eosin Methylene Blue agar (EMB) (Oxoid) at 37°C/24h. Conventional techniques, such as Gram stain and gross colony morphology on media cultures, were used to identify the strains of *E. coli*. According to Collee et al. (1996) specialized biochemical tests were used to identify strains of *E. coli*.
All positive *E. coli* isolates were examined using the disk diffusion method against ten antibiotic discs belonging to eight antibiotic classes (Oxoid, UK) on Muller-Hinton agar plates (Oxoid, Ltd, Basingstoke, Hampshire, UK) according to Abdeen et al. (2020). Following the Clinical Laboratory Standards Institute (CLSI, 2015), the evident clear zone of inhibition that restricted the growth of bacterial isolates was measured to determine the extent of antimicrobial sensitivity against bacteria. The antimicrobial medications under investigation classified the *E. coli* isolates as susceptible or resistant. Based on field use, antibiotics used according to antibiotic class Beta-lactams inhibitor, Amoxicillin/ clavulanic acid (AMC); Aminoglycosides, such as Gentamicin (CN), and streptomycin (S); Tetracyclines, such as Doxycycline; Fluoroquinolones, as Ciprofloxacin (CIP) and Norfloxacin (NOR); Phenicols, as Chloramphenicol (C) Penicillins, as Penicillin G; Cephalosporins, as Cefotaxime (CTX) and Sulfonamides, as Sulfamethoxazole/ Trimethoprim (SXT).

2.4. *Treatment protocols of CM cases*

Before starting the treatment trial, the udder, collected milk samples, and the general body condition of animals were clinically examined. The owner was advised to isolate mastitic cows and fully milk out the animal to eliminate any potential toxins, debris, milk clots, and bacteria content. All animals admitted to veterinary clinics showed an inflammation of the udder quarter.
This study focused on the efficacy of field-applied antibiotics against *E. coli* -diseased cows, consequently 64 milk samples from 64 infected cows with a typical CM were collected for detection of *E. coli* pathogen. After the application of antibiotic sensitivity medication, these animals were treated specifically against the *E. coli* infection. Treatment was with marbofloxacin (Vetoquinol), a fluoroquinolone drug that has been developed specifically for veterinary use. It is very effective against diseased animals suffering from gram-negative and occasionally gram-positive. Marbofloxacin has been recommended for the treatment of acute mastitis and metritis at a dose of 1 ml/50 kg B.wt, administered I/M once a day for three days in successive days. The following additional symptomatic therapies were administered: that were used in both groups, NSAIDs such as flunixin meglumine (Norbrook); for bovine mastitis, a dose of 2 mL/45 kg B.wt should be administered by I/M injection once a day for a maximum of three days. Also, ilium Meloxicam 20 (Amriya) may be administered as a single dose at a dose of 2.5 mL/100 kg S/C. As recorded by Smulski et al. (2020) and Hossain et al. (2017), additional supportive measures, included various combinations of antihistaminic drugs, vitamin and mineral mixtures, calcium, and glucose. These were administered according to animal case status, especially in severe cases involving severe general health conditions. Following five days of therapy, the cows underwent clinical examinations. When the udder and teat sizes were normal during the follow-up inspection and there were no flakes or the milk color returned to normal, the clinical cure was said to have been achieved, furthermore, to ascertain that the animals had been

cleared of any *E. coli* infection, milk samples were gathered from the treated cow.

2.5. Evaluation of Biochemical markers

Milk samples from non-mastitis cows and mastitic cows (before and after treatment) were collected. These samples were then used to detect biochemical markers such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total protein (TP), triglycerides (TG), malondialdehyde (MDA), and total antioxidant capacity (TAC), sodium, and calcium levels using the colorimetric method according to kits supplied by Chema Diagnostica (Monsano, Italy).

2.6. Statistical analysis

We utilized GraphPad INSTAT software (Version 2) to conduct the statistical analysis. The statistical assessment involved applying a one-way analysis of variance (ANOVA) and Tukey's multiple range test. The results comprise the presentation of standard error and mean differences. Significance was determined by considering a p-value of 0.05 or less.

3. RESULTS

3.1. Clinical observations of CM cows

Examining diseased cows admitted to veterinary clinics revealed that typical signs of CM such as inflammation of one quarter or more as illustrated in (Fig. 1A); in some cases, udders are hot and painful during clinical examinations; additional clinical signs affect negatively health body condition of diseased animal include fever, recumbence, weakness; in addition, typical symptoms of CM during milk examinations include flakes or clotted milk as in (Fig.1B) as well as clear serum-like; in some cases, milk is tinged with blood or has pussy milked discharge with an unpleasant odor. Mastitic cows revealed decreased average daily milk yield (2.5-3.5 kg/day/cow) compared to 6-8 kg/day/cow for healthy cows.

Table 1 illustrates how CM cases admitted to both clinics were divided into three groups based on the degree of infection and the existence of a systemic reaction. Of these, mild, moderate, and severe CM cases accounted for 14.1%, 15.6% and 70.3% of cases, respectively with a significant difference (*P*<0.0001). About 45.3% of instances of CM included udder tissue inflammation and milk alterations, these cases also had poor general health conditions such as pyrexia, recumbence, in-appetence, elevated respiratory rate, and seven cases with metritis. 54.7% of CM cases, in contrast, have an acceptable general health status and there is non-significant difference (p>0.999). Certain variables might cause mastitis to recur, and they can also make a mild case into a severe one. 87.5% of the cases admitted to our clinic study had a history of mastitis, which might be attributed to the animal having been exposed to the disease during a prior lactation season. (Table 1) shows how other potential risk factors, such as age and the frequency of lactating seasons, which are strongly correlated with animal age, affect the occurrence of CM in a proportionate way. 37.5% of instances had one or two lactating seasons; conversely, 62.5% involved four or more lactating seasons.

3.2. Laboratory diagnosis and antibiotic sensitivity test

Eleven (17.1%) of the 64 milk samples that were obtained tested positive for *E. coli* while they had been examined bacteriologically on McConkey agar medium, displaying distinctive pink colonies, and growing colony subcultures on EMB medium have a distinct metallic green color as in (Fig. 1C).

Fig .1. Clinical inflammation in left quarter of udder (A), clotted milk indicative of CM (B), *E. coli* colonies (metallic green) on Eosin Methylene Blue plates(C).

According to the results of figure 2, all strains of *E. coli* were completely (100%) resistant to classes penicillins, tetracyclines, and beta-lactam antibiotics. Additionally, the strains exhibited intermediate susceptibility to Aminoglycosides (streptomycin), sulfamethoxazole-trimethoprim, and cephalosporins (cefotaxime (CTX) (27.3%, 63.6%, and 72.7%, respectively). Moreover, *E. coli* strains were 72.7% sensitive to gentamicin and 77.8% chloramphenicol which belonged to antibiotic classes of aminoglycosides and phenicols, respectively. Fortunately, *E. coli* strains exhibited a high degree of sensitivity to the fluoroquinolone antibiotic class, with percentages for ciprofloxacin and norfloxacin of 90.9% and 100%, respectively.

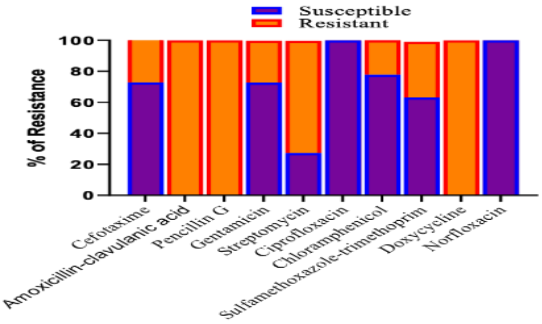


Fig .2. Antimicrobial sensitivity testing of *E. coli* isolated from milk.

3.3. Therapeutic management of CM cases

Based on the outcomes of the treated animals with systemic reaction and marked abnormal milk secretion and coloration, were cured with antibiotics against infected and isolated *E. coli* pathogens and were administered along with other NSAIDs for symptomatic treatment. A synthetic quinolone was used to treat *E. coli* mastitis in all eleven cows after fluoroquinolones showed sensitivity in vitro antibiotic sensitivity testing. According to the case, other symptomatic medications such as NSAIDs as flunixin meglumine or, ilium Meloxicam 20 are also given in Egypt along with commercial marbofloxacin. Other medications were also given as antihistaminic drugs, fluid therapy, and calcium therapy, particularly to animals that showed signs of anorexia, dullness, and recumbence. Following prescriptive medication, only nine cows (81.8%) were completely cured, the cow's milk and slightly inflamed quarters returned to normal no flakes or blood in the milk, and udder sizes were normal during the follow-up test. Furthermore, milk samples were taken from cows in response to therapy, and when these samples were analyzed bacteriologically using specific EMB media, no evidence of colony growth was observed.

3.4. Effect of treatment options on milk enzymes levels in mastitis cases

Results showed that milk from udders with mastitis (before treatment) significantly increased LDH and ALP levels by 214 % and 68 %, respectively, compared to that from non-mastitis udders.

After treatment, the LDH and ALP levels were markedly reduced by 67 % and 83 % related to their levels in mastitis milk. Treatment levels of these indices were within the range of non-mastitis milk (Fig. 3A and B).

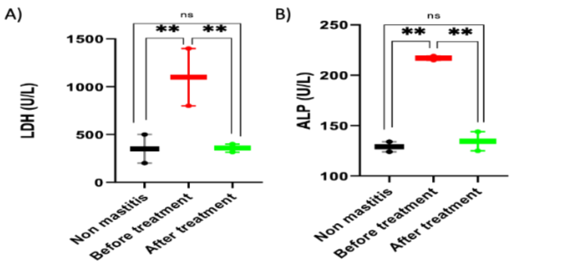
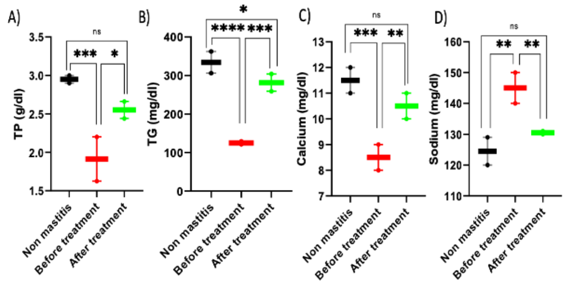


Fig.3. Effect of therapy on milk enzymes in mastitis cases. A) LDH, and B) ALP. Data are presented as means ± SD. $^{**}P < 0.01$ vs. before the treatment group.

3.5. Influence of therapy on milk compositions in mastitis cases

The mastitis group (before treatment) exhibited a significant fall in TP, TG, and calcium by 35%, 62%, and 26%, respectively, and an increase in sodium levels by 16% in milk samples in comparison to the non-mastitis group. Treatment of mastitis cases (after treatment) induced a rise in TP, TG, and calcium by 33%, 125%, and 23%, respectively, and a decline in sodium levels by 10% in milk samples versus the mastitis group (before treatment). Compared to the non-mastitis group, milk composition was insignificantly different in the after-treatment group (Fig.



4A-D).

Fig.4: Changes in milk compositions in mastitis cases exposed to therapy. A) TP: B) TG: C) Calcium and D) Sodium. Data are presented as means ± SD. $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ vs. before treatment group

3.6. Effect of treatment on the oxidant/antioxidant parameters in milk

The mastitis group (before treatment) showed a notable increase in MDA levels in the milk samples by 81% and a decrease in TAC levels by 18% in comparison to the non-mastitis group. On the other hand, the after-treatment group led to a significant 47% decrease in MDA concentration, and an increase in TAC levels by 14% compared to the before-treatment group. Surprisingly, there were no notable differences in the above indices between rats administered non-mastitis group and mastitis cows that received treatments (Fig. 5A and B).

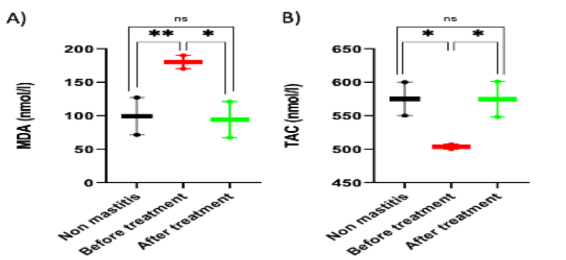


Fig.5. Effect of therapy on oxidative status in milk of mastitis cases. A) MDA, and B) TAC. Data are presented as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. before the treatment group.

4. DISCUSSION

During an examination of clinically infected cows admitted to the veterinary clinic, the site of our studies, and when the immune system reacts to intramammary infection, visually aberrant milk may develop with or without secondary signs, which is known as CM. It is evident and easily detected by visible abnormalities in the udder tissue or milk samples, such as red and swollen udder, loss of milk yield, the milk of the cow appearing watery with the presence of flakes and clots, and visual abnormal coloration of milk. Similar signs were also reported by Constable et al. (2017) and Ruegg and Erskine (2020).

The severity of CM is usually categorized as mild, which just affects the milk and slight inflammation of udder tissue; moderate, which also includes inflammatory indications of the affected quarter and a slightly systemic reaction; and severe, which also involves signs of systemic illness (Sears and McCarthy, 2003), In severe cases, CM can occasionally be fatal. Although there are several criteria for classifying cases of severe CM, most agree that the cow has to be systemically impacted (Wenz, 2006), which can be determined by clinical indications such as lethargy, anorexia, hyper- or hypothermia, or recumbence (Gruet et al., 2001). In this investigation, the percentage of cases with mild-and-moderate CM cases was 14.1% and 15.6%, respectively, while the percentage of cases with severe CM cases was 70.3%. This is in contrast to the findings of Tomazi et al. (2018), who reported that non-severe cases of mastitis presenting with abnormal milk or abnormal milk accompanied by localized swelling of the udder, account for approximately 85% of all CM cases. These findings may be related to our study locations, which included the veterinary clinics in villages and small breeders who primarily consult a veterinarian when their animal's condition is advanced. So once there are changes in the udder tissue or changes in the milk, it's a starting point to begin treatment, as delaying treatment leads to a more expensive process and difficulty in the complete curing of an animal's udder. When the animals were examined and the owners were questioned, it was found that 87.5% of the cases had previously suffered from mastitis and a decrease in milk production during previous lactating seasons. Additionally, 62.5% of the cases had more than four lactating seasons, most likely as a result of a wider or long-term partially open teat canal from frequent milking. Additionally, the permeability of the mammary epithelium in older cows has increased, primarily due to irreversible damage from prior inflammations. These findings are consistent with Kibebew (2017). The dry period is another cause of recurring udder inflammatory outbreaks, especially in elderly cows (Ricci et al., 2017). All owners of infected cases in this study, during taking the history of cases, neglect the appropriate dry period time and do not employ dry period treatment with long-acting antibiotics to eradicate and prevent future infections, which aids in the recovery of the udder tissue and general animal health. In addition any infection during the dry period can have an impact on subsequent birth (Biggs, 2017).

Early detection of CM signs can lead to effective treatment and illness control (Hulsen et al., 2008). The principal mastitis-causing bacteria pathogens (Bhosale et al., 2014). Thus, as a result of this, since bacterial infection is the main cause of mastitis, antimicrobial medications are the main strategy to treat mastitis conditions (Hossain et al., 2017; Hayashi et al., 2023). The overuse of antibiotics for growth promotion and feed efficiency is widespread in Egypt, which contributes to the emergence of illnesses resistant to antibiotics. Farmers mistreat animals and people by giving them excessive amounts without the necessary, and the misuse of antibiotics has contributed to the appearance of antimicrobial-resistant bacteria (Zhang et al., 2018). Speedy bacteriological diagnosis would make it easier to determine the appropriate antimicrobial treatment, so in the current investigation, 17.1% (11/64) of the milk samples from cows with CM illness contained isolates of *E. coli*. This percentage is higher than that reported by Cheng and Han, 2020, and Kareem et al., 2023, who reported that 4.6% and 14.1%, respectively, of *E. coli* isolates were recovered from cattle with CM. Conversely, El-Sayed et al. (2017) and Bag et al. (2021) reported that *E. coli* isolates from cows with CM accounted for 40% and 35.8% of the isolates, respectively. The number of *E. coli* isolates, the duration of milk sample collection, and the animal immune system may have contributed to the differences observed in previous studies.

The antibiotic sensitivity test results in the present study revealed that the obtained *E. coli* isolates were 100% resistant to the antibiotic classes of penicillins, tetracyclines, and beta-lactam antibiotics (amoxicillin/clavulanic acid), followed by streptomycin (class aminoglycosides) at 72.2% and sulfamethoxazole/trimethoprim at 36.4%. The lowest percentage of resistance was detected against class fluoroquinolones (either ciprofloxacin or norfloxacin was 9.1% and 0%, respectively). Results in agreement with Cheng et al. (2020), who detected 95.5% of *E. coli* isolates were susceptible to oxytetracycline and streptomycin, and Kareem et al. (2023), who informed that *E. coli* is highly resistant to streptomycin (aminoglycosides) and sulphathiazole + trimethoprim (sulphonamides), relatively agree with Pascu et al. (2022), who reported 71.4% of *E. coli* resistance to tetracycline, and Abed et al. (2021), who detected 81% and 88% of *E. coli* isolates resistance to amoxicillin/clavulanate, respectively. Verma et al. (2023) indicated that fluoroquinolones demonstrated intermediate sensitivity to the *E. coli* pathogen. Due to endotoxin-induced shock, cases of mastitis caused by *E. coli* require to be treated quickly after diagnosis (Persson et al., 2015). The class of fluoroquinolones is recommended for treating *E. coli* in cases of mastitis based on an in vitro antibiotic sensitivity test. This finding is consistent with studies by Suojala et al. (2013) and Krömker and Leimbach (2017), which reported that parenteral administration of fluoroquinolones, such as enrofloxacin, danofloxacin, and marbofloxacin, is recommended to treat *E. coli* mastitis. Fluoroquinolones are very effective and have several advantages, including their broad-spectrum antibacterial action and wide volume of distribution. Fluoroquinolones are commonly used in clinics to treat both Gram-negative and positive bacterial infections. Because of their therapeutic significance in the fields of human and veterinary medicine, fluoroquinolones are regarded by the WHO as critically important antibiotics (Patel and Goldman, 2016). Parental antibiotic therapy is strongly advised in cases of serious infections in which the antibiotic's diffusion into the parenchyma is required, as well as in cases involving life-threatening conditions or general signs (Grandemange et al., 2012).

In the current investigation, all eleven cows received marbofloxacin, a component of the fluoroquinolone class, in addition to additional supportive treatments and symptomatic care, particularly in severe cases. Only nine (81.9%) of the cows responded to the medication; the other two had complex cases, were unable to move, and were in a recumbent position. Although the general condition of the cows improved after the course of treatment, the udder and milk didn’t return to normal during the follow-up. This might be because the disease was treated too late and its condition was already severe, or it could be because the animal had already been exposed to a lot of infections and had received antibiotics regularly, which caused the disease to become resistant to the treatments. According to Suojala et al. (2013), Oliveira and Ruegg (2014), Kromker and Leimbach (2017), and Verma et al. (2023), this result is in line with their recommendation that parenteral administration of fluoroquinolones be used instead of intramammary drugs due to the risk of uncontrolled bacterial growth in the mammary gland and consequent control of bacteremia. Additionally, NSAIDs are effective in treating *E. coli* mastitis and are also suggested as supportive treatment for CM.

We performed the biochemical analysis of milk samples to further confirm the potential role of treatment protocol in alleviating CM in our study. The study revealed that the milk from cows with mastitis had substantially lower levels of TP, TG, and calcium and significantly higher concentrations of LDH, ALP, and sodium than milk from non-mastitis cows. These findings are consistent with those of Lebeaux et al. (2014). Milk's elevated enzyme levels are primarily attributed to leukocytes, degenerative parenchyma cell leaks, and increased microcirculatory channel permeability in inflammatory zones. The reduction in TG levels could potentially be attributed to the lipolysis of milk globule membranes by leucocyte lipases or plasmin, thereby diminishing the secretory capacity and fat content of the mammary gland. Mastitis in dairy cows can result in higher milk conductivity and elevated sodium levels. The presence of inflammatory cytokines can also impact the secretion of parathormone, which may ultimately lead to a decrease in calcium levels (Shehata et al., 2024). Results showed that treatment decreased enzyme activities and restored milk composition. This may suggest that the therapy may reduce the number of recruited leukocytes and damaged udder epithelial cells.

Udder health assessment relies on milk oxidation level, measured through MDA, and understanding the body's total antioxidant capacity provides insights into its ability to combat oxidative stress (Farghali et al., 2021). Our findings were in line with those of Zigo et al. (2019). During inflammatory processes in the mammary gland, lipid peroxidation increases, leading to higher MDA levels, while TAC activity in milk may decrease due to the depletion of free radicals. Furthermore, we found a significant decrease in MDA and an increase in TAC in the milk of the therapeutic group. The protective benefits of a rapid and accurate treatment protocol are thought to be caused by a reduction in cellular damage from peroxidases and oxygen radicals, as well as an improvement in the effectiveness of the enzymes responsible for intracellular killing mechanisms.

5. CONCLUSIONS

This study was designed to investigate and assess the field treatment of *E. coli* infected cases among bovine clinical mastitic cases admitted to two veterinary clinics in Sharkia and Behiera governorates, examine the antimicrobial susceptibility patterns of *E. coli* isolates, and changes in biochemical markers were measured to further confirm the potential role of treatment protocol in alleviating CM in our study. Eleven milk samples (17.1%) tested positive for *E. coli* with complete resistance to antibiotic classes of penicillin, tetracyclines, and beta-lactam antibiotics. These findings of moderately high isolation rate of *E. coli,* and the increasing development rate of antibiotic resistant strains mainly to commonly used beta-lactam group could explain the clinical observations of the poor *E. coli* infected bovine cases response to treatment and severe aggressive nature of clinical mastitis associated with *E. coli* infection. In line with treatment, milk from treated cows restore the normal biochemical status following considerably greater concentrations of LDH, ALP, sodium chloride, and MDA and significantly lower levels of TP, TG, calcium, and TAC.

RECOMMENDATION

A further study focusing on early diagnosis of bovine mastitis based on milk examination, and/or measuring biochemical markers are of high economic need. Also, preventing new infections during the dry period as it is a critical period in increasing bacterial infections of the udder via immunization against *E. coli* needs further evaluation for the produced protective level of antibodies in both milk, bloodstream and around the teat canal.

-In the recent studies, the uncontrolled and over usage of veterinary field level of antibiotics induce the spread of antibiotic resistance resulting in failure of antibiotic therapy in cases of CM, so a further study about alternatives protocols to antibiotics drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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