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Antimicrobial Residues in Raw Milk of Mastits Cattle Treated with Ethnoveterinary Product: A Pilot Study

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Abstract
Of all the infectious diseases of cattle, mastitis is the most predominant disease that affects the dairy cattle globally. Mastitis in cattle is common in laccating cattle but also recorded during dry period. The condition is caused by various infectious pathogens that not only affects the udder tissue but also the quality and quantity of the milk in the affected animal thus rendering huge economic loss to the farmer. Milk from mastitis treated cattle often contains residues of antimicrobials following their administration through different routes. As per the European Commission, antibiotic residues are "pharmacologically active substances and their metabolites which remain in foodstuffs obtained from animals to which the veterinary medicinal products have been administered." Tetracyslines, Beta lactams and fluoroquinolones are the most common antibiotic residues detected in certain states of India. The present study was carried out to evaluate the presence of antimicrobial residues in the raw milk of mastitis affected cattle vis a vis antibiotic treated ones. 10 cattle with 18 affected quarters whose milk was found positive for *Staphylococcus sps. and E.coli* were treated with topical ethno-veterinary product, *Herbolact* for 3-5 days and milk samples were evaluated for the presence of antimicrobial residues on day 5,10 and 15. From the present study all the samples from the cattle that were treated with ethnoveterinary product were found negative for antimicrobial residues on day 5, 10 and 15.

Keywords: Mastitis, Antimicrobial residues, Ethnoveterinary treatment, Raw milk, Staphylococcus spp. and E. coli

Introduction
Milk being a complete balanced nutrition, is one of the most consumed animal origin food for human. In Indian scenario, milch animal rearing has been one of the common livelihood for small and marginal livestock farmers. Among the various infectious diseases, mastitis is one of the most important diseases that not only affects the health and production of the milch animals but also affects the economy of the livestock farmer in general. Mastitis in general is being treated with various antimicrobials which lead to the presence of their residues in raw milk and in its products too that cause a serious threat to the human health. Misuse of antibiotics both in humans and animals leading to antimicrobial resistance, thus tackling AMR as a global priority (Zhang *et al*., 2022) [18]. Several antimicrobial residues were identified in bovine milk samples even after the withdrawal period of drugs (Natalia *et al*., 2024) [14]. In this scenario, ethnoveterinary products plays a major role not only in treating the diseases of livestock with herbal therapeutic agents but also combating the presence of antibiotic residues in their products. The present study was carried out to assess the presence of antimicrobial residues in mastitis affected cattle milk samples that were treated with ethnoveterinary product, *Herbolact*.

Materials and Methods: The pilot study on the presence of antimicrobial residues in the raw milk of the mastitis cattle treated with ethnoveterinary product was carried out in farmer-owned crossbred cattle of Warangal, Mahaboobnagar and Jagityal districts of Telangana State. A total of 10 crossbred cattle with clinical mastitis were selected for the present investigation. Milk samples from the affected quarter were collected in sterile vials to identify the specific etiology. Depending on the severity of the condition, the ethnoveterinary product *Herbolact* @ 20 g was applied topically on the affected udder thrice daily for 3 - 7 days. The product was thoroughly mixed with lukewarm water (100 ml) and applied all over the affected quarters and healthy quarters with gentle massage.

Before each application, milk was completely stripped out and the udder was cleaned and dried. Milk from all the affected quarters was collected on day 5, 10 and 15 and were subjected for detection of the presence of antimicrobial residues using lateral flow assay kit, TriTest BTS Test Kit (β-Lactams& Tetracyclines & Sulfonamides) supplied by M/S Ring Biotechnology Co., Ltd., China. However, milk from mastitis cattle that were treated with beta lactams and tetracyclines were also evaluated to establish comparison.

Results and Discussion

A total of 10 crossbred adult cattle with signs of clinical mastitis of 18 affected quarters were identified for the present investigation. All these affected animals were presented with swollen hot and painful udder that has engorged teats, flaky/watery milk with clots. The dairy cattle globally are affected with the most common clinical entity, the mastitis that is caused by a variety of infectious pathogens resulting in either clinical or subclinical forms. While affecting the quality and quantity of the milk in the affected animal resulting a huge economic loss to the farmer, mastitis has been considered as one of the most important infectious diseases that affects the livelihood of middle and small-scale livestock farmers (Mushtaq *et al.*, 2018) [12]. The clinical form of mastitis is characterised by certain significant signs such as, hard and swollen udder and teats with the presence of clots that even affect the quality and quantity of the milk (Kholif *et al.*, 2017) [8]. Mastitis in severe cases and forms not only cause huge economic loss to the farmer, but may also cause death of the affected cattle. In the present study, the common bacteria that were isolated from the milk samples were *Staphylococcus sps* and *E.coli* (Krishnamoorthy *et al*., 2021) [9]. Apart from CMT, somatic cell count, milk pH and detection of pathogenic bacteria in the milk samples, mastitis has been diagnosed by a recently developed Immuno Chromatographic Strip coated with anti-ribosomal protein L7/L12 for detecting staphylococcus aureus associated bovine mastitis (Nagasawa *et al.*, 2020) [13]. Some of the common bacteria like *Streptococcus* sp., and *Staphylococcus aureus* that was normally seen on the skin are common pathogens apart from other environmental microorganisms like *E.coli* and other coliforms. Mastitis always exists a positive correlation between high milk yield and its occurrence (Cheng and Han 2020) [6]. Mastitis is predominantly a bacterial disease of the mammary gland of high-yielders (Sharun *et al*., 2021) [17]. Antibiotics are often used or misused in the dairy sector for therapeutic and prophylactic purposes (Manyi-Loh *et al*., 2018) [11]. Antibiotics are also eliminated in the milk till 7 days post-treatment. However, there is an absence of compliance to milk withdrawal periods following antibiotic administration, which leads to antibiotic residues in milk. Hence, improper use of antibiotic therapy in milch animals poses a threat to the public, especially if milk is consumed unpasteurized (Anika *et al*., 2019; Kumar *et al*., 2021) [1, 10].

Following treatment with the ethnoveterinary product, improvement in the signs along with absence of abnormalities in the milk was noticed in all the affected quarters by day 5-7. Further, none of the milk samples on days 5, 10 and 15 revealed positive for the presence of antimicrobial residues in the milk by Tritest BTS test kit. However, the milk samples from the animals treated with beta lactams and tetracyclines revealed positive reaction for the antimicrobial residues on day 5 and 10 but negative on day 15. Antimicrobials remain the mainstay of mastitis treatment in bovines, that raised a concern for human consumers due to the presence of antimicrobial residues in the milk even after recovery. Thus, the role of alternative medicine is emerging and most of the diseases including the mastitis is now being treated with various herbal drugs. The modern ethnoveterinary practices has been used by rural people traditionally. This alternate medicine involves the utilisation of whole plant or its components as medicine that has antibacterial, antifungal and antioxidant properties and thus help in preventing or curing the disease (Bakare *et al.*, 2020) [3]. The present ethnoveterinary medicine, Herbolact contains aloe vera, curcuma longa and calcium hydroxide acts synergistically and provides an effective cure in clinical mastitis owing to its wide spectrum of antimicrobial, antioxidant, anti-inflammatory and immunomodulator affects, that are similar which can be attained by use of an antimicrobial. The herbal ingredients, aloe vera, curcuma longa and calcium hydroxide improve the status of metabolism, reduces the inflammation and microbial load, cleanse the toxins of various parts of the body including udder and lactiferous tissue and pacify the aggravated pitta dosha and raktha doosha (Charaka Samhitha, 1992) [5]. Following topical application of the herbolact for 5-7 days, all the affected milk samples were found negative for culture with no recurrence till 6 months. Antimicrobial residues are of most important concern in different products like meat and milk particularly from mastitis-treated cows, resulting in antibiotic resistance a most significant public health risks. Following prescribed milk withdrawal periods the farmers can prevent having these residues in milk. However, as it affects the economic status of particularly small-scale livestock farmers, these residues that are pharmacologically active substances are seen in milk. Poor hygiene and management on farms, coupled with a lack of farmer education on proper antibiotic use and withdrawal, contribute to the prevalence of these residues in the milk supply. The best ways to avoid consuming this contaminated milk are to discard the milk until the recommended withdrawal period and to minimize the use of antibiotics. The primary reason for its presence in milk is incorrect antibiotic use for treating diseases, especially mastitis (Sachi *et al*., 2019) [15].

Milk from mastitis treated cattle often contains residues of antimicrobials following their administration through different routes. As per the European Commission, antibiotic residues are “pharmacologically active substances and their metabolites which remain in foodstuffs obtained from animals to which the veterinary medicinal products have been administered.” There is a milk withdrawal period that extends to 7 days following antibiotic therapy. Tetracyslines, Beta lactams and fluoroquinolones are the most common antibiotic residues detected in certain states of India (Sahoo *et al*., 2023) [16]. From the available literature, *Staphylococcus* spp. emerged as the most prevalent mastitis-causing bacteria, accounting for 45% of cases in India. Moreover, vide variety of antimicrobials including that of penicillin, streptomycin, erythromycin, tetracycline and ampicillin developed resistance against *S. aureus* in mastitis cattle. Studies also shown *S. aureus* isolates that were resistant to various β-lactams like penicillin, amoxicillin, and methicillin, as well as oxytetracycline (Chandrasekaran *et al*., 2015; Hamid *et al*., 2017) [4, 7]. In a similar study, the resistance pattern of *S.*

aureus to ampicillin, methicillin, oxacillin, ceftriaxone, rifampicin, vancomycin, amoxiclav, oxytetracycline, erythromycin, nitrofurantoin, cefuroxime, gentamicin, norfloxacin, ciprofloxacin, and levofloxacin was observed in milk of cows and buffaloes (Baghel *et al*., 2018) [2].

Conclusion

The ethno-veterinary practice is one of the most sustainable alternative medical practices that incorporate traditional beliefs to scientific knowledge on medicinal plants is a simple, easy and cost effective alternate approach with much less to no side effects. It also provides a promising therapeutic efficacy with minimal side effects, lack of AMR to these phytochemicals, and reduced drug residues in animal products. From the present study, it may be concluded that the antibiotic residues are present in raw milk that were treated with the antimicrobials, but with no residues among the milk samples that were treated with ethnoveterinary compound, *Herbolact*. Hence, the pilot study puts on record about its therapeutic efficacy including anti-inflammatory and antibacterial properties of the herbolact, along with no antimicrobial residues in the milk of the affected cattle both during and after treatment. However, further studies involving a larger sample are warranted.

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