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Antibiogram pattern of sub clinical mastitis milk samples of dairy cattle in Devanahalli taluk

Pramod KS, M Narayana Bhat, Ramesh PT and Paramesha SC

Abstract
The present study was undertaken to find out the presence of Subclinical mastitis (SCM) in pooled samples collected from milking dairy cattle and to track the SCM cases in individual herds of selected villages. Further, the antimicrobial resistance pattern of the bacteria associated with subclinical mastitis of dairy cattle were studied. Out of 1330 pooled milk samples screened for SCM, 101 household samples were positive for SCM by California mastitis test (CMT) with overall prevalence of 7.59 per cent. Later the 101 positive samples were tracked back to individual households and all the animals in the herd were screened for SCM by CMT. Out of 413 animals screened for SCM, 127 (30.75%) animals were found positive. Positive samples were subjected to Antibiotic Sensitivity test (ABST). Milk samples were highly sensitive to Ceftriaxone and least sensitive to Penicillin-G.

Keywords: SCM, CMT, ABST, Sensitivity, ceftriaxone, penicillin - G

Introduction
Mastitis is an inflammation of the mammary gland that affects all domestic animal species and is a major concern for the dairy industry. Mastitis is a prevalent disease in cattle in both developed and developing countries, and it is an economically significant disease affecting dairy cow breeding since it occurs frequently and lowers milk output. Mastitis is divided into four types: acute, subclinical, clinical, and peracute (Kader *et al*., 2003) [5]. According to Bradley (2002) [2], 75-80% of mastitis is subclinical, defined by a significantly elevated leukocyte count in milk. According to (Radostits *et al*., 2007) [11], there are no evident clinical indications such as inappropriate milk, udder enlargement or discomfort, or systemic signs such as fever or depression in subclinical mastitis (SCM). Instead, there is an increase in somatic cell proliferation.

When Penicillin was introduced into Veterinary Medicine in 1944 (Kakavas, 1944) [6], many practitioners assumed that mastitis would become a thing of the past. In truth, even after 75 years and despite the discovery of new medicines, mastitis persists, and the importance of antibiotics in mastitis control is diminishing. For the control of SCM, significant progress has been made in the fields of sanitation, milking, and immunology. Nonetheless, antibiotics are an essential component of most mastitis therapy and control plans.

In a study conducted by (Sankar, 2016) [12], he stated that for the past 40 years, a wide spectrum of antibiotics have been employed in the management of bovine mastitis. Some of the reasons for the poor cure rate in mastitis were (i) low intracellular uptake of commonly used medications, (ii) non-diffusion of acidic antibiotics at neutral extracellular or cytoplasmic pH via the lysosomal membrane, and (iii) very poor retention in cells.

According to (Biswas *et al*., 2008) [1], drug resistance is a natural phenomenon that arises over time, mainly as a result of genetic changes acquired through mutation and selection (vertical gene transfer) and/or gene acquisition between strains and species (horizontal gene transfer). Antimicrobial resistance is a major concern for both animal and human health in S. aureus infections.

According to (Ter Kauil *et al*., 2016) [17], inappropriate antibiotic use can hasten the selection of drug resistance strains. He also claims that administering modest quantities of antimicrobial medicines to domestic animals can select bacteria with antimicrobial resistance genes (ARGs) or multidrug-resistant plasmids, causing the genes to multiply and spread across strains.

The success in the treatment of bovine mastitis by antimicrobials depends collectively on judicious use of these antibiotics, immune status of animals and appropriate hygienic measures. However, most of the research works have been conducted on the prevalence studies by screening individual animals.

There is a need to go for systematic screening of pooled milk samples so that vast population can be covered.
In the present study the vast population of dairy cattle were covered by subjecting the pooled milk samples for CMT at milk producer's co-operative societies from herds of dairy cattle and tracking back the positive samples into individual households/herds to check each and every animal in the herd, further the animals which were positive for SCM through CMT were considered for the present study.

Materials and Methods

The current study was under taken to diagnose the SCM in dairy cattle to study the present status of pathogens involved in mastitis and to find out the antimicrobial resistance pattern in bacteria associated with subclinical mastitis of dairy cattle.

❖ **CMT reagent (M/s Ruchi pesto chem (India) Pvt. Ltd. Pune)**

Composition

- Sodium hydroxide 1.5% w/v
- Teepol 0.5% v/v
- Bromothymol blue 0.01% w/v

Milk samples were screened using California Mastitis test according to the procedure given by Quinn *et al.* (1999) [10].
Pooled household milk samples were tested for SCM by CMT. Positive samples were tracked to household and 127 samples are collected from individual animals. Milk samples were collected at evening milking. The first three foremilk streams were discarded. The teats were then meticulously cleansed with cotton and 70% ethanol. In sterile vials, 15 mL of milk was collected aseptically. The samples were promptly delivered to the laboratory and refrigerated.
On Muller Hinton agar plate, milk samples were tested for antimicrobial sensitivity using the disc diffusion method described by Cruickshank *et al*. (1975) [3].
For bacteriological analysis, 0.01 mL of milk sample was put onto Nutrient broth and incubated at 37 ^{0}C for six to eight hours to allow organisms to develop.
Using a sterile cotton swab, the broth culture was placed to the surface of a Muller Hinton agar plate, and the plate was covered for 15 minutes at room temperature to allow the inoculum to dry. Antimicrobial discs were carefully removed from their separate vials and placed 20 mm apart on the plates using flamed forceps. Plates were chilled for 15 minutes to allow antibiotic pre-diffusion before being incubated at 37^{0}C for 18 to 24 hours.
The sensitivity pattern of isolates to several antimicrobial discs was read by measuring the diameter of the zone of inhibition in millimeters using the manufacturers' chart.
The antibiotic discs used in this study included Ceftriaxone, Cefoxitin, Doxycycline, Erythromycin, Enrofloxacin, Gentamicin, Kanamycin, Methicillin, Oxacillin, Penicillin, Rifampicin, Streptomycin, Tetracycline, Tobramycin and Vancomycin.

Results and Discussion

A total of 1330 pooled household milk samples were screened for subclinical mastitis by employing California mastitis test at MPCS of 17 villages of Devanahalli taluk of Bangalore rural district, Karnataka state. Later all the animals in the positive herds were screened for SCM by CMT. CMT positive samples were subjected to ABST.
Milk samples collected from 127 subclinical mastitis cows were subjected to antimicrobial sensitivity test. The results indicate that the pathogens involved in SCM in these villages were highly resistant to most of the antibiotics. They were 100 per cent resistant to Penicillin-G, Rifampicin, Methicillin. The resistance to other antibiotics ranged from 63.72 per cent to 98.43 per cent. None of the antibiotics had more than 50 per cent sensitivity.
Ceftriaxone had the highest sensitivity in this trial, followed by Doxycycline, Tobramycin, Tetracycline, and Enrofloxacin. This is consistent with the findings of Mohanty *et al*. (2013) [8], who discovered the highest sensitivity to Levofloxacin, Enrofloxacin, and Chloramphenicol. Similarly to Joshi and Gokhale (2006) [4], Kumar and Bhat (2016) did a study on the antibiogram pattern of bacterial isolates from clinical mastitis cases and found that the majority of the isolates were sensitive to Enrofloxacin.
In the present work, milk samples showed lowest sensitivity to Penicillin, Erythromycin, Vancomycin, Kanamycin, Gentamicin, Methicillin, Rifampicin and Streptomycin indicating very high antibiotic resistance. This is consistent with the findings of Mohanty *et al*. (2013) [8], who revealed that none of the isolates were susceptible to Penicillin-G. Similarly, Sumathi *et al.* (2008) [16] reported that Resistance to Cephalexin, Penicillin, Streptomycin, and Sulpha-diazine was found in a greater frequency of isolates. Bacterial isolates were also shown to be extremely sensitive to chloramphenicol and least sensitive to penicillin-G, according to Sulthana *et al*. (2015) [15].
Mubarack *et al*. (2012) [9], on the other hand, reported the highest sensitivity to Gentamicin. The ineffectiveness of these antibiotics against bacterial isolates may be due to their indiscriminate usage un animals. Furthermore, repeated use of certain antibiotics, underdosage, inadequate course, and resistant bacterium genes are some of the factors contributing to antibiotic resistance.
Antibiotic resistance is a major problem throughout the world. Antibiotic resistant bacteria can be zoonotically important especially in immune compromised patients.

Antibiogram pattern of subclinical mastitis milk samples (n=127)

Antibiotic disc	Sensitive	Sensitivity (%)
Ceftriaxone	46	36.28
Doxycycline	33	25.98
Tetracycline	29	22.83
Tobramycin	29	22.83
Enrofloxacin	28	22.04
Erythromycin	10	7.87
Streptomycin	9	7.08
Gentamicin	7	5.51
Vancomycin	7	5.51
Kanamycin	2	1.57
Methicillin	0	0
Rifampicin	0	0
Penicillin – G	0	0

Conclusion

The present study reveals that the indiscriminate use of antibiotics in the treatment of mastitis and subclinical mastitis will lead to resistance against most of the available antibiotics, hence selecting the antibiotic based on the results of ABST of the milk samples will be the more beneficial than treating randomly.

Conflict of interest

The authors declare that they have no known competing

financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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