Cultural and biochemical studies of sub-clinical mastitis in cows “in and around Hyderabad”

Anusha Dasohari, Ayodhya Somasani and Nagaraj P

Abstract
The present study “Cultural and Biochemical studies of subclinical mastitis in cows “ in and around Hyderabad” was undertaken to diagnose the subclinical mastitis in cows, was carried out during a period of 7 months i.e., February to August, 2015. To diagnose subclinical mastitis (SCM), a total of 534 quarter milk samples from 136 apparently healthy cows of three local dairy farms and individual holdings were collected and subjected to cultural and biochemical (IMViC) test, for isolation of etiological agents. According to cultural and biochemical (IMViC) test Staphylococcus spp., were most prevalent followed by Streptococcus spp., and Escherichia coli. Among the Staphylococcus spp. isolated, coagulase positive organisms were more prevalent (37.31%) compared to coagulase negative Staphylococcus spp. (CoNS) (19.4%)

Keywords: Subclinical mastitis, cultural, (IMViC) test, Staphylococcus, Streptococcus, Escherichia coli

1. Introduction
Subclinical mastitis is bereft of any obvious manifestation of inflammation and is characterized by having no visible signs either in the udder or in the milk, but the milk production decreases and there is change in milk composition [5]. Subclinical mastitis is 3–40 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds [1]. Besides causing huge losses to milk production, the sub clinically affected animals remain a continuous source of infection to other herd mates [7]. The subclinical form of mastitis in dairy cow’s is important because it is 15 to 40 times more prevalent than the clinical form and is difficult to detect, reduces milk production and adversely affects milk quality [12]. The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell counts (SCC) and microbiological status of the quarter. Though bacteriological culture of milk samples is the standard method for identifying mastitis, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted [14]. The present study “Cultural and Biochemical Studies of Sub-Clinical Mastitis in Cows “in and around Hyderabad” was undertaken to study therapeutic efficacy of two different antibiotics for the treatment of subclinical mastitis in cows.

2. Material & Method
The study was carried out on cases of subclinical mastitis from three different dairy farms and individual holdings during the period from February 2015 to August 2015.

Screening of Animals for Subclinical Mastitis: Total of 534 quarter milk samples from 136 apparently healthy cows were collected and subjected to California mastitis test (CMT), White side test (WST) and Surf field mastitis test (SFMT) and Somatic Cell Count (SCC) to differentiate subclinical mastitis from clinical mastitis. Based on the above test results the milk samples were collected by aseptic precautions into sterile vials and then subjected to bacteriological examination for isolation of etiological agents.

Primary identification of bacteria was done based on colony morphology, type of hemolysis and Gram’s staining and pure cultures were identified up to genus level as per the Bergey’s Manual of Determinative Bacteriology [4]. Gram staining, Motility test, Catalase activity, coagulase test, sugar fermentation test, Haemolysin test, Indole test, Methyl Red test, Voges – Proskauer test, Citrate utilisation tests were done on a 24-48 hour old pure culture for the identification of bacteria.
3. Result

The 115 quarter milk samples collected from 30 animals which were subjected to all the diagnostic tests were only cultured. Out of 115 quarter milk samples, 59 were culturally positive (51.30%). Examination of gram stained milk smears revealed gram positive coci in 49/59 (83.05%) samples. Hence, for further isolation, they were streaked on blood agar, nutrient agar, Edward’s medium and mannitol salt agar plates. The gram positive coci in 38/49 quarter samples produced hemolysis after 24 hours of incubation at 37°C when streaked on blood agar plates (fig. 1. Small, round, cream coloured colonies appeared on nutrient agar after incubation for 24 hours. The coci from 38 quarter samples fermented mannitol that was present in the medium and turned the color of the medium to yellow. Colonies appeared on MSA plates were round, smooth and glistening and had a golden-yellow pigment (fig. 2). Hence, they were considered as *Staphylococcus* and tested further biochemically.

![Fig 1: Haemolytic colonies of Staphylococcus on blood agar](image)

![Fig 2: Staphylococcus showing golden yellow pigment on MSA on blood agar](image)
Gram stained milk smears of broth culture revealed the gram negative, medium sized rods in 15/59 quarters (25.42%). Hence, the culture was streaked on nutrient agar, Mac Conkey and EMB agars. On nutrient agar the colonies appeared as greyish in color which were round and shiny. Round, shiny and bright pink color colonies appeared in 13/15 samples (86.67%) on Mac Conkey agar plates incubated at 37°C for 24 hours (fig. 7). On EMB agar plates, the bacteria produced metallic sheen (fig. 8). When the isolates were subjected IMViC test, the bacteria showed a positive reaction for Indole production as well as for Methyl Red (MR) test and was negative to Voges-Proskauer (VP) test and citrate utilization (fig. 9). Hence, they were considered as *E.coli* (fig. 10).

Two out of fifteen (13.33%) gram negative isolates produced capsulated, large and highly mucoid colonies on Mac Conkey agar plates when incubated for 24 hours at 37°C (fig. 11). When the isolate was subjected to IMViC test, isolates were negative for Indole production and Methyl Red (MR) test and positive for Voges-Proskauer (VP) test and citrate utilization (fig. 12). Hence it was considered as *Klebsiella* spp. Out of the 59 quarter samples examined, 13/59 (22.03%) and 2/59 (3.39%) were *E.coli* and *Klebsiella* spp., respectively.
The Pharma Innovation Journal

5. References


3. Musoke MG, Owiny DO, Persson Y. Prevalence and distribution of Staphylococcus aureus, Escherichia coli and Streptococcus spp. in milk from dairy cows in Mbarara, Western Uganda.


5. Saini al [8]. reported that the major pathogens among the Staphylococci indicates unhygienic milking practices as this pathogen is mainly spread during milking via milkers’ hands.

6. Harini and Sumathi [6] and Patnaik [17] reported Staphylococcus aureus (58%) and Escherichia coli (23.5%) followed by Staphylococcus epidermidis (8%), Streptococcus spp. (5.5%), Klebsiella spp. (3%) and Bacillus spp. (2%). Mir et al [8]. reported Staphylococcus aureus (41.04%), Corynebacterium (30.60%), Streptococcus spp. (21.27%), E. coli and others (7.09%). The higher incidence of Staphylococci indicates unhygienic milking practices as this pathogen is mainly spread during milking via milkers’ hands.

from cases of subclinical mastitis in dairy cattle in Kampala, Uganda. Irish Veterinary Journal, 2014; 67(12).