

Prevalence of sub-clinical mastitis, identification of causative agents and sensitivity profile of isolates in Northern Pakistan

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Abstract: Mastitis is considered priciest disease causing multimillion dollar losses to dairy industry worldwide annually. Surf Field Mastitis Test (n=200) revealed almost one forth (n=57, 28.6%) positive. Similarly, highest infestation was recorded in Sonikot (n=30, 75%) followed by Kashrote (n=10, 25%), Yadgar Muhallah and Nagaral (n=8, 20%) each and Baseen (n=1, 2.5%) being the least. The various pathogens isolated by culturing and identified by biochemical tests from Surf Field Mastitis Test positive milk samples. *Staphylococcus* was found 12 (21.5%), *Streptococcus* 10 (17.54%), *Pseudomonas* 8 (14.3%), and *Escherichia coli* 10 (17.54%). The infestation of *Staphylococcus*, *Streptococcus* and *Escherichia coli* was high as compare to *Pseudomonas*. Most of the isolates found sensitive against Pefloxacin, partial resistant with Ampicillin. All isolates were resistant with Erythromycin and Amoxicillin. It is recommended that the milking cows should be kept in dry and clean areas, teats must be dipped in any antiseptic after milking process to avoid bacterial infections and treatment of the infected cows is important with an appropriate antibiotic.

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Key words: Microbiology, cow milk, bacterial contamination, prevalence, Gilgit, antibiotic sensitivity pattern.

Introduction

Bovine mastitis is one of the most prevailing and longtime infection intricate to detect. It is important due to the fact that it adversely affects milk quality and production of dairy animals and constitutes basin for dairy animals that can affect other animals with in the herd due to its contagious nature. It is a worldwide problem produce physical, chemical and microbiological changes in the milk and pathological changes in the glandular tissue of the udder.

As compared to the developed countries, it is the most common issue in developing countries like Pakistan. Mastitis is documented as the most costly disease of dairy animals (Lightner *et al.*, 1988; Ali *et al.*, 1989). It is an inflammatory condition of the udder irrespective of the cause. It results in huge economic loses (Ahmad, 2001). This disorder also carries risk for the zoonotic transfer of tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to humans (Radostits *et al.*, 2000).

Mastitis represents a serious problem to be considered just because economic loses for which it is responsible (Ahmad, 2001). Arshad (1999) warned that the losses due to mastitis might be higher in Pakistan because of lacking of mastitis prevention techniques like teat dipping and dry period antibiotic therapy. If this disease is identified at very early stage the loss can be lessen to a greater extent. The common primary causative agents of mastitis are bacteria,

viruses, fungi and algae. Among these, bacteria are considered the major cause (i.e. *Staphylococcus aurea*, *Streptococci*, *Corynebacterium pyogenes* and *coliform spp.*) and minor pathogens include (Coagulase negative *Staphylococci* and *Corynebacterium bovis*) (Hameed *et al.*, 2008). This disease is the upshot of the association of host, pathogen and the environment.

Mastitis is a contagious disease and spreads through milker's hands, contaminated towels used to dry or clean the udder and also due to the flies. Mastitis has two forms, clinical mastitis and Sub-clinical mastitis. In clinical mastitis udder shows all the five major signs (redness, heat, swelling, pain and loss of milk production) of inflammation and hence can be diagnosed without any laboratory test.

All immunization techniques developed against the disease have remained ineffective due to its multiple causative agents. The abuse of antibiotics at low levels over longer periods may cause the therapeutic failure and the development of drug resistance. Therefore, regular studies on antibiotic sensitivity of bacterial isolates are mandatory for effective and economical treatment of the disease. The present study was conducted to check the prevalence of Sub-clinical mastitis, its causative agents and antibiotic sensitivity profiles in Gilgit city.

Materials and Methods

Study was conducted in five towns (Sonikot, Kashrote, Yadgar Muhalla, Nagral and Baseen) in and around Gilgit city in Northern Pakistan. A total of two hundred samples (N=200) were investigated, forty (n=40) from each village or town. A single cow for sample collection was randomly selected from each household following National Mastitis Council (1990) procedure. Labeled test tubes were transported in cooler and conducted SFMT. Positives samples were cultured on MacConkey agar Oxoid code (CM 0007) and incubated at 37°C for 24 hours. Next day cultures were observed and processed the bacteria for identification National Mastitis Council Inc., USA (1987) protocols.

Antibiotic sensitivity of confirmed organisms was performed by disk diffusion method (Bauer *et al.*, 1966). One ml of each identified bacterial isolate was prepared from an overnight culture and adjusted to 0.5 McFarland Standard. A sterilized wooden swab was soaked in each culture and used to streak on Mueller-Hinton agar (MHA) and allowed to dry at room temperature. Commercially available sterile disks of specific concentrations of Ampicillin (10µg), Amoxicillin (10µg), Erythromycin (10µg) and Pefloxacin (10µg) were placed on the pre streaked agar plates with the help of sterilized forceps. Sensitivity recorded by measuring the zone of inhibition around the discs for each of the isolated cultures and documented in millimeter (mm).

Results and Discussion

SFMT:

Pathogens identified:

Prevalence of Mastitis was assessed using Surf Field Mastitis Test (SFMT) revealed 28.5% (n=57)

positive while 71.5% (n=143) negative. Among others Sonikot area exhibited highest incidence with 75% (n=30) positive out of forty (N=40) followed by Kashrote 25% (n=10), Yadgar Muhalla 20% (n=08), Nagral 20% (n=08) and Baseen 2.5% (n= 01) being the least (Figure 1).

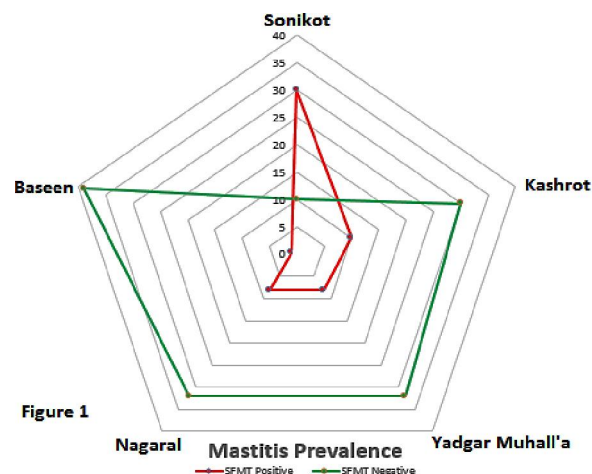


Figure 1

Highest infestation (n=12; 21.5%) recorded with *Staphylococcus spp.* followed by *Streptococcus spp.* (n=10; 17.54%), *Escherichia coli* (n=10; 17.54%) and *Pseudomonas spp.* (n=8; 14.03%). The mixed infestation was also found in (n=17; 29.83%) milk samples.

Antibiogram:

All isolates found resistant with the commonly used antibiotics i.e. Amoxicillin, Erythromycin and Ampicillin. However, exhibited sensitive with Pefloxacin (Table 1).

Table 1. In vitro antibiogram of various isolates.

Antibiotics	<i>E. coli</i> No. 10	<i>Pseudomonas</i> No. 07	<i>Staphylococcus</i> No. 12	<i>Streptococci</i> No. 09
Amoxicillin: S (10 µg)	00	00	00	00
I	07 (70%)	00	00	00
R	03 (30%)	07 (100%)	12 (100%)	09 (100%)
Erythromycin: S (10 µg)	00	00	00	00
I	10 (100%)	07 (100%)	12 (100%)	09 (100%)
R	00	00	00	00
Ampicillin: S (10 µg)	03 (30%)	00	00	00
I	00	00	00	00
R	07 (70%)	7 (100%)	12 (100%)	09 (100%)
Pefloxacin: S (10 µg)	10 (100%)	07 (100%)	12 (100%)	07 (77.8%)
I	00	00	00	02 (22.2%)
R	00	00	00	00

Discussion

Our findings about Mastitis prevalence (28.05%) are in close agreement with Mustafa *et al.* (2012) who reported 30.63% from District Lahore and endorse Iqbal *et al.*, (2004) report of 15.16% in dairy buffaloes in Faisalabad, Pakistan. Moreover, present study validate Pitkala *et al.*, (2004) reported microbial growth in 21-33% in milk samples.

Anwar *et al.* (2013) conducted a study in Khyber Pakhtunkhwa, Pakistan and isolated more *Staphylococcus aureus* and *Streptococcus agalactiae* in cows and buffaloes as compare to other pathogenic bacteria. Ashfaq and Muhammad (2008) also isolated *Staphylococcus aureus* and Streptococcus in their study conducted in Faisalabad.

In Gilgit there is no concept of cleaning the floor of cow bedding and it always remains dirty causing higher incidence of mastitis (Joseph, 1996).

Iqbal *et al.*, (2008) found amoxicillin only 16.1% in their study conducted in Faisalabad. While Serkan *et al.*, (2013) found the sensitivity of Amoxicillin 16.6%, Ampicillin 8.33% and Erythromycin 33.3% in their study conducted in Marmara Region of Turkey.

It is concluded that cows should be kept in neat and clean environment and milked aseptically to minimize the chances of infection of cows with the pathogenic organisms. The Surf Field Mastitis Test should be conducted on regular basis to check the health status of animals. The diseased cows should be separated from the herd and treat with an appropriate antibiotic.

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