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BACTERIAL ETIOLOGY OF SUBCLINICAL MASTITIS IN DAIRY GOATS AND MULTIPLE DRUG RESISTANCE OF THE ISOLATES

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ABSTRACT

Milk samples (n=200) of dairy goats from D. G. Khan and Lahore districts (n=100, each), Punjab, Pakistan were screened with Whiteside Test (WST) for sub-clinical mastitis. Samples positive for mastitis were cultured for bacterial growth on blood agar. Bacterial growth was obtained in 45 % milk samples (90/200). From WST positive milk samples, 146 bacterial isolates were identified on the basis of colonial, microscopic and biochemical profiles. Highest prevalence was of *Staphylococcus aureus* (61.64 %) followed by *Escherichia coli* (10.96 %), *Streptococcus spp*. (9.59 %), *Pseudomonas spp.*, *Bacillus spp*. (6.85 %, each) and *Corynebacterium spp*. (4.11 %). *Staph. aureus*, *E. coli and Strep. spp*. isolates (n=10, each) were tested for antibiotic resistance against ten selected antibiotics used for treatment of mastitis in field. Highest resistance (58.69%) was recorded against Penicillin. Percent resistance of bacterial isolates to more than two antibiotic classes was 44.44, declared as multiple drug resistance (MDR). Most of the MDR isolates were sensitive (83.33%) to Amoxicillin and Clavulanic acid combination out of the four tested. It was concluded that consumption of goat milk by children may transfer antibiotic resistance to the normal micro-flora that may lead to super infection.

Key words: Goat, Milk, Mastitis, Multiple Drug Resistance and White side Test.

INTRODUCTION

Goat (*Capra hircus*) is among one of the oldest domesticated animals. In Pakistan, dairy goat is the main livelihood source for poor farmers. Goat population in the country is 63.1 millions producing 0.779 million tons of milk and mutton production is 0.629 million tons (Anonymous, 2012). Goats are susceptible to variety of bacterial infections especially on rearing under high stocking density. Mastitis is one of the most frequent and multifactor disease of goats resulting in production losses. Mastitis has been recognized as the most important economical factor affecting the dairy animals worldwide (Ali *et al.*, 2011). Different bacteria frequently isolated from mastitis in goats include *Streptococcus spp*., *Staphlococcus spp.*, *Pasteurella spp*. and *E. coli* (Contreras *et al.*, 2007). *Proteus spp*., *Salmonella spp*. and *Bacillus spp*. have been isolated from milk samples collected from goats suffering from mastitis (Iqbal *et al.*, 2004). Diagnosis in clinical as well as sub-clinical cases largely depends on the presence of significantly higher leukocytes count in the milk from affected glands. In context of milk, these leukocytes are called somatic cells. It may be mentioned that the sub clinical mastitis is reasonable for greater pecuniary losses to the goat farmers than its clinical counterpart. The effective prevention and etiological therapy of this disease requires precise bacteriological diagnosis along with sensitivity testing of microbial agents against various antimicrobials (Malinowski *et al.*, 2002).

Concurrent resistance of bacterial species to antimicrobials of different structural classes is increasing in multitude complicating therapeutic management of infections (Iqbal *et al.*, 2002). Bacterial pathogens not responding to more than two antibiotic classes at therapeutic dose are declared multiple drug resistant (MDR). It is one of the emerging problems of mastitis causing bacteria (Hameed *et al.*, 2007). The presence of multiple drug resistant bacteria in goat mastitis milk deteriorates its quality and its use may lead to the transfer of resistance to normal flora of consumers.

Present research plan was to determine percent infection level in dairy goats of districts D.G. Khan and Lahore. Milk samples collected from dairy goats were screened by White Side Test (WST). Purified bacterial isolates were identified on the basis of microscopic morphology, culture characteristics and biochemical profiles. MDR bacterial isolates were checked for sensitivity against four different combinations of antibacterial drugs.

MATERIALS AND METHODS

Milk sampling: Milk samples (n=200) of dairy goats from D. G. Khan and Lahore districts (n=100, each) were collected in sterile containers and screened by Whiteside Test (WST) as described by Barnumt and Newbouldt (1960). Clinical examination criterion of Biffa *et al.* (2005) was followed for goat udder. Milk samples declared positive by WST were transported to the Bacteriology Laboratory, Department of Microbiology, University of Veterinary and Animal Sciences, Lahore at 4 ˚C.

Bio-characterization of bacteria: Each milk sample (0.5 ml) declared positive by WST (n=90) was cultured on blood agar using spreading technique and plates incubated at 37˚C for 24 hours (Lafi and Hailat, 1998). Bacterial colonies with different colony characters were purified by multiple streaking. Purified bacterial isolates were visualized to observe microscopic morphology post stained by gram's technique at 1000X magnification under bright field compound microscope (Scales, 1922). Isolates were characterized biochemically using appropriate selective/differential media and fermentation of different sugars following the identification flow charts provided in Bergey's Manual for Determinative Bacteriology (Holt *et al*., 2000). Bacteria were identified on the basis of pooled observations of colony characters, microscopic morphology and biochemical profiles. The results were recorded.

Multiple drug resistance: Biochemically characterized bacterial isolates were tested for sensitivity against different antibiotics including Penicillin (10IU), Amoxicillin (30µg), Gentamycin (10µg), Oxytetracyclin (30µg), Ceftizole (30µg), Streptomycin (30µg), Ciprofloxacin (10µg), Enrofloxacin (5µg), Norfloxacin (5µg) and Trimethoprim plus Sulfamethoxazole (1.25, 23.75 µg) following the procedure described by Bauer *et al.*, (1966). Briefly, broth activated bacterial cultures of each isolate were swabbed over the surface of Mueller-Hinton agar, allowed to dry for five minutes, antibiotic discs placed on under sterilized conditions and plates incubated at 37˚C for 24 hours. Diameter of bacterial inhibition zone around each applied disc was measured and compared with standard. Bacterial isolates showing no inhibition zone around the antibiotic disc used and well-developed colonies within the zone were rechecked against same drug for confirmation of resistance. Bacteria exhibiting resistance to more than two antibiotic classes were checked for sensitivity against different combinations of antibiotics including Penicillin + Streptomycin, Gentamycin + Ceftizide, Amoxicillin + Clavulanic acid and Oxytetracyclin+Tylosin. Sensitivity pattern of these antibiotics combinations were checked for MDR isolates.

RESULTS AND DISCUSSION

Dairy goats are susceptible to a variety of bacterial infections. Mastitis is one of the most important and frequent disease of goat. Mastitis is characterized by pathological changes in mammary glandular tissue (Chineme and Addo, 1984) with significant increase in somatic cell count (Contreras *et al*., 1996). A wide range of physical, chemical changes in milk and pathological anomalies in glandular tissue characterizes this complex disease. Mostly, higher leukocyte counts are detected in milk of affected animals (Radostits *et al*., 2006).

Present plan was to determine percent level of mastitis in dairy goats reared under field conditions including D. G. Khan and Lahore districts. By screening collected milk samples with Whiteside mastitis Test, 45 percent (90/200) were declared positive for sub-clinical disease. Recorded sub-clinical mastitis was little higher in goats of D. G. Khan than Lahore district. In corroboration, infection rate of mastitis in goats had been reported by Iqbal *et al.* (2004). Higher prevalence of mastitis was reported 47 and 53.5% in dairy goats by Poutrel *et al*. (1997) and Ali *et al*. (2010).

In contrast, Mahmood (1996) declared 22 percent clinical mastitis in goats on the basis of microbiological screening and somatic cell count. This difference may be due the fact that Mahmood (1996) included healthy and diseased goat population in experimental plan. Goat breeds, pattern of farming, housing conditions, milking practices and load/type of microorganisms in surrounding environment play key role in occurrence of mastitis in dairy goats.

Major bacteria involved in etiology of dairy goat clinical or sub-clinical mastitis are *Staphylococcus aureus, Escherichia coli, Streptococcus, Corynebacterium, Brucella, Bacillus* and *Pseudomonas species*. Among these *Staph. aureus* is at top rank in causing mastitis of dairy goats. However, prevalence and relative importance of different etiological agents of mastitis may differ in different geographical regions (Contreras *et al*., 1995). In present study, 146 bacterial isolates were obtained from 90 WST positive milk samples (Table 01). Highest percent bacteria revealed were *Staph. aureus* (61.64) followed by *E. coli* (10.96), *Streptococcus spp*. (9.59), *Pseudomonas/Bacillus spp*. (6.85) and *Corynebacterium spp*. (4.11). *Staphylococcus aureus* had been reported most frequent etiological agent (45.34%) in cases of dairy goat mastitis (Ali *et al*., 2010). Similar findings were declared by Contreras *et al*. (1995) and Bedidi-Madani *et al*. (1998).

In a recent study, *Staph. aureus, Strep. spp.* and *E. coli* revealed in milk collected from subclinical mastitis were 61, 15 and 5 percent, respectively (Aydin *et al*., 2009). These results are in agreement with present research findings. Significantly, different percent

infection rate of *E. coli* (25%) was reported in dairy goat mastitis cases by Iqbal *et al*. (2004).

In-vitro antibiotic sensitivity test was carried out on all bacterial isolates using Penicillin (10IU), Amoxicillin (30µg), Gentamycin (10µg), Oxytetracyclin (30µg), Ceftizole (30µg), Streptomycin (30µg), Ciprofloxacin (10µg), Enrofloxacin (05µg), Norfloxacin (05µg) and Trimethoprim Sulfamethoxazole (1.25, 23.75µg). *E. coli* were found most resistant to Streptomycin (87.5%) followed by Norfloxacin/ Ciprofloxacin (75%), Ceftizole/ Penicillin (62.5%), Oxytetracycline (50%), Amoxicillin/ Enrofloxacin/ Trimethoprim+ Sulfamethoxazole (37.5%) and Gentamycin (25%). Four *E. coli* isolates were resistant to more than two antibiotic classes and declared as MDR. Highest resistance of *Streptococcus* species determined by disc diffusion test was against Streptomycin/ Oxytetracycline/ Ciprofloxacin (71.42%) followed by Penicillin/ Norfloxacin (57.14%), Ceftizole (42.85%) and Amoxicillin/ Enrofloxacin/ Gentamycin/ Trimethoprim plus Sulfamethoxazole (28.57%). Two S*treptococcus* isolates were resistant to more than two antibiotic classes and designated MDR. *Staph. aureus* were found most resistant to Ciprofloxacin (75%) followed by Streptomycin/ Oxytetracycline/ Norfloxacin (62.5%), Ceftizole (50%), Penicillin/ Enrofloxacin/ Trimethoprim + Sulfamethoxazole (37.5%), Amoxicillin (25%) and Gentamycin (12.5%). Three *Staph. aureus* tested isolates were MDR. Most of the MDR bacteria were sensitive to Amoxicillin + Clavulanic acid combination.

Aydin *et al*. (2009) reported 100 percent resistance of *E. coli* isolates to Ceftizole and Norfloxacin which is in contrast with findings of present study. However, percentage of MDR bacteria determined in present study is in agreement with the published results of subclinical mastitis in goats by Boscos *et al*. (1996) and Ndegwa *et al*. (2000).

It was concluded that there are some MDR pathogens responsible for causing mastitis in dairy goats. Milk consumed from these goats suffering from clinical or sub-clinical mastitis may transmit antibiotic resistance genes to micro flora of human beings. This situation may be a biggest human beings health hazard.

Table 1. Prevalence of bacteria isolated and identified from milk of dairy goats with subclinical mastitis

Sr. No	Bacteria	Isolates from District D. G. Khan	Isolates from District Lahore	Total No of Isolates	Percentage
1	*Staphylococcus aureus*	50	40	90	61.64
2	*Escherichia coli*	10	6	16	10.96
3	*Streptococcus*	6	8	14	9.59
4	*Bacillus*	6	4	10	6.85
5	*Corynebacterium*	4	2	6	4.11
6	*Pseudomonas*	4	6	10	6.85
	Total	80	66	146	**100.00**

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