openaccess Journal
SCIENCE LETTERS
2013 | Volume 1 | Issue 1 | Pages 17-20

In vitro antimicrobial sensitivity pattern of mastitis causing bacterial pathogens isolated from cattle in arid zones of Punjab, Pakistan

Sajid Umar[1*], Fozia Sarwar[2], Muhammad Usman[2], Muhammad Ali A Shah[1], Abdul Ghafar[3], Asif Ali[1], Sajjad Asif[4]
[1]Department of Pathobiology, PMAS, Arid Agriculture University, Rawalpindi, Pakistan
[2] Poultry Research Institute, Rawalpindi, Pakistan
[3]NUST Center of Virology &Immunology, Islamabad
[4] Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Abstract
Mastitis is economically most significant disease of lactating animals. Mastitis is a multi-etiological complex disease; however, bacteria are among major pathogens. Bacteria related to mastitis in cattle were identified biochemically and their liability to different antibiotics was judged. Milk samples (n=436) were screened for sub-clinical mastitis by California Mastitis Test. Positive samples (149/436) were cultured on different media and bacterial isolates were identified biochemically. Maximum isolates identified were *Staphylococci* (44.39%) followed by *Streptococci* (31.03%), *E. coli* (12.5%), *Bacillus spp. (5.17%), Pseudomonas* (3.45%) and *Corynebacterium* (3.45%). Sensitivity of bacterial isolates was tested for commonly used antibiotics. Gentamicin, enrofloxacin, amoxicillin and cephradine showed best efficacy against the mastitis causing bacteria amongst the antibiotics tested. Ampicillin streptomycin and kanamycin showed moderate sensitivity for mastitis causing bacteria where as least sensitivity was observed to cloxacillin and penicillin. It was concluded that prior to treatment of mastitis, effective antibiotic should be determined through antibiotic sensitivity testing.
Key words: Cattle, Mastitis, California Mastitis Test, Antibiotic sensitivity, antibiotics

Received July 15, 2013; **Revised** August 22, 2013; **Accepted** September 04, 2013
***Corresponding author:** Sajid Umar, Email: Sajncvi@gmail.com

Introduction

Mastitis is a complex and most economic loss causing disease of dairy animals [1[. It may be due to infection or trauma. The predominant infectious cause of bovine mastitis is *Staphylococcus aureus* followed by *Streptococci* [2]. Mastitis is considered as the most important disease in cattle from economic point of view. The complexity of the disease is due to the multi-etiological factors that affect the udder as well as composition of the milk. Due to complexity of the disease, there is decrease in milk yield and quality as well as pathological changes in the mammary tissue [3]. It causes severe economic losses to dairy industry worldwide affecting quality of the milk and production losses [4,5]. Mastitis in dairy cattle is a global issue as it puts lethal effects on animal health, quality and yield of milk resulting in economic losses of dairy industry especially in developing countries it causes huge economic losses [6]. Over the last many years, mastitis has been a serious problem of dairy animals in Pakistan. As teat dipping at post milking and antibiotic therapy during dry period are not common precautionary measures for mastitis in Pakistan, economic losses caused by mastitis can be much higher [7]. Mastitis is considered main culling reason in dairy animals of Pakistan.

According to a survey 22% lactating animals are being culled every year in Pakistan [8]. Milk from those cattle having mastitis also has significant health hazard for human being in the form of drug residue and pathogenic bacteria [9]. Bacteria and their toxins in mastitic milk render milk unsuitable for human consumption and act as a major source for the spread of bacterial infections such as tuberculosis, sore-throat, brucellosis, leptospirosis etc. and has zoonotic importance [10]. California Mastitis Test (CMT) is considered as gold standard in detection of intramammary infection due to its high specificity (80.6%) and sensitivity (82.4%) in early lactating cows especially against gram positive cocci [2, 11]. This study has three purposes. First, it is designed to detect mastitic milk by California mastitis. Second, it is aimed to isolate prevailing bacteria from mastitis positive milk finally, to perform in vitro culture sensitivity test for those antibiotics which are being used in daily practices.

Materials and Methods

Collection of milk samples

Milk samples from 436 lactating cattle were collected aseptically in sterile plastic vials. Before collection of milk samples, cattle teats were sanitized with 70% ethyl alcohol [12]. The collected milk samples were transported to the Laboratory in ice box The prevalence of infected cattle from mastitis was diagnosed by using California Mastitis Test (CMT) as described by Islam [4]. Bacterial isolation was directly processed from mastitic milk samples as documented by National Mastitis Council Inc [13].

Antimicrobial susceptibility of bacterial isolates

Morphological, cultural and biochemical characteristics were utilized for the identification and isolation of 149 milk samples positive for mastitis. Cultural examination was studied by inoculating positive milk samples in different bacterial growth media using standard laboratory procedures. The cultured plates were incubated under aerobic conditions at 37°C for 48 hours. Identification of bacterial isolates were determined by using different characteristics i.e cultural, morphological and biochemical characteristics [14]. Hemolysis, morphological and staining characters were evaluated for different bacterial isolates. Disc diffusion method was used for the determination of susceptibility of individual bacterial isolates [15]. Nine different antibiotics were tested for the susceptibility of bacterial isolates in the current study. After streaking of bacterial suspension, antibiotic discs were placed with the help of antibiotic disc dispenser selectively in a clock wise manner. Culture plates were incubated at 37°C for 24 hours after putting antibiotic diffusion discs in an inverted position [16]. Diameters of zone of bacterial growth inhibition were measured in millimeters (mm) by using antibacterial zone gauge for the determination of antibiotic sensitivity for treatment purpose (Table 1).

Table 1: Antibiotic sensitivity and inhibition zones parameters.

Indication	Inhibition zone	Sensitivity
+++	>12 mm	High sensitivity
++	8-12 mm	Moderate sensitivity
+	5-8 mm	Least sensitivity
0	<5 mm	Resistant

Table 2: Concentration of antibiotics used for antibiogram.

Name of antibiotic	Abbreviation	Concentration (µg)
Ampicillin	A	10
Cepharidine	C	30
Cloxacillin	Cx	5
Gentamicin	G	10
Kanamycin	K	30
Enrofloxacin	En	10
Norfloxacin	Nx	10
Penicillin	P	10 unit
Streptomycin	St	10

Statistical analysis

The data was statistically analyzed by applying descriptive statistics [17].

Results and Discussion

Out of total milk samples (436) collected from lactating cows, 149 samples declared mastitis positive. The prevalence of mastitis was 34.17%. These findings are in line with those of Ali [18]. who reported 32 to 58% prevalence of mastitis in organized farms and individual herds respectively. On the other hand our results disagree from Ikhwan [19], who claimed 9.53% prevalence of mastitis in milk producing ruminants. Mustafa et al. [20] reported the prevalence of mastitis in cow and buffalo milk was 24.71 % and 20.98 % respectively. Amongst, the cases of clinical and sub clinical mastitis 40.35 % & 59.64% in buffalo and in cow 61.26% and 30.63%.

Table 3: Mastitis Positive cases (%).

Number of milk samples tested	Positive samples	Positive cases (%)
436	149	34.17

In present study, 113 (75.83%) samples showed bacterial growth out of the total 149 milk samples considered positive for mastitis, while 36 samples (24.16%) yielded no bacterial growth.

Table 4: Number of milk samples showed microbial growth.

Milk samples	Milk samples with growth	Positive samples (%)
149	113	75.83
	Milk samples with no growth (%)	Negative samples (%)
	36	24.16

Premedication of lactating cows with antibacterial drugs and type of media that did not suit for the growth of all types of mastitis causing bacteria may be the cause of no growth of milk samples [17]. A total of 232 bacterial isolates were obtained from 149 mastitis positive milk samples, from these *Staphylococcus aureus*103 (44.39%), *Streptococcus agalactiae* 72(31.03%), *Escherichia coli* 29 (12.5%), Bacillus spp. 12 (5.17%), *Corynebacterium* 8 (3.45%), *Pseudomonas* and mixed growth 8(3.45%) were identified and isolated. Khan reported 48% frequency of *Staph. aureus* as highest in his study [21]. The present findings are in line with Memon who showed that *Staphylococcus aureus* (38%), *Streptococcus uberus* (13%) and *Escherichia coli* (11%) are the major pathogenic bacteria that cause mastitis [25]. The findings also supported by Farooq [19] who described *Staphylococcus aureus* as most prevalent (44%), f *Streptococcus agalactiae* (22%), *E.coli* (16%) and *Bacillus spp.* (4%). Bhalerao [22] claimed *Staphylococcus aureus* (54.55%) and *Streptococci* (36.36%) are the main mastitis causing bacterial pathogens. Khan and Muhammad [23] has

described the prevalence of *Staphylococcus aureus*, *Streptococcus agalactiae*, *E. coli* and *Bacillus spp*. from buffalo milk samples which was as 45%, 23%, 18% and 14%, respectively. Amongst cow samples the prevalence of these bacteria was seen to be 48%, 30%, 13% and 8% respectively.

Table 5: Bacteria identified from mastitic milk samples of lactating cows.

Name of Bacteria	Isolates	Occurrence (%)
Staphylococcus spp.	103	44.39
Streptococcus spp.	72	31.03
E.coli	29	12.5
Bacillus spp.	12	5.17
Corynebacterium spp.	8	3.45
Pseudomonas spp.	8	3.45

Table 6: Antibiotic sensitivity against mastitis causing bacteria in cattle.

Name of antibiotic	*Staphylococcus* sensitivity (%)	*Streptococcus* sensitivity (%)	*E. Coli* sensitivity (%)
Gentamicin	98	96.5	93.5
Enrofloxacin	94.1	93.4	90
Amoxicillin	88.4	89.7	96
Cephradine	84.4	82	85
Ampicillin	49.2	75	62.3
Streptomycin	37	28.5	46.3
Kenamycin	35.7	67.4	55
Cloxacillin	36.3	42.8	81.2
Pencillin	45	49	R

Nine commonly available antibiotics were evaluated during this experiment. It is found that gentamicin, enrofloxacin, amoxicillin and kanamycin are more efficacious antibiotics *in vitro* than other antibiotics. According to the present study, *Staphylococci* (44.39%) found more prevalent following *Streptococci* (31.03%), *E. coli* (12.5%), *Bacillus* (5.17%), *Corynebacterium* (3.45%), *Pseudomonas* with mixed organisms (3.45%) as documented by Rehman.[29] The highest percentage of *Staphylococci* (44.39%), and *Streptococci* (31.03%) indicated poor management practices at dairy farms of Pakistan. *Staphylococci* are the most common environmental contaminants, which becomes pathogenic if animal environment become unhygienic. According to the results of Antibiotic sensitivity test, it was shown that the mastitic causing pathogenic isolates were sensitive against gentamycin (49%), enrofloxacin (43.45%) amoxicillin (35.2%) kanamycin (33.9), ampicillin (18.2), streptomycin(12.3%), cephradine (9.8%), cloxacillin (9.3%), and penicillin G (8.1%), which is closely related to as reported by Anjum and Rehman [24, 26] Similar findings were described by Iqbal, who claimed gentamycin, enrofloxacin and kanamycin were the most useful drugs against mastitis in vitro [27]. Farooq found highest effectiveness of norfloxacin, gentamycin and chloromphenicol against mastitic pathogens in vitro [19]. Khan analyzed the antibiogram pattern as mastitic pathogens show high sensitivity against gentamycin and relatively low against cloxacillin and penicillin [25].

It was concluded from present study that *Staphylococci* (44.39%), and *Streptococci* (31.03%) are major mastitis causing bacteria in the lactating cows of Pakistan and gentamycin, enrofloxacin, amoxicillin and kanamycin are the possible best antibiotics for treatment. From the prevention point of view, regular testing of subclinical mastitis and use of above mentioned antibiotics is suggested to reduce the economic losses of mastitis.

Reference

[1] Bilal MQ, Iqbal MU, Muhammad G, Avais and Sajid MS (2004) Factors Affecting the Prevalence of Clinical Mastitis in Buffaloes Around Faisalabad District (Pakistan). *Int. J. Agri. Bio* 6 (1):185–187

[2] Workineh S, Bayleyegn M, Mekonnen H and Potgieter LND (2002) Prevalence and etiology of Mastitis in Cows from Two Major Ethiopian Dairies. Trop Anim Health and Pro34(1): 19-25

[3] Radostits OM, Blood DC, Gay CC, Hinchiff KW and Handerson JA (2000) Veterinary Medicine. 9th Ed., W. B. Saunders Company, London, U.K.

[4] Islam MA, Rahman A, Rony SA and Islam MS (2010) Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari milk shed area of sirajganj. Bangl J Vet Med 8(2) : 157 – 162

[5] Sharma N, Maiti SK and Sharma KK (2007) Prevalence, etiology and antibiogram of microorganisms associated with Sub-clinical mastitis in buffaloes in Durg, Chhattisgarh State (India). Int J Dairy Sci 2(2):145-151

[6] Sharma N, Maiti SK and Singh R (2004) Mastitis in dairy animals-A global problem. Dairy Planner. 1: 40-41

[7] Hussain M, Malik MA, Fatima Z and Yousaf MR (2005) Participatory surveillance of livestock diseases in Islamabad capital territory. Intern J Agri Biol 7(4): 567-570

[8] Sammiullah, Syed MUD, Arif M, and Khan M (2000) Frequency and causes of culling and mortality in Holstein Friesian cattle in NWFP (Pakistan). J Anim Health Pro 20: 22-24

[9] Iqbal M, Amjed M, Khan MA, Qureshi MS and Sadique U (2006) Comparative study of various laboratory diagnostic tests of sub-clinical mastitis in dairy cows and buffaloes. Pak Vet J 73-79

[10] Petrovski K R, Trajcev M and Buneski G (2006) A review of the factors affecting the costs of bovine mastitis. J South Afr Vet Assoc 77(2): 52–60

[11] Randy T, Dingwell, Leslie KE, Schukken YH, Sargeant JM and Timms LL (2003). Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can Vet J (44): 413–416

[12] Boddie RL, Owens WE, Ray CH, Nickerson, and Boddie NT (2002) Germicidal Activities of Representatives of Five Different Teat Dip Classes Against Three Bovine Mycoplasma Species Using a Modified Excised Teat Model. J Dairy Sci 85:1909–1912

[13] National Mastitis Council Inc (1987) Microbiological Procedure for the Diagnosis of Udder Infection, Wilson Boulevard Arlington, V.A. 22201, USA
[14] John GH (2000) Bergey's manual of determinative Bacteriology Actinomycetales. 9th ed. Williams and Wilkins, Baltimore.
[15] Arshad M, Muhammad G, Siddique M, Ashraf M and Khan HA (2006) Staphylococcal Mastitis In Bovines And Some Properties Of Staphylococcal Isolates, Pak Vet J 26 (1): 20-22
[16] Arshad M, Qamar FK, Afzal H and Sidique M (1998) Epidemiological studies of bovine mastitis in district Gujrat. Proc. Intern. Seminar on Microbial Disease of Livestock and Poultry, CVS, Lahore. pp: 23
[17] Farooq AA, Inayat S, Akhtar MS and Mushtaq M (2008) Prevalence Of Mastitis And Antibiotic Sensitivity Of Bacterial Isolates Recovered From Nili Ravi Buffaloes. J Anim Pl Sci 18: 76-77
[18] Ali MA, Ahmad MD, Muhammad K and Anjum AA (2011) Prevalence of sub clinical mastitis in dairy buffaloes of Punjab, Pakistan. J Anim Pl Sci 21(3): 477-480
[19] Ikhwan K, Shah M, Anwar M, Rehman S, Hafeez M and Qureshi MB (1989) To detect the evidence of mastitis in cows and buffaloes and to study the anti-biogram (in vitro) of the causative organisms. Proc: 2nd Inter Provincial Seminar on Animal Health and Production. Organized by Pak. Vet. Med Assoc. Peshawar. pp: 27-32
[20] Mustafa et al Mustafa YS, Awan SA, Zaman T, Chaudhry SR and Zoyfro V (2011) Prevalence and antibacterial susceptibility in mastitis in buffalo and cow in and around the district lahore-Pakistan. Pak J Pharm 24 (1 - 2): 29-33
[21] Memon MI, Mirbahar KB, Memon MR, Akhtar N, Soomro SA and Dewani P (1999) A study on the etiology of subclinical mastitis in buffaloes. Pak J Agri Agri Engg Vet Sci 15: 34- 36
[22] Khan AZ, Khan A, Hayat CS, Munir Z and Ayaz U (2004) Prevalence of Mastitis in Buffaloes and Antibiotics Sensitivity Profiles of Isolates. Pak j life soc sci 2(1): 73-75
[23] Khan AZ, and Muhammad G (2005) Quarter-Wise Comparative Prevalence Of Mastitis In Buffaloes And Crossbred Cows. Pak Vet J 25(1): 9-12
[24] Rehman FU (1995). Study on: (i) evaluation of SURF FIELD MASTITIS TEST for the detection of subclinical mastitis in buffaloes and cattle, and (ii) antibiotic susceptibility of the pathogens. M. Sc. Thesis, Deptt. Vet. Clinical Medicine and Surgery, Univ. Agri., Faisalabad
[25] Bhalerao DP, Jagadish S, Keskar DV, Dangore AD and Sharma LK (2000) Antibiogram and treatment of bovine subclinical mastitis. Ind Vet J 77: 244-246
[26] Anjum AA, Qamar MF, Ali MA, Usman M and Khan TM (2010) Bacteriology of mastitic milk of cattle and In vitro antibiogram of the pathogens isolated, in District Hafizabad, Pakistan. Biologia 56: 117-122
[27] Iqbal M, Khan MA, Daraz B and Siddique U (2004) Bacteriology Of Mastitic Milk And In Vitro Antibiogram Of The Isolates. Pak Vet J 24(4): 161-164