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Incidence and Pattern of Antibiotic Resistance of *Staphylococcus aureus* Isolated from Clinical and Subclinical Mastitis in Cattle and Buffaloes

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ABSTRACT

The main objective of the present study was to report the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) among bovines used for milk production in Mathura, India. A total of 80 milk samples were collected from clinical and subclinical cases of mastitis from cows (40) and buffalos (40). Milk samples were processed for isolation and identification of *S. aureus* using standard bacteriological procedures. *Staphylococcus aureus* were isolated from only 27 samples showing the overall incidence of *Staphylococcus aureus* in clinical as well as sub clinical mastitis was 33.75%. The incidence of *Staphylococcus aureus* was higher (50.00%) in clinical mastitis in comparison to that of subclinical mastitis (17.50%). The results revealed that the incidence of *Staphylococcus aureus* in clinical as well as sub-clinical mastitis was higher in cattle in comparison to buffaloes. Drug sensitivity revealed the 100% resistance against penicillins followed by vancomycin (88.89%), nalidixic acid (77.78%), cefixime, methicillin, novobiocin (66.67% each), amoxiclav, colistin, pipemidic acid (55.56% each), ofloxacin, streptomycin, sulphamethizole (44.44% each), ampicillin/sulbactam, cefalexin, cefazolin, cefoperazone, enrofloxacin, floxidin, meropenem (33.33% each), cefuroxim, ciprofloxacin, clindamycin, gentamicin, levofloxacin, norfloxacin, tetracycline (22.22% each). Eighteen isolates were found to be methicillin-resistant, while the remaining (09) were methicillin-susceptible. Similarly, twenty four *S. aureus* isolates were intermediate to vancomycin while three were vancomycin susceptible and no isolate was resistant to vancomycin. Thus, the findings are useful for formulating specific control programs for bovine mastitis caused by *S. aureus* in this region.

Key words: Antibiogram, bovine mastitis, methicillin resistance *Staphylococcus aureus*

INTRODUCTION

Mastitis (inflammation of mammary gland) is one of the most devastating disease conditions leading to significant economic losses globally (Kumar *et al.*, 2010a; Abd Ellah, 2013) because of

reduced milk production, treatment costs, increased labor, milk withholding following treatment, death and premature culling (Lightner *et al.*, 1988; Kaneene and Hurd, 1990; Miller *et al.*, 1993; Szweda *et al.*, 2014). Due to multiple etiologies, it always remained a challenge to veterinarian worldwide. Approximately, 140 species of microorganisms have been identified as etiological agents of bovine mastitis. Of these various etiological agents, *Staphylococcus aureus* is a major pathogen associated with bovine clinical and subclinical mastitis (Wilson *et al.*, 1997; Brito *et al.*, 1999; Tenhagen *et al.*, 2006; Piepers *et al.*, 2007; Bhatt *et al.*, 2011; Cervinkova *et al.*, 2013).

At present, there is paucity of reports about occurrence of these virulence factors among *S. aureus* isolates from India and about the possible distribution of single *S. aureus* clones as causative agents of bovine mastitis. Since the introduction of $-lactamase-stable antimicrobial drugs in clinical use, *Staphylococcus aureus* strains have emerged worldwide as important nosocomial pathogens. Their prevalence in the community is increasing substantially. The indiscriminate use of antibiotics like ampicillin, penicillin, oxacillin and methicillin may contribute to the increasing occurrence of antibiotic resistant strains in cows with mastitis. These strains in intramammary dissemination often produce incurable severe intra herd infections (Moon *et al.*, 2007). Resistance of *S. aureus* to antimicrobial agents can complicate treatment of its infections (Lowy, 2003). Among *S. aureus*, Methicillin-resistant strains (MRSA) has recently emerged as a serious life-threatening infective agent which does not respond to a lot of antimicrobial treatments (Kamal *et al.*, 2013). The mastitis caused by *S. aureus* is characterized by significantly lower cure rates compared with infections caused by other microorganisms, which may be either as a result of unusually frequent acquisition of antibiotic resistance mechanisms among this group of bacteria or also their ability to form biofilm (slime) (Cramton *et al.*, 1999). Considering the potential of the area and the economic significance of dairy production to the local community, the present study was carried out to detect the incidence of *Staphylococcus aureus* infection in mastitis cases and their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Sample collection: A total of 80 milk samples from healthy, subclinical and clinical mastitis cases of cattle and buffaloes 10, 20 and 20 each, respectively were collected either from Instructional Livestock Farm Complex (ILFC) or cases presented to Teaching Veterinary Clinical complex (TVCC), DUVASU, Mathura or during animal health camps etc. All samples were kept at 4°C in insulated ice box and transferred to the laboratory, College of Biotechnology, DUVASU, Mathura and analyzed within 4 h of collection.

Examination for mastitis: The cases of Clinical Mastitis (CM) were diagnosed on the basis of history, clinical signs, physical examination of udder (swelling and pain) and milk (colour-yellow or blood tinged and consistency-watery, etc), while subclinical mastitis (SCM) was diagnosed on the basis of California Mastitis Test (CMT) (Schalm *et al.*, 1971).

Bacterial isolation and identification: Each of the thoroughly mixed milk sample (Mastitis/subclinical mastitis) was transferred to 10 mL of nutrient broth and incubated at 37°C for 15-18 h to resuscitate the organisms. Thereafter, a loopful of inoculum from the nutrient broth was streaked on to nutrient agar plates and incubated at 37°C for 24 h. Presumptive Staphylococcus colonies (golden/white, round, smooth, glistening, opaque) were picked up and characterized biochemically as per Barrow and Feltham (1993). The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's Method of staining. The

stained smear was observed under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes were considered to be *Staphylococci.* A battery of biochemical tests viz., Catalase test, Oxidase test, Voges Proskauer (VP) test, Oxidation-Fermentation (OF) test etc were carried out as per Barrow and Feltham (1993).

Antibiotic resistance: All the confirmed *S. aureus* isolated under study were examined for their antibiotic resistance pattern by disc diffusion method (Bauer *et al.*, 1966) using 38 antibiotic discs (Hi-Media, Mumbai) viz., amikacin (30 µg), amoxiclav (10 µg), ampicillin/sulbactam (10/10 µg), Azithromycin (15 µg), Cefalexin (30 µg), cefazolin (30 µg), cefixime (5 µg), cefoperazone (75 µg) cefotaxim (30 µg), ceftriaxome (10 µg), cefuroxim (30 µg), chloramphenicol (30 µg), ciprofloxacin (30 µg), clindamycin (2 µg), colistin (10 µg), erythromycin (10 µg), floxidin (30 µg), gentamicin (10 µg), imipenem/cilastin (10/10 µg), levofloxacin (5 µg), meropenem (10 µg), methicillin (10 µg), nalidixic acid (5 µg), nitrofurantoin (300 µg), novobiocin (5 µg), ofloxacin (5 µg), penicillin (10 U), pipemidic acid (30 µg), piperacillin/Tazobactam (100/10 µg), rifampicin (5 µg), streptomycin (10 µg), sulfamethizole (300 µg), tetracycline (10 µg), vancomycin (30 µg) etc following (NCCLS., 2004).

RESULT AND DISCUSSION

The species wise incidence of *Staphylococcus aureus* in clinical and subclinical mastitis were shown in Table 1. Overall incidence of *Staphylococcus aureus* in clinical as well as sub clinical mastitis, was 33.75%. The incidence of *Staphylococcus aureus* was higher (50.00%) in clinical mastitis in comparison to that of subclinical mastitis (17.50%) and the incidences of *Staphylococcus aureus* in clinical as well as sub-clinical mastitis were higher in cattle in comparison to buffaloes. These results are almost in the concurrence of previous study conducted in the region in 2010, which revealed *S. aureus* as a major pathogen in the cases of mastitis in Mathura and its surroundings. The incidence of *S. aureus* was 37.03 and 31.70% in cattle and buffaloes, respectively (Kumar *et al.*, 2010a). It clearly indicated the presence of *S. aureus* as most prevailing pathogen in the cases of mastitis in dairy animals. Moreover, it is persisting in the similar pattern not only in clinical cases but also in subclinical cases. Various studies have been conducted in different parts of country to assess the prevalence status of bacterial pathogens in mastitis of dairy animals. Similar to the present findings, Purohit (1990) also reported the staphylococcal mastitis in cows to be 31.94% while Ranjan *et al.* (2011), Mengistie (2003) and Kivaria *et al.* (2005) reported the incidence to be comparatively as 27.37% in Jharkhand, 27.1% and 21.0%, respectively. However, higher incidence of staphylococcal mastitis was reported by Wani and Bhatt (2003), Patel (2007) and Thennarrasu *et al.* (2003), who reported the incidence of staphylococcal mastitis in cows to be 45%, 44% and 47.06% respectively. The high prevalence of staphylococci has been reported by several workers in India (Tuteja, 1999; Kaya *et al.*, 2000; Sharma *et al.*, 2007) and abroad (Hawari and Dabas, 2008; Tenhagen *et al.*, 2009; Nickerson, 2009; Zutic *et al.*, 2012). This

Table 1: Species wise incidence of *Staphylococcus aureus* in mastitis among dairy animals

| Animal | Clinical mastitis (CM) | | | Subclinical mastitis (SCM) | | | | | |
| | Total | Positive | | Total | Positive | | Total | Positive | |
		No.	(%)		No.	(%)		No.	(%)
Cows	20	11	55.00	20	04	25.00	40	15	37.50
Buffaloes	20	09	45.00	20	03	15.00	40	12	30.00
Total	40	20	50.00	40	07	17.50	80	27	33.75

difference in the prevalence/incidence of pathogens is influenced by parity, type of sample, season and place (Sharma *et al.*, 2007). Distribution of pathogens in mastitis changes over time, therefore, bacteriological examination at herd level must be taken regularly to monitor udder health. Incidence of *Staphylococcus aureus* from clinical cases of mastitis was found to be 50.00% which was slightly higher than that previous studies conducted by Thennarrasu *et al.* (2003) and Patel (2007), who reported an incidence of *Staphylococcus aureus* from clinical cases of mastitis as 47.06 and 40.40%, respectively. While incidence of *Staphylococcus aureus* from subclinical cases of mastitis was found to be 17.50%, which differed from that reported earlier by Purohit (1990) and Goswami (1998), who found the incidence of staphylococcal subclinical mastitis to be 21.64 and 51.29%, respectively. These findings are suggestive of improper management and failure to maintain good managemental practices in Indian dairy animals particularly in Mathura region. Being commensal to skin *S. aureus* are supposed to be first and foremost bacteria to enter in teat canal. However, the incidences can be reduced by maintaining proper hygienic conditions and pre and post milking sanitation of udder and its surroundings.

All of the 27 *Staphylococcus aureus* isolates (Fig. 1 and 2) were found catalase positive, oxidase negative, fermentative by O-F test, urease positive, failed to grow on Mac conkey agar, Voges Proskauer (VP) positive and coagulase positive on being subjected to above mentioned biochemical tests. The presence of coagulase enzyme is considered as criteria for the pathogenicity of *S. aureus* which is assessed to differentiate between pathogenic and nonpathogenic *S. aureus*. In present study, all the *Staphylococcus aureus* isolates from clinical and subclinical mastitis cases 27 (100.00%) were found positive for coagulase production. Similarly, previous studies conducted by Pandya (1991) and Patel (2007) also reported high percentage positivity of *S. aureus* for coagulase production i.e. 100.00%, where as lower percent positivity of *S. aureus* for coagulase production were also reported earlier by Kato and Kume (1980) 34.50%, Boerlin *et al.* (2003) 50.00% and Wani and Bhatt (2003) 51.11%. The presence of 100% coagulase positive isolates in present study further suggests the increase in the number of pathogenic *S. aureus* in dairy animals. This is an alarming condition as in general *S. aureus* are supposed to be non pathogenic commensal organisms.
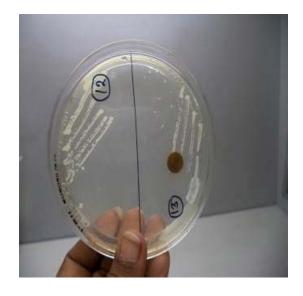

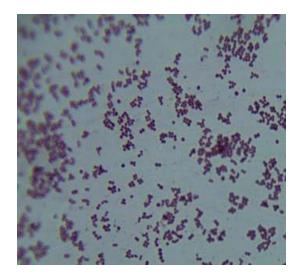Fig. 1: *Staphylococcus aureus* on Nutrient agar

Fig. 2: *Staphylococcus aureus* after Gram's staining

Fig. 3(a-b): Antibiotic sensitivity test of *Staphylococcus aureus* on Mueller Hilton agar showing resistance to antibiotics

The *in vitro* antimicrobial sensitivity (Fig. 3a and 3b) patterns of isolates recovered from mastitis and subclinical mastitis cases revealed 100 percent sensitivity to amikacin, azithromycin, imipenem and nitrofurantoin and high sensitivity (88.89%) towards cefotaxim, ceftriaxone, chloramphenicol, erythromycin, furidic acid, piperacillin/tazo, rifampicin and tylosin. As far as resistance is concerned, all the isolates were resistant to penicillin (100.00%), followed by vancomycin (88.89%), nalidixic acid (77.78%), cefixime, methicillin, novobiocin (66.67% each), amoxiclav, colistin, pipemidic acid (55.56% each), ofloxacin, streptomycin, sulphamethizole (44.44% each), ampicillin/sulbactam, cefalexin, cefazolin, cefoperazone, enrofloxacin, floxidin, meropenem (33.33% each), cefuroxim, ciprofloxacin, clindamycin, gentamicin, levofloxacin, norfloxacin and

tetracycline (22.22% each). Eighteen isolates were found to be methicillin-resistant, while the remaining (09) were methicillin-susceptible. Similarly, twenty four *S. aureus* isolates were intermediate to vancomycin, while three were vancomycin susceptible. None of the isolate was resistant to vancomycin.

Studies conducted by several workers (Sharma *et al.*, 2007; Chavan *et al.*, 2007; Roychoudhury and Dutta, 2009) have showed increased resistance towards different traditional and newly introduced antibiotics. Appearance of resistance against a particular antibiotic in a specific region may be due to its frequent and long-term use (Sabour *et al.*, 2004; Moon *et al.*, 2007; Kumar *et al.*, 2010a, b). The results of the present study revealed that a significant number of isolates showed resistance to antibiotics (penicillin-G, amoxiclav, cefalexin, cefazolin, cefuroxim, gentamicin, streptomycin, ampicillin, enrofloxacin, ciprofloxacin and many others) that are frequently used in mastitic animals. These resistance patterns are alarming as in comparison to the previous study (Kumar *et al.*, 2010a) conducted in similar region in the cases of dairy animals suffering from mastitis revealed almost entirely reversed drug resistance pattern. As per Kumar *et al.* (2010a) majority of *S. aureus* isolates (18 out of 23) were sensitive to ciprofloxacin and only 9 out of 23 were sensitive to amikacin. Thus, the continuous use of specific antibacterials might be the cause of this change of drug resistance pattern. In late nineties and the first decade of 21st century, quinolones have been the drug of choice of clinicians and this is similarly followed by laymen in field condition. Thus, indiscriminate use might have changed this drug sensitivity pattern within few years. Similarly, for last few years amikacin has not been drug of choice for veterinarians in large animal due to higher dose and twice a day application.

Antimicrobial resistance represents a serious problem in the treatment of infectious diseases including mastitis. In recent times, an increasing antimicrobial resistance rate has been recognized in *S. aureus* from bovine mastitis (Saini *et al.*, 2012; Wang *et al.*, 2013). Due to antibacterial usage over many decades, multiple drug resistance among the mastitis causing agents is a major problem in controlling intra-mammary infections. This is generally attributed to indiscriminate and continuous use of antibacterial drugs without prior drug susceptibility testing or selection pressure of antimicrobials on pathogens or colonization of the mammary gland by resistant strains. Such antimicrobial resistant organisms can pose serious health related problems to animals as well as human beings.

Moreover, there is an increased incidence of Methicillin Resistant *S. Aureus* (MRSA) all over the world. MRSA is the term used for any strain of *Staphylococcus aureus* that has developed resistance to $-lactam antibiotics, which include the penicillins (methicillins, oxacillin, dicloxacillin etc.,) and cephalosporins. Synonyms are multi- drug resistant *Staphylococcus aureus* and oxacillin resistant *Staphylococcus aureus*. These strains act as reservoir for multiple drug resistant genes. Due to antibiotic resistance, sometimes it is called as "superbug" (Batabyal *et al.*, 2012). This methicillin resistance seems to be widely spread among *S. aureus* isolates from bovine milk, which is in accordance with this study results as well (Zutic *et al.*, 2012). MRSA first emerged as a serious pathogen in human medicine during late 1970s and has been reported in animals during the past 10 years (Leonard and Markey, 2008). In India, scanty of information is available, however prevalence of MRSA in cattle in India is reported to be 13.1% (Kumar *et al.*, 2011). However, the present study revealed a higher incidence of MRSA (66.67%) as compared with those in similar reports in the literature from other countries (Lee, 2003; Moon *et al.*, 2007; Van den Eede *et al.*, 2009). The higher prevalence of MRSA in present study clearly indicates the increase in the MRSA.

With the emergence of MRSA, methicillin became ineffective against them and vancomycin became the drug of choice for MRSA (Ng *et al.*, 2011). The excessive use of vancomycin against MRSA led to the emergence of two types of glycopeptides resistant *Staph. aureus*, vancomycin intermediate *Staphylococcus aureus* and vancomycin resistant *Staphylococcus aureus* (Courvalin, 2006). Almost after 40 years of methicillin resistance, the resistance of vancomycin evolved among the *Staphylococcus aureus*. In 1996, first Glycopeptides-Intermediate *Staphylococcus Aureus* (GISA) isolate was described from a pediatric patient in Japan (Hiramatsu *et al.*, 1997) and another one with high resistance to vancomycin was first diagnosed in a patient in the USA, which contained both V and A gene from enterococci and methicillin resistance mecA gene. World's sixth VRSA had been isolated in Kolkata (India) in 2005 (Chakraborty *et al.*, 2011). Thus, it can be concluded that VRSA isolate is rare but still is an emerging pathogen and it has been appeared in India. There is no report available regarding the vancomycin resistant *S. aureus* in dairy animals in India. The present study also revealed the presence of vancomycin intermediate *S. aureus*. The presence of twenty four vancomycin intermediate *S. aureus* isolates out of 27 isolates clearly indicated the reduction in drug sensitivity and it might be an indication of future vancomycin resistance in existing *S. aureus* population.

CONCLUSION

The incidence of *Staphylococcus aureus* in clinical as well as sub clinical mastitis, was 33.75%. Overall, antimicrobial resistance to penicillin, cefixime, methicillin, amoxiclav, ofloxacin, streptomycin, ampicillin/sulbactam, cefalexin, cefazolin, cefoperazone, enrofloxacin and tetracycline was found in bovine mastitis cases. Systematic records regarding the epidemiology of mastitis in dairy animals including status of infection and antibiogram studies would provide useful information to the producer, farmer and veterinarian for management of farms. High incidence of Methicillin resistant *S. aureus* among bovine mastitic milk represents major threats for transmission of this multidrug resistant to human beings. Routine surveillance for antibiotics resistance patterns of MRSA isolated from clinical as well as subclinical cases of mastitis from dairy animals (cattle and buffaloes) could be an important measure for detection of the emergence and spread of such resistance. It further highlighted the necessity of enforcement of hygienic implementations and practices within dairy facilities.

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REFERENCES

Abd Ellah, M.R., 2013. Role of free radicals and antioxidants in mastitis. J. Adv. Vet. Res., 3: 1-7.

Barrow, G.I. and R.K.A. Feltham, 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edn., Cambridge University Press, Cambridge, pp: 140-43.

Batabyal, B., G. Kundu and S. Biswas, 2012. Methicillin-resistant *Staphylococcus aureus*: A brief review. Int. Res. J. Biol. Sci., 1: 65-71.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bhatt, V.D., M.S. Patel, C.G. Joshi and A. Kunjadia, 2011. Identification and antibiogram of microbes associated with bovine mastitis. Anim. Biotechnol., 22: 163-169.

Boerlin, P., P. Kuhnert, D. Hussy and M. Sehaellibaum, 2003. Methods for identification of *Staphylococcus aureus* isolates in cases of bovine mastitis. J. Clin. Microbiol., 41: 767-771.

Brito, M.A.V.P., J.R.F. Brito, M.T. Ribeiro and V.M.O. Veiga, 1999. [Dairy herds pattern of intramammary infection: Evaluation of all mammary quarters of lactating cows]. Arq Bras Med. Vet. Zootec, 51: 129-135.

Cervinkova, D., H. Vlkova, I. Borodacova, J. Makovcova and V. Babak *et al.*, 2013. Prevalence of mastitis pathogens in milk from clinically healthy cows. Vet. Med., 58: 567-575. 2013. Prevalence of mastitis pathogens in milk from clinically healthy cows. Vet. Med., 58: 567-575.

Chakraborty, S.P., S. KarMahapatra, M. Bal and S. Roy, 2011. Isolation and identification of vancomycin resistant *Staphylococcus aureus* from post operative pus sample. Al Ameen J. Med. Sci., 4: 152-168.

Chavan, V.V., S.U. Digraskar, S.N. Dhonde and P.B. Hase, 2007. Observation on bubaline subclinical mastitis in and around Parbhani. Indian J. Field Vet., 3: 50-50.

Courvalin, P., 2006. Vancomycin resistance in gram-positive cocci. Clin. Infect. Dis., 42: S25-S34.

Cramton, S.E., C. Gerke, N.F. Schnell, W.W. Nichols and F. Gotz, 1999. The intercellular adhesion (ica) locus in *Staphylococcus aureus* and is required for biofilm formation. Infect. Immun., 67: 5427-5433.

Goswami, S.N., 1998. Comparative study for detection of bovine subclinical mastitis by direct and indirect tests. M.Sc. Thesis, Gujarat Agricultural University, Anand.

Hawari, A.D. and F. Al-Dabbas, 2008. Prevalence and distribution of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Jordan. Am. J. Anim. Vet. Sci., 3: 36-39.

Hiramatsu, K., H. Hanaki and T. Ino, 1997. Methicillin-resistant *staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J. Antimicrob. Chemother., 40: 135-136.

Kamal, R.M., M.A. Bayoumi and S.F.A. El Aal, 2013. MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. Food Control, 33: 49-53.

Kaneene, J.B. and H.S. Hurd, 1990. The national animal health monitoring system in Michigan. III. Cost estimates of selected dairy cattle diseases. Preventive Vet. Med., 8: 127-140.

Kato, E. and T. Kume, 1980. Enterotoxigenicity of bovine *Staphylococci isolated* from California mastitis test-positive milk in Japan. Japanese J. Vet. Res., 28: 75-85.

Kaya, O., S. Kirkan, M. Gulal and B. Unal, 2000. Identification and antibiotic susceptibility of microbes causing mastitis in dairy cows. Vet. Bull., 70: 290-290.

Kivaria, F.M., J.P.T.M. Noordhuizen, A.M. Kapaga and H. Hogeveen, 2005. Risk indicators associated with *Staphylococcus aureus* subclinical mastitis in smallholder dairy cows in Tanzania. Proceedings of the 4th IDF International Mastitis Conference, June 12-15, 2005, Maastricht, The Netherlands, pp: 722-727.

Kumar, A., A. Rahal, S.K. Dwivedi and M.K. Gupta, 2010a. Bacterial Prevalence and Antibiotic Resistance Profile from Bovine Mastitis in Mathura, India. Egypt. J. Dairy Sci., 38: 31-34.

Kumar, R., B.R. Yadav and R.S. Singh, 2010b. Genetic determinants of antibiotic resistance in *Staphylococcus aureus* isolates from milk of mastitic crossbred cattle. Curr. Microbiol., 60: 379-386.

Kumar, R., B.R. Yadav and R.S. Singh, 2011. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. J. Biosci., 36: 175-188.

Lee, J.H., 2003. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Applied Environ. Microbiol., 69: 6489-6494.

Leonard, F.C. and B.K. Markey, 2008. Meticillin-resistant *Staphylococcus aureus* in animals: A review. Vet. J., 175: 27-36.

Lightner, J.K., G.Y. Miller, W.D. Hueston and C.R. Dorn, 1988. Estimation of the costs of mastitis, using National animal health monitoring system and milk somatic cell count data. J. Am. Vet. Med. Assoc., 192: 1410-1413.

Lowy, F.D., 2003. Antimicrobial resistance: The example of *Staphylococcus aureus*. J. Clin. Invest., 111: 1265-1273.

Mengistie, A.Z., 2003. Molecular Epidemiology of *Staphylococcus Aureus* and Streptococcus Agalactiae Isolated from Bovine Mastitis in Ethiopia. Mensch and Buch-Verlag, USA., ISBN: 9783898205122, Pages: 139.

Miller, G.Y., P.C. Bartlett, S.E. Lance, J. Anderson and L.E. Heider, 1993. Costs of clinical mastitis and mastitis prevention in dairy herds. J. Am. Vet. Med. Assoc., 202: 1230-1236.

Moon, J.S., A.R. Lee, H.M. Kang, E.S. Lee and M.N. Kim *et al.*, 2007. Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J. Dairy Sci., 90: 1176-1185.

NCCLS., 2004. Performance standards for antimicrobial disk susceptibility testing: Fourteenth informational supplement. NCCLS document M100-514, Wayne, PA.

Ng, S.T., C.Y. Lim, C.S. Tan, A.A. Karim, H. Haron, N.S. Ahmad and V. Murugaiyah, 2011. Emergence of Vancomycin-Resistant Staphylococcus Aureus (VRSA). Webmed Central Infect. Dis., Vol. 2. 10.9754/journal.wmc.2011.002773

Nickerson, S.C., 2009. Control of heifer mastitis: Antimicrobial treatment-an overview. Vet. Microbiol., 134: 128-135.

Pandya, K., 1991. Incidence of *Staphylococcus aureus* in cow milk and assessment of characteristics associated with its virulence. M.Sc. Thesis, Gujarat Agricultural University, Sardarkrushinagar.

Patel, N.P., 2007. Determination of virulence factors in *Staphylococcus aureus* isolated from clinical cases of mastitis in sheep, goats, cattle and buffaloes. M.Sc. Thesis, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

Piepers, S., L. De Meulemeester, A. de Kruif, G. Opsomer, H.W. Barkema and S. de Vliegher, 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J. Dairy Res., 74: 478-483.

Purohit, J.H., 1990. Isolation and characteristics of *Staphylococcus aureus* from bovine milk. Ph.D. Thesis, Gujarat Agricultural University, Sardarkrushinagar.

Ranjan, R., M.K. Gupta and K.K. Singh, 2011. Study of bovine mastitis in different climatic conditions in Jharkhand, India. Vet. World, 4: 205-208.

Roychoudhury, P. and T.K. Dutta, 2009. Prevalence and antibiotic sensitivity pattern of bacteria from bovine mastitis in Mizoram. Indian J. Anim. Sci., 79: 483-484.

Sabour, P.M., J.J. Gill, D. Lepp, J.C. Pacan, R. Ahmed, R. Dingwell and K.J. Leslie, 2004. Molecular typing and distribution of *Staphylococcus aureus* isolates in Eastern Canadian dairy herds. Clin. Microbiol., 42: 3449-3455.

Saini, V., J.T. McClure, D.T. Scholl, T.J. de Vries and H.W. Barkema, 2012. Herd-level association between antimicrobial use and antimicrobial resistance in bovine mastitis *Staphylococcus aureus* isolates on Canadian dairy farms. J. Dairy Sci., 95: 1921-1929.

Schalm, O.W., G. Carroll and N.C. Jain, 1971. Bovine Mastitis. Lea and Fibiger, Philadelphia, USA., Pages: 360.

Sharma, H., S.K. Maiti and K.K. Sharma, 2007. Prevalence, etiology and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in durg, Chhattisgarh state (India). Int. J. Dairy Sci., 2: 145-151.

Szweda, P., M. Schielmann, A. Frankowska, B. Kot and M. Zalewska, 2014. Antibiotic resistance in *Staphylococcus aureus* strains isolated from cows with mastitis in Eastern Poland and analysis of susceptibility of resistant strains to alternative nonantibiotic agents: Lysostaphin, nisin and polymyxin B. J. Vet. Med. Sci., 76: 355-362.

Tenhagen, B.A., G. Koster, J. Wallmann and W. Heuwieser, 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J. Dairy Sci., 89: 2542-2551.

Tenhagen, B.A., I. Hansen, A. Reinecke and W. Heuwieser, 2009. Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. J. Dairy Res., 76: 179-187.

Thennarrasu, A., M.R. Muralidharan and M. Murugan, 2003. Incidence of clinical mastitis in bovines-a study in Chennai city. Cherion, 32: 140-141.

Tuteja, F.C., 1999. Studies on mastitis in buffaloes with reference to serum selenium status and control by treating teat canal infections. Ph.D. Thesis, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Van den Eede, A., A. Martens, U. Lipinska, M. Struelens and A. Deplano *et al.*, 2009. High occurrence of methicillin-resistant *Staphylococcus aureus* ST398 in equine nasal samples. Vet. Microbiol., 133: 138-144.

Wang, S., C. Wu, J. Shen, Y. Wu and Y. Wang, 2013. Hypermutable *Staphylococcus aureus* strains present at high frequency in subclinical bovine mastitis isolates are associated with the development of antibiotic resistance. Vet. Microbiol., 165: 410-415.

Wani, S. and M. Bhatt, 2003. An epidemiological study on bovine mastitis in Kashmir valley. Indian Vet. J., 80: 841-844.

Wilson, D.J., R.N. Gonzalez and H.H. Das, 1997. Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects on somatic cell count and milk production. J. Dairy Sci., 80: 2592-2598.

Zutic, M., I. Cirkovic, L. Pavlovic, J. Zutic, J. Asanin, O. Radanovic and N. Pavlovic, 2012. Occurrence of methicillin-resistant *Staphylococcus aureus* in milk samples from Serbian cows with subclinical mastitis. Afr. J. Microbiol. Res., 6: 5887-5889.