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Original Research Article

Incidence of *Staphylococcus aureus* Mastitis in Cows of Faizabad and Sultanpur Districts of Eastern Plain Zone of Uttar Pradesh

Sandeep Yadav, Dinesh Kr. Yadav, S.V. Singh, Ramakant, Naveen Kr. Singh, Vibha Yadav, R.P. Diwakar and Ram Jatan

College of Veterinary Science and Animal Husbandry
Narendra Deva University of Agriculture and Technology
Kumarganj-224 229, Faizabad (U.P.), India
**Corresponding author*

A B S T R A C T

Staphylococcus aureus is an opportunistic organism and has gain antibiobiotic resistance against several drugs. Cow milk samples (100) were subjected to cultural examination, out of which 68 (68%) samples were found positive for *S. aureus* mastitis. Hemolytic activity on blood agar and biochemical tests viz. catalase test, coagulase test, nitrate test and sugar fermentation tests were performed.

Keywords

Cow, mastitis, *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*., 2010; AbdEllah, 2013) because of reduced milk production, treatment costs, increased labor, milk with holding following treatment, death and premature culling (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 (Piepers *et al*., 2007; Tenhagen *et al*., 2009; Bhatt *et al*., 2011; Cervinkova *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).

Materials and Methods

Milk samples were collected from cows of Sultanpur and Faizabad districts of Eastern Plain Zone of Uttar Pradesh. Initially udders of the cows were examined by visual inspection and palpation for the presence of any lesion, pain, heat and swelling. In addition, milk from each quarter was withdrawn and checked for any change in

colour and consistency. Battery of tests viz California mastitis test and white side tests were employed to test milk for the presence of mastitis.

The affected quarter was washed with tap water, dried properly and the teat was swapped with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk was then collected aseptically from a mastitic quarter into sterile bottle after discarding the first 3 to 4 milking streams. Sample from each quarter were transported on ice packs to the bacteriology laboratory and were immediately cultured and stored at 4°C for a maximum of 24 hr until cultured on standard bacteriological media. In this way a total of 100 milk samples showing strong positive reaction were collected for isolation of *Staphylococcus aureus*.

Bacteriological examination of samples

The milk samples were subjected to bacteriological examination as per the method prescribed by National committee for clinical laboratory standards (NCCLS, 1997). Isolates of *Staphylococcus aureus* were obtained after subjecting samples to Mannitol salt Agar (MSA) plates followed by nutrient agar slants. The slants were incubated aerobically at 37°C and stored at 4°C until further use. All the samples of *S. aureus* yielded characteristic pink-yellow color colonies on the Mannitol Salt Agar. The isolates obtained as above were subjected to Gram staining. The culture showing violet coloured gram positive cocci, in grape like clusters were primarily considered as of *Staphylococcus aureus*. They were further subjected to hemolytic activity on blood agar and biochemical tests viz. catalase test, coagulase test nitrate test and sugar fermentation tests as per the procedure described by Cappuccino and Sherman (1982).

In Slide coagulase test clumping of cocci within 5 to 10 seconds in the test suspension was taken as positive. In tube coagulase test formation of clot in the test tube indicated positive coagulase test. The method described by Quinn *et al.* (1994) was followed to determine Catalase test. The production of gas bubbles or effervescence within a few seconds was considered as catalase positive reaction. Development of a distinct red colour which returned to brown rapidly was considered as positive test for Nitrate reduction test. In Sugar fermentation test the sugars used for fermentation reaction were glucose, mannitol and maltose. The observations were made based on colour change and gas production.

Results and Discussion

A total of 100 samples were subjected to bacteriological examination out of which 68 samples (68 %) were found positive for *S. aureus*. Among these isolated *S. aureus* isolates from mastitic milk, 62 (91.12%) isolates were tested positive for catalase. A total of 54 (79.41%) isolates were positive in slide coagulase test and 56 (82.35%) isolates were positive in tube coagulase test. Beta hemolytic property was exhibited on sheep blood agar by 50 (73.53%) isolates and nitrate was reduced by 57 (83.82%) *S. aureus* isolates. Methyl reduction (MR) and Voges-Proskauer (VP) test was given by 60 (88.24%) and 58 (85.30%) isolates respectively. In our study 56 (82.35%) isolates of *S. aureus* were found fermenting glucose along with acid production while 46 (67.64%) were gas producer. In Mannitol fermentation 64 (94.12%) isolates were acid producer and 62 (91.18%) were gas producer. In Lactose fermenters, the number of isolates exhibited acid and gas production were 55 (80.88%) and 52 (76.47%) respectively. The details of these tests have been mentioned in table 1 and table 2.

Table.1 Results of Biochemical Test

	Name of the Biochemical test	Positive *S. aureus* isolates	
		Number	Percentage (%)
1.	Methyl Red test	60	88.24
2.	Voges-Proskaur test	58	85.30
3.	Catalase	62	91.12
4.	Coagulase		
a.	Slide Coagulase	54	79.41
b.	Tube Coagulase	56	82.35
5.	Nitrate reduction	57	83.82
6.	β –haemolysis	50	73.53

Table.2 Results of sugar fermentation

S. No.	Sugars	Acid Positive Isolates		Gas Producer Isolates	
		Number	Percentage	Number	Percentage
1.	Glucose	56	82.35	46	67.64
2.	Mannitol	64	94.12	62	9.18
3.	Lactose	55	80.88	52	76.47

The high prevalence of this organism may be associated with its frequent colonization of teats, its ability to exist intracellularly and localize within micro abscesses in the udder and hence resistant to antibiotic treatment (MacDonald, 1997). The bacteria usually establish chronic, subclinical infections and are shed in the milk, which serves as a source of infection for other healthy cows during the milking process. Transmission among cows increases whenever there is lack of effective udder washing and drying, post milking teat dip and drying, inter-cow hand-washing and disinfection, washing clothes and milking machine cups (Radostitis *et al*., 2007). Therefore, the *S. aureus* occurrence at a considerable high percentage indicates the alarming situation for dairy sector. Our findings are closely related to the findings of Ankita (2015) who also reported 90.66% Catalase positive *Staph aureus* in Faizabad district. Ali *et al*. (2015) also recorded 60% incidence of *Staphylococcus aureus* mastitis from Bareilly, U.P. Large number of workers from different states of India have also reported *Staph aureus* to be the main etiological agents of mastitis (Harini *et al*., 2011; Patel *et al*., 2012; Sharma *et al*., 2012; Abhishek Kumar *et al*., 2013; Awandkar *et al*., 2013; Mohanty *et al*., 2013; Charaya *et al*., 2014; Patnaik *et al*.,

2014; Chandrasekaran *et al.*, 2015 and Jena *et al*., 2015).

In recent times, an increasing antimicrobial resistance rate has been recognized in *Staph aureus* from bovine mastitis (Saini *et al*., 2012; Wang *et al*., 2013). Moreover, there is an increased incidence of Methicillin Resistant *S. aureus* (MRSA) all over the world. Further studies are therefore required to study the incidence of methicllin resistant Staphylococcus aureus in this part of eastern plain zone of Uttar Pradesh.

References

AbdEllah, M.R. (2013). Role of free radicals and antioxidants in mastitis. *J. Adv. Vet. Res.,* 3:1-7.

Abhishek Kumar, Haque, S., Sahay, S., Singh, K.K. and Roy, B.K. (2013). Bacteriology and antibiogram of bovine mastitis in ranchi and its vicinity. *Int. J. Agrl.Sc&Vet.Med.*

Ali, Z., Dimri, U. and Jhambh, R. (2015). Prevalence and antibiogram of bacterial pathogens from subclinical mastitis in buffaloes. *Buffalo Bulletin,* 34(1): 41-44.

Ankita, Y. (2015). Characterization of *Staphylococcueaureus* associated with bovine mastitis in reference to Methicillin resistance and antibiogram. M.V.Sc. thesis submitted to N.D. University of Agriculture and Technology, Kumarganj, Faizabad (UP), India.

Awandkar, S.P., Bhikane, A.U., and Kulkarni, M.B. (2013). Antibiotic resistance trends in clinical bovine mastitis. *Biolife.,* 1(3):139-143.

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

Cappuccino, J.G. and Sherman, N. (1992). Biochemical activities of microorganisms. In : Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co. California, USA.

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

Chandrasekaran, D., Nambi, A.P., Thirunavukkarasu, P.S., Venkatesan, P., Tirumurugaan, K. G., and Vairamuthu, S. (2015). Incidence of resistant mastitis in dairy cows in tamilnadu, india. *J. Appl. & Nat. Sci.*, 7 (1): 304 – 308.

Charaya, G., Sharma, A., Kumar, A., Singh, M., Goel, P. (2014). Pathogens isolated from clinical mastitis in Murrah buffaloes and their antibiogram. Veterinary World, 7(11): 980-985.

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

Harini, H. and Sumathi, B.R. (2011). Screening of bovine milk samples for sub-clinical mastitis and antibiogram of bacterial isolates. *Veterinary World,* 4(8):358-359.

Jena Biswadeep, Pagrut Nilesh Kumar, Sahoo Abhishek and Ahmed Abrar (2015). Subclinical Bovine Mastitis in Rural, Peri-Urban and Suburban Regions of Jaipur District of Rajasthan. *India Journal of Animal Research,* 5 (1): 175-182.

Kumar, A., Rahal, A., Dwivedi, S.K. and Gupta, M.K. (2010). Bacterial prevalence and antibiotic resistance profile from bovine mastitis in Mathura, *India. Egypt. J. Dairy Sci.,*

38: 31-34.

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

Mohanty, N.N., Das, P., Pany, S.S., Sarangi, L.N., Ranabijuli, S. and Panda, H.K. (2013). Isolation and antibiogram of Staphylococcus, Streptococcus and E. coli isolates from clinical and subclinical cases of bovine mastitis, Veterinary World, 6(10): 739-743.

NCCLS (1997). National Committee for Clinical Laboratory Standards. Performance standard for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals and humans. Approved Standard. *NCCLS document M 31-A, NCCLS, Villanova, PA.*

Patel, J.V., Bhingaradia, B.V., Patel, B.B., Patel, S.B., Patel, P.B. and Vahora S.P. (2012). Study on P Study on Prevalence of Mastitis and Antibiotic evalence of Mastitis and Antibiotic Sensitivity of Bacterial Isolates Recovered from Crossbred Cows of Anand District of Gujarat. *Indian J. Dairy Sci.,* 65(6).

Patnaik, S., Prasad, A., and Ganguly, S. (2014). Biochemical characterization and antibiogram of staphylococcal microorganisms associated with subclinical mastitis in lactating crossbred cows. Animal Science Reporter, 8(4): 123-129.

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

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. (1994). *Clin. Vet. Microbiol.,Wilfe Publishing, London.* 95-101.

Saini, V., McClure, J.T., Scholl, D.T., Vries, T.J. de and Barkema, H.W. (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.

Szweda, P., Schielmann, M., Frankowska, A., Kot, B. and Zalewska, M., (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., Hansen, I., Reinecke, A.and Heuwieser, W. (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.

Wang, S., Wu, C., Shen, J., Wu, Y. and Wang, Y. (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.