The Pharma Innovation Journal 2018; 7(4): 500-502

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277- 7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.03**
TPI 2018; 7(4): 500-502

www.thepharmajournal.com
Received: 02-02-2018
Accepted: 04-03-2018

**Shiva Jyothi**
College of Veterinary Science, P. V. N. R. Telangana Veterinary University, Rajendrangar, Hyderabad, Telangana, India

**Kalyani Putty**
Department of Veterinary Biotechnology, College of Veterinary Science, P. V. N. R. Telangana Veterinary University, Rajendrangar, Hyderabad, Telangana, India

**Narasimha Reddy Y**
College of Veterinary Science, P. V. N. R. Telangana Veterinary University, Rajendrangar, Hyderabad, Telangana, India

**Ramani Pushpa**
NTR College of Veterinary Science, Gannavaram, SVVU, Tirupati, Andhra Pradesh, India

**Dhanalakshmi K**
College of Veterinary Science, P. V. N. R. Telangana Veterinary University, Rajendrangar, Hyderabad, Telangana, India

**Correspondence**
**Kalyani Putty**
Department of Veterinary Biotechnology, College of Veterinary Science, P. V. N. R. Telangana Veterinary University, Rajendrangar, Hyderabad, Telangana, India

# Characterization of *Staphylococcus* sp isolates from milk of cross bred cows on the day of drying

**Shiva Jyothi, Kalyani Putty, Narasimha Reddy Y, Ramani Pushpa and Dhanalakshmi K**

**Abstract**
The dry period is a crucial phase in the lactation cycle of a dairy cow. It is a time of nutritional, metabolic and mammary change that will profoundly impact health and productitvity in next lactation. It is during the first and last 2weeks of the dry period that is when the teat canal kertain plug is forming and then dissolving that the cow is especially susceptible to new infections. The currnt study was conducted with am aim to isolate and charecterize *Staphylococcus sp* from milk of crossbred dairy cows on the date of drying. Two hundred and eighty one samples were collected from crooss bred dairy cows on the date of drying. Two hundred *Staphylococcus* isolates were obtained from the tested samples and a comparitive study was performed with regard to biofilm formation on CRA, haemolysis, coagulase production and methicillin resistance. Out of 200 *Staphylococcus* isolates, 6% were coagulase positive, 21.5% isolates haemolysed sheep blood agar, 77.5% of isolates were biofilm producers, and 82.5% of isolates were methicilin resisstant. Present study showed phenotypic relation between the biofilm production and methicillin resistance i.e., 86.6% of the methicillin resistant isolates were biofilm producers on CRA. The present study therefore shows high prevalence of methicillin resistance and biofilm forming ability in *Staphylococcus sps* in milk at the time of drying, understanding of which is essential to design better therapeutic measures against mastitis.

**Keywords:** *Stphylococcus*, dry period, biofilm.

## Introduction
India is the largest milk producing country in the world. The Indian dairy industry reported a market size of USD 48.5 billion for 2011. With a Compound Annual Growth Rate of 16 percent, it is anticipated to reach USD 118 billion in 2017 (National Mastitis Council report 2013) [9]. Mastitis is the inflammation of the mammary gland which is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissues (Sharma *et al*., 2006 and Sharma, 2007) [14, 13]. Subclinical mastitis was believed to be more prevalent, about 19 to 78 per cent than clinical in most countries (Tuteja *et al*., 1993) [15]. Dry period is the time between the last milking of one lactation and calving at the start of the next during which the mammary gland undergoes a series of changes that influence the cow's resistance to bacterial infection (Green *et al*., 2002) [6]. Woolford *et al*. (1998) [17] showed that 97% of dry period mastitis infections were in open quarters i.e. quarters that had not developed a good teat seal, and that cows given dry cow therapy formed a good seal, as teat canal organisms degraded keratin and their removal with dry cow therapy lead to more effective seal. Dry period infections may not be presented as clinical cases during the dry period, but there is a high risk that subclinical cases would become clinical after calving. Few studies however, have recorded the quarter wise prevalence of IMIs at the time of drying and the estimates varied from 5% to 28% (Eberhart *et al*., 1986; Oliver *et al*., 1988; Hogan *et al*., 1989; Schukken *et al*., 1993 and Dingwell *et al*., 2002) [5, 10, 7, 12, 4]. The most prevalent pathogen isolated at drying off was Coagulase negative *Staphylococcus* (CNS) according to the studies of Petzer *et al*. (2009) [11]. Similar finding was also previously reported by Aarestrup *et al*. (1995) [1] who reported 70% of CNS from heifers prior to calving. Hence in the present study, we have aimed at looking at the prevalence of *Staphylococcus sps* at the time of drying and also the comparison of the isolates with regards to the methicillin resistance, biofilm formation, haemolysis on blood agar, and coagulase activity.

## Materials and Methods
The dairy farms in and around Hyderabad, India were visited weekly and cows were enrolled

in the study on the day of drying off. Milk samples were centrifuged at 2000 g at 37$^0$C for 10 minutes, supernatant was discarded, and 5 ml of BHI broth was added to the sediment and incubated at 37$^0$C for 24 h (Cruickshank *et al*., 1975) [3]. After incubation of milk samples in BHI broth, the morphology of the organisms was studied with Gram's stain and cultural characters of the isolates was studied using Mannitol Salt Agar (MSA). The isolates were also subjected to various biochemical tests as per the methods described by Cruickshank *et al*. (1975) [3] and Bailey and Scott's (2007).

**Coagulase test**

Several colonies of test organism were emulsified in 0.5ml rabbit plasma (1:4 in PBS) till a milky suspension appeared. The tube was incubated in ambient air for 4 h and if no clot formation was seen the tubes were further incubated for 24 h and clot formation time was recorded.

**Haemolysis on sheep blood agar**

A single colony of *S. aureus* was streaked on blood agar plate and incubated at 37°C for 48 h and the hemolytic pattern was recorded around the colonies.

**Biofilm detection**

Slime production was evaluated by cultivation of *Staphylococcus* isolates on Congo Red Agar (CRA) plates (Mathur *et al.,* 2006) [8]. Isolates were interpreted according to their colony phenotypes as described by Vasudevan *et al* (2003) [16].

**Results and Discussion**

In the present study, we have attempted looking for the prevalence of the *Staphylococcal sps* at the time of drying from 281 milk samples collected from cross bred dairy cows on the day of drying. We could isolate around 200 *Staphylococcus sps* which is quite an higher prevalence. This higher numerical made us even more curious to look into the further characters of the *Staphylococcus* species such as if the isolates were coagulase positive, hemolytic in nature, methicillin resistant, and biofilm formation capability. Interestingly, our study showed that out of 200 *Staphylococcal* isolates 6% were coagulase positive, 21.5% of isolates were haemolytic, 77.5% of isolates were biofilm producers, and 82.5% of isolates were methicillin resistant (Table 1). Further, on comparison of coagulase positive and negative isolates with haemolysis production, biofilm formation and methicillin resistance (Table 2), we can state that majority of the coagulase producers were hemolytic, and biofilm positive. These findings suggest a positive pathogenic relation of coagulase positive *Staphylococcus sps* with biofilm formation and haemolysis. Even more interesting finding in our study is the higher incidence of the *Staphylococcus sps* in milk samples at the time of drying which has to be looked into as the presence of these might result in postpartum mastitis. Furthermore, we would like to state that implementation of dry cow therapy would be at a greater benefit as it would decrease the clinical mastitis postpartum and also the usage of antibiotics at milking stage which would ultimately result in fewer antibiotic residues in the milk, a matter of greater concern from the consumer point of view.

**Table 1:** Characterization of *Staphylococcus sp.* isolates (N=200)

| Properties | Isolates Positive (%) |
|---|---|
| Coagulase Test | 6 |
| Haemolysis on Blood agar | 21.5 |
| Biofilm production on Congo Red Agar | 77.5 |
| Methicillin Resistance | 82.5 |

The identified isolates had higher methicillin resistance (82.5%), followed by biofilm production (77.5%), haemolysis nature (21.5%), and coagulase activity (6%).

**Table 2:** Phenotypic characterization of *Staphylococcus sp* isolates (N=200)

| Characters | Coagulase Positive (%) | Coagulase Negative (%) |
|---|---|---|
| Haemolysis Positive | 91.60 | 16.00 |
| Biofilm Positive | 75.00 | 78.20 |
| Methicillin Resistance | 25.00 | 17.10 |

The coagulase positive isolates had higher ability of hemolytic nature (91.6%), biofilm formation (75%), and methillicilln resistance (25%) when compared with coagulase negative isolates of the current study.

**Acknowledgements**

The authors are thankful for the dairy farm owners for samples obtained.

**Conclusion**

The presence of high prevalence of methicillin resistance and biofilm forming ability *Staphylococcus sps* in milk at the time of drying urges the need and the use of dry cow therapy. Our study also suggests that the emergence of higher number of antibiotic resistant isolates in the current scenario could be due to biofilm formation by the pathogens which could be reduced by the use of antibiofilm agents in conjunction with antibiotics.

**References**

1. Aarestrup FM, Dangler CA, Sordillo LM. Coagulase gene polymorphism in *Staphylococcus aureus* isolated from dairy cattle with mastitis. Canadian Journal of Veterinary Research. 1995; 59:124.
2. Bailey, Scotts Diagnostic Microbiology, 14th Edition BY by Patricia Tille.
3. Cruickshank R, Duguid JP, Marmion BP, Swain RHA. Medi*ca*l microbiology 12thedition, Churchill Livingstone, Edinburgh, 1975.
4. Dingwell RT. Management strategies for the prevention and elimination of intramammary infections in non-lactating dairy cows. DVSc thesis, University of Guelph, 2002.
5. Eberhart RJ. Management of dry cows to reduce mastitis. J Dairy Sci. 1986; 69:1721-1732.
6. Green MJ, Green LE, Medley GF, Schukken YH, Bradley AJ Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. Journal of Dairy Science. 2002; 85:2589-2599.
7. Hogan JS, Smith KL, Hoblet KH. Field survey of clinical mastitis in low somatic cell count herds. Journal of Dairy

Science. 1989; 72:1247-1556.
8. Mathur Singhal TS, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clin*ica*l isolates of staphylococci: an evaluation of three different screening methods. Indian Journal of Medical Microbiology. 2006; 24(1):25-29.
9. National Mastitis Council. The value and use of dairy herd improvement somatic cell count. Accessed, 2013, 21.
10. Oliver SP, Sordillo LM. Udder health in the periparturient period. J Dairy Sci. 1988; 71:2584-2606.
11. Petzer IM[1], Lourens DC, van der Schans TJ, Watermeyer JC, van Reenen R, Rautenbach GH, Thompson P. Intramammary infection rate during the dry period in cows that received blanket dry cow therapy: efficacy of 6 different dry-cow intra-mammary antimicrobial products. JS Afr Vet Assoc. 1988-2009; 80(1):23-30.
12. Schukken YH, VanVliet J, VandeGeer D, Grommers FJ. Arandomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. Journal of Dairy Science. 1993; 76:1915-1930.
13. Sharma N, Maiti SK, Sharma KK. Prevalence, etiology and antibiogram of microorganisms associated with subclinical mastitis in Durg, Chattisgarh State (India). Int J Dairy Sci. 2007; 2:145-151.
14. Sharma N, Gautam A, Upadhyay SR, Hussain K, Soodan JS, Gupta SK. Role of antioxidants in udder health: a review. Indian J Field Vet. 2006; 2(1):73-76.
15. Tuteja FC, Kapur MP, Sharma A, Vinajaka AK. Studies on bovine subclinical mastitis: Prevalence and microflora. Indian Vet. J 1993; 70:787-79.
16. Vasudevan P, Nair MKM, Annamalai T, Venkitanarayanan KS. Phenotypic and genotypic characterization of bovine mastitis isolates of Staphylococcus aureus for biofilm formation. Veterinary Microbiology. 2003; 92(1-2):179-185.
17. Woolford MW, Williamson JH, Henderson HV. Change in electrical conductivity and somatic cell count between milk fractions from quarters subclinically infected with particular mastitis pathogens. J Dairy Res. 1998; 65:187-198.