Indian J Microbiol (July–Sept 2013) 53(3):315–320
DOI 10.1007/s12088-012-0336-1

ORIGINAL ARTICLE

Incidence of Subclinical Mastitis and Prevalence of Major Mastitis Pathogens in Organized Farms and Unorganized Sectors

Raveendra Hegde · Shrikrishna Isloor · K. Nithin Prabhu · B. R. Shome · D. Rathnamma · V. V. S. Suryanarayana · S. Yatiraj · C. Renuka Prasad · N. Krishnaveni · S. Sundareshan · D. S. Akhila · A. R. Gomes · Nagendra R. Hegde

Received: 1 September 2012 / Accepted: 12 November 2012 / Published online: 23 November 2012

Abstract Subclinical mastitis (SCM) represents a major proportion of the burden of mastitis. Determining somatic cell count (SCC) and electrical conductivity (EC) of milk are useful approaches to detect SCM. In order to correlate grades of SCM with the load of five major mastitis pathogens, 246 milk samples from a handful of organized and unorganized sectors were screened. SCC ($>5 \times 10^5$/mL) and EC (>6.5 mS/cm) identified 110 (45 %) and 153 (62 %) samples, respectively, to be from SCM cases. Randomly selected SCM-negative samples as well as 186 samples positive by either SCC or EC were then evaluated for isolation of five major mastitis-associated bacteria. Of the 323 isolates obtained, 95 each were *S. aureus* and coagulase-negative staphylococci (CoNS), 48 were *E. coli* and 85 were streptococci. There was no association between the distribution of organisms and (a) the different groups of SCC, or (b) organised farms and unorganised sectors. By contrast, there was a significant difference in the distribution of CoNS, and not other species, between organized farms and unorganized sectors. In summary, bacteria were isolated irrespective of the density of somatic cells or the type of farm setting, and the frequency of isolation of CoNS was higher with organized farms. These results suggest the requirement for fine tuning SCC and EC limits and the higher probability for CoNS to be associated with SCM in organized diary sectors, and have implications for the identification, management and control of mastitis in India.

Keywords Subclinical mastitis · Somatic cell count · Electrical conductivity · Major bacterial pathogens causing mastitis

R. Hegde · S. Isloor · K. N. Prabhu · D. Rathnamma · S. Yatiraj · N. Krishnaveni · S. Sundareshan · D. S. Akhila
Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University,
Hebbal 560024, Bengaluru, India

R. Hegde · C. R. Prasad · A. R. Gomes
Institute of Animal Health & Veterinary Biologicals,
Hebbal 560024, Bengaluru, India

B. R. Shome
Project Directorate on Animal Disease Monitoring and Surveillance, Hebbal 560024, Bengaluru, India

V. V. S. Suryanarayana
Indian Veterinary Research Institute, Hebbal 560024, Bengaluru, India

N. R. Hegde (✉)
Ella Foundation, Genome Valley, Turkapally, Shameerpet Mandal, Hyderabad 500078, Andhra Pradesh, India
e-mail: hegden@ellafoundation.org

Mastitis is the major lactation-associated disease that causes serious losses to the dairy industry globally, including India. Clinical mastitis is easy to detect and hence amenable for immediate treatment. Subclinical mastitis (SCM), on the other hand, is an invisible malady, and routine surveillance and monitoring is necessary for its detection. Unfortunately, SCM represents a significant proportion (20–25 %) of the burden of mastitis in modern dairy management [1]. The cost of SCM is very difficult to quantify, but is definitely more than that due to clinical mastitis. Approximately 70 % of the cost is associated with reduction in milk production [2]. A survey conducted about 20 years ago estimated that in India, the average decrease in milk yield due to clinical and subclinical mastitis was 50 and 17.5 %, respectively [3]. The same survey estimated the economic loss to be Rs. 6,038.7 and 4,831 millions due to subclinical mastitis and Rs. 2,856.4 and Rs. 2,345.9

millions due to clinical mastitis in cattle and buffaloes, respectively. The total loss due to both forms of mastitis has increased almost five-fold from Rs. 16,072 millions [3] to Rs. 71,655 millions [4] in a span of just 15 years.

While the cross-breeding programme was a major reason for thrusting India to be the highest milk producer in the world, the associated decrease in threshold for susceptibility to diseases has possibly increased the incidence of mastitis. The dairy owner needs to be cognizant of this fact and needs to perform routine screening of milk samples to devise appropriate management practices. Tests such as somatic cell count (SCC) and electrical conductivity (EC) can be used as reliable tools for the detection of SCM in individual herds [5–7]. However, it is not clear whether the cut-off values used for these tests to declare positivity in other countries are correlative for local breeds, crossbreds and non-descript cattle and buffaloes in different husbandry settings in India. Based on the impact on cow health, milk quality and productivity, five pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Strep. dysgalactiae*, *Strep. uberis* and *Escherichia coli*) are considered to be the major mastitogens in most countries, and similar observations have been made with SCM cases in India [8–12]. However, a comprehensive study investigating the correlation between the grade of SCM and the type and density of organisms involved either alone or in combination is lacking anywhere in the world, let alone in India.

The present study was carried out to examine the association between the extent of SCM, different groups of SCC, distribution of the major mastitis pathogens in bovine milk samples and the kind of dairy sector viz., organized or unorganized. This approach is of significance in detection and effective management of SCM in India.

Materials and Methods

Following strict aseptic measures, 246 cattle milk samples were collected over a period of 13 months (September 2009 to October 2010) from four organized farms and three unorganized sectors from various geographic locations in and around the metropolitan setting of Bengaluru (see Table 1). The milk samples were immediately transported to the laboratory in cold chain and screened for SCM by SCC and EC, using Nucleocounter (ChemoMetec A/S, Denmark) and Milk Checker (Oriental Instruments Ltd., Japan), respectively.

All bacteriological media (Colloids Impex Pvt. Ltd., Bengaluru), and reagents for biochemical tests (HiMedia Laboratories, Mumbai) were obtained from commercial sources.

For isolation of staphylococci, milk samples were initially enriched in brain heart infusion (BHI) broth for 6 h at 37 °C and then streaked onto mannitol salt agar and incubated at 37 °C for an additional 24 h. After recording the colony morphology, the colonies were re-streaked onto BHI agar for further identification procedures.

Isolation of streptococci was carried out by enrichment in streptococcus selection broth for 6 h followed by streaking onto blood agar plates and incubating in 5 % CO_2. After recording the pattern of haemolysis and the colony morphology, the colonies were re-streaked onto blood agar plates and incubated further at 37 °C for 48 h in 5 % CO_2 to obtain pure cultures. The pure cultures were then streaked onto BHI agar for further identification procedures.

For isolation of *E. coli*, initial enrichment was carried out in tryptone phosphate broth for 18 h at 37 °C, followed by streaking onto MacConkey agar and incubation at 37 °C for 24 h. The lactose fermenting colonies were further streaked onto Eosin Methylene Blue agar and incubated at 37 °C for 24 h. The metallic sheen colonies were streaked onto BHI agar for further identification procedures.

Pure cultures were subjected for various biochemical tests as per standard procedures [13]. For identification of streptococci, catalase, Voges Proskauer (VP), pyrrolidonyl arylamidase, hippurate hydrolysis, esculin hydrolysis and sugar fermentation tests were employed. For staphylococci, catalase, coagulase, thermonuclease, urease, and VP tests

Table 1 Number of samples under various SCC and EC values

Setting	No. of milk samples	SCC values				EC values	
		0–1 × 10^5/mL	1–2 × 10^5/mL	2–5 × 10^5/mL	>5 × 10^5/mL	<6.5 mS/cm	>6.5 mS/cm
Organised farm A	10	Nil	Nil	1	9	1	9
Organised farm B	17	1	1	1	14	9	8
Organised farm C	61	9	3	11	38	7	54
Organised farm D	27	10	4	4	9	4	23
Unorganised sector A	75	38	7	9	21	43	28
Unorganised sector B	30	11	1	8	10	12	18
Unorganised sector C	26	9	3	4	10	16	10
Total	246	78	19	38	111	92	150

Table 2 Distribution of organisms in different settings

Farm/Sector code	No. of milk samples	Streptococci	*S. aureus*	CoNS	*E. coli*
Organised farm A	10	14	4	8	8
Organised farm B	17	2	3	11	1
Organised farm C	61	16	19	40	6
Organised farm D	27	10	5	14	5
Unorganised sector A	75	8	24	14	7
Unorganised sector B	30	19	22	5	14
Unorganised sector C	26	16	18	3	7
Total	246	85	95	95	48

were employed and for *E. coli,* nitrate, indole, methyl red, VP, citrate and urease production tests were employed.

The distribution of isolates was subjected to two-way analysis of variance with Bonferroni post tests at $P < 0.05$, $P < 0.01$ and $P < 0.001$ against various groups of SCC using GraphPad Prism software version 5 (GraphPad Software Inc., USA).

Results and Discussion

Since direct microbiological investigation is not feasible, indirect tests are necessary to identify intra-mammary infections (IMI). The gold standard is to measure inflammation through cytological investigation [6], i.e., counting somatic cells. In addition, "cow side" tests such as the California Mastitis Test and measuring the EC of milk can also be used. In order to test for correlations between SCC and/or EC results and SCM of specific aetiology, or for the ability of the results to predict the incidence of SCM in organized or unorganized sectors, clinical diagnostic (SCC and EC) and microbiological analyses were performed on 246 milk samples in this study.

The International Dairy Federation recommends that the diagnosis of mastitis be based on the SCC and microbiological status. The acceptability break point for SCC is 5×10^5 cells/mL [14], but different cut-off values are adopted by different countries. In the European Union, Australia and New Zealand, the penalty limit for saleable milk is 4×10^5 cells/mL, whereas Canada, the US and Sweden use 5×10^5, 7.5×10^5 and 2×10^5 cells/mL limit, respectively [15]. Since no standards are adopted in India, the cut-off of 5×10^5 cells/mL was applied in this study, when 45 % of the 246 milk samples were positive for SCM. Further, 78 (31.7 %), 19 (7.7 %), 38 (15.4 %) and 111 (45 %) samples grouped under 0–1 $\times 10^5$, 1–2 $\times 10^5$, 2–5 $\times 10^5$ and >5 $\times 10^5$cells/mL, respectively (Table 1). With the same samples, 92 (38 %) were negative and 150 (62 %) were positive for SCM by EC, considering >6.5 mS/cm as the cut-off (Table 1). When either of the two tests was applied, 186 samples were positive for SCM. These results show that in the sampled location, SCM was prevalent anywhere between 45 % (by SCC alone) and 75.6 % (when both methods were combined). Further analyses revealed that on an average 61 % of the samples from organized farms showed SCM,

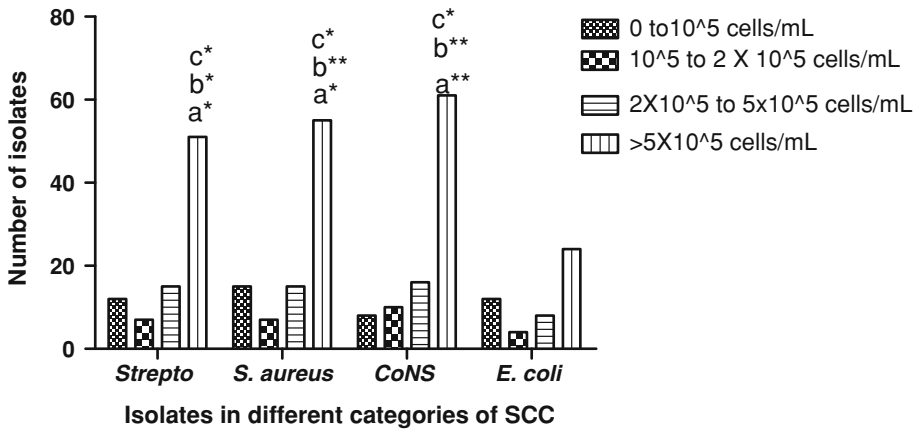

Fig. 1 Distribution of organisms in different groups of SCC. Milk samples were subjected to SCC, which was then categorized arbitrarily into four different groups as shown by the different bars. Milk samples were subjected to microbiological evaluation and distribution of the four major bacterial species/groups in each SCC group is shown. *a* compared with 0–1 $\times 10^5$ cells, *b* compared with 1 $\times 10^5$–2 $\times 10^5$ cells, *c* compared with 2 $\times 10^5$–5 $\times 10^5$ cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

although the level varied from 33 to 90 % between individual farms.

All the samples positive for SCM by either of the methods, as well as random negative samples were subjected to microbiological evaluation. Of the total 323 bacterial isolates recovered from the 186 milk samples (Table 2), staphylococci were the most frequent (95 isolates each of *S. aureus* and CoNS) followed by streptococci (85 isolates) and *E. coli* (48 isolates). In general, milk samples with SCC $>5 \times 10^5$/mL, irrespective of their source from organised or unorganised sector, showed high prevalence of bacterial pathogens (Table 2, Fig. 1). The number of isolates at 5×10^5 SCC was statistically significant from all the other SCC categories for streptococci ($p < 0.05$ for all the pair-wise comparisons), *S. aureus* ($P < 0.05$ for $>5 \times 10^5$ with $0–1 \times 10^5$ as well as $2–5 \times 10^5$; $p < 0.01$ for 5×10^5 with $1–2 \times 10^5$), and CoNS ($p < 0.05$ for $>5 \times 105$ with $02–5 \times 10^5$ and $p < 0.01$ for 5×10^5 with $0–1 \times 10^5$ as well as $1–2 \times 10^5$). No significant difference was observed for *E. coli* (Fig. 1). In organized farms, the only significant difference observed was with CoNS when the $>5 \times 10^5$ SCC category was compared to $0–1 \times 10^5$ ($p < 0.01$), $1–2 \times 10^5$ or $2–5 \times 10^5$ ($p < 0.05$) categories (Table 3; Fig. 2a). In the unorganized sector, the only significant difference observed was with *S. aureus* when the $>5 \times 10^5$ SCC category was compared with $1–2 \times 10^5$ ($p < 0.05$) category (Table 3, Fig. 2b).

Surprisingly, pathogens could be isolated even with very low SCC of 10,000–25,000/mL. Out of 17 such milk samples, five isolates of streptococci, ten isolates of staphylococci, five isolates of CoNS and eight isolates of *E. coli* were obtained. Similar observations were made with EC, which increases variably from the normal range of 5.5–6.5 mS/cm during SCM [16–19]. Out of 58 samples with EC values < 6.5 mS/cm, nine isolates of streptococci, 32 isolates of staphylococci, 21 isolates of CoNS and six isolates of *E. coli* were obtained. Thus, lower SCC or EC could be misleading in accurately reflecting the bacteriological status or udder health, although it is noteworthy that we did not assess the potential of these isolates to be pathogenic. However, the analyses are limited to a few hundred samples from a small region in South India. Moreover, no effort was made to restrict the sampling to species (cattle, buffalo), breed, age, parity or the time of lactation. Since India has different agro-climatic conditions under various kinds of husbandry practices, it would be difficult to extrapolate these data to other parts of the country.

The distribution of *S. aureus*, CoNS, streptococci and *E. coli* observed in this study is in accordance with earlier reports from India [8–10, 12] and elsewhere [8, 20, 21]. The presence of *S. aureus* in almost a third of the samples buttressed its importance in SCM. Indeed a recent metagenomic study of SCM revealed the preponderance of *E. coli* in the two indigenous breeds and *S. aureus* in cross-bred cattle in India [22]. Streptococci could not be identified to species level despite conducting a thorough biochemical investigation. Similar confounding observations have been reported by others, especially with the hydrolysis of esculin and the Christie Atkins Munch Peterson (CAMP) test [23–27]. Furthermore, since several atypical streptococci also test positive in biochemical tests, the identification of *Streptococcus* at species level is difficult.

Prior to the 1970, CoNS were regarded as contaminants in clinical specimens [28]. Of the more than 40 staphylococcal species described [29], CoNS are often considered as minor pathogens, with insignificant or little impact on udder health [28]. However, recent reports suggest that CoNS have become the predominant pathogens isolated from SCM and may cause substantial herd problems in

Table 3 Distribution of isolates according to SCC

Farm	somatic cell count (SCC)															
	$0–1 \times 10^5$/mL				$1–2 \times 10^5$/mL				$2–5 \times 10^5$/mL				$>5 \times 10^5$/mL			
	Strepto	Sau	EC	CoNS	Strepto	Sau	EC	CoNS	Strepto	Sau	EC	CoNS	Strepto	Sau	EC	CoNS
Organised farm A	0	0	0	0	0	0	0	0	1	0	0	0	13	4	8	11
Organised farm B	0	0	0	0	0	0	0	0	0	0	0	0	2	6	1	11
Organised farm C	1	0	0	0	0	1	1	1	5	1	0	7	10	8	5	29
Organised farm D	3	3	1	2	1	0	1	4	1	0	3	2	5	2	0	6
Total for organised farms	4	3	1	2	1	1	2	5	6	1	3	9	30	20	14	57
Unorganised A	1	5	1	3	2	2	0	5	0	5	1	3	5	15	5	3
Unorganised B	5	4	7	3	1	1	0	0	5	7	3	2	8	10	4	0
Unorganised C	2	3	3	0	3	3	2	0	3	2	1	2	8	10	1	1
Total for unorganised sector	8	12	11	6	6	6	2	5	8	14	5	7	21	35	10	4

Strepto streptococci, *Sau Staph. aureus*, *EC E. coli, CoNS* Coagulase negative staphylococci

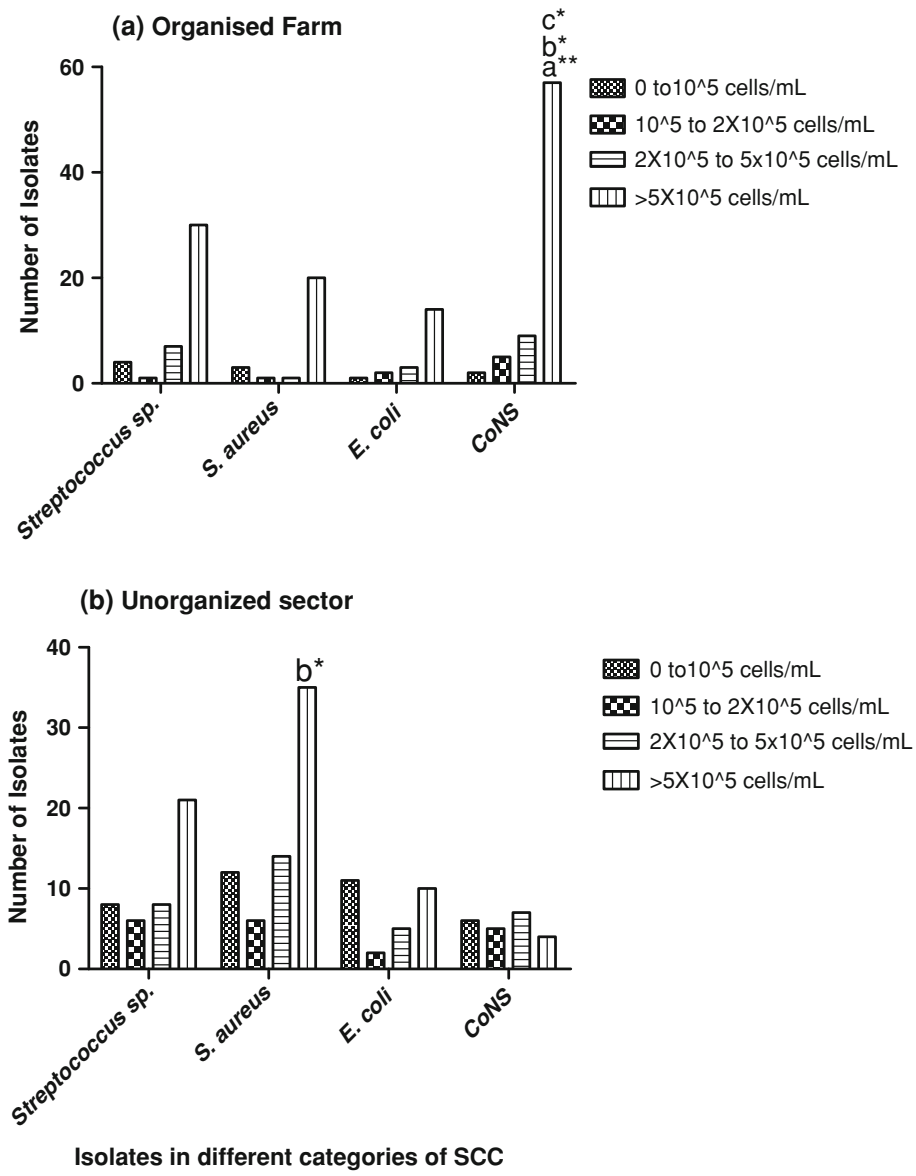

Fig. 2 Distribution of organisms in organized and unorganized sectors according to groups of SCC. Milk samples obtained from organized farms (**a**) and unorganized sector (**b**) were subjected to microbiological evaluation and the number of isolates of each of the major bacterial species/ groups are shown under four different arbitrary SCC groups. *a* compared with 0-1 $\times$ 10^5 cells, *b* compared with 1 $\times$ 10^5–2 $\times$ 10^5 cells, *c* compared with 2 $\times$ 10^5–5 $\times$ 10^5 cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

many countries [1, 30–32]. In Finland, CoNS were isolated from 17 % of all the samples and from 50 % of the bacteriologically positive quarters [33]. In two dairy research herds in Ontario, Canada, CoNS were the most common (51 %) bacteria causing IMI at drying off [29]. In the present study, 29.4 % of the isolates were CoNS, a finding which is in agreement with other reports [31, 34]. In organized farms, a significant difference ($p < 0.01$ or $p < 0.05$) was observed in the distribution of CoNS between the different SCC groups, whereas distribution of other organisms was similar. Interestingly, in unorganized sector, there was a random distribution of the organisms, except that more number of *S. aureus* isolates was recovered, the only significant difference being that between 5 $\times$ 10^5 and 1–2 $\times$ 10^5 SCC categories ($p < 0.05$). If extended and confirmed, the higher frequency of CoNS in organized farms and that of *S. aureus* in unorganized sectors could form one of the criteria for management of SCM in organized versus unorganized sectors in India.

In summary, although generally accepted cut-off values for SCC and EC may be applied, baseline parameters need to be established and/or fine-tuned for each breed and/or agro-climatic region in India. Simultaneous quantitative estimation, and not merely the qualitative detection, of the major pathogens as well as determining their virulence potential may be required. This would enable a reasonably accurate and realistic determination of the status of SCM and in turn enable implementation of mastitis control programmes to ensure quality milk production.

Acknowledgments This work was funded by the Indian Council of Agricultural Research under the National Agriculture Innovation Projects Scheme. We thank Avinash Bhat, Dept. of Pharmacology, Veterinary College, Bengaluru, for assistance with statistical analysis.

References

1. Wilson DJ, Gonzales RN, Das HH (1997) Bovine mastitis pathogens in New York and effects on somatic cell count and milk production. J Dairy Sci 80:2592–2598
2. Oliver SP, Calvinho LF (1995) Influence of inflammation on mammary gland metabolism and milk composition. J Anim Sci 73:18–33
3. Singh PJ, Singh PB (1994) A study of economic losses due to mastitis in India. Ind J Dairy Sci 47:265–272
4. Bansal BK, Gupta DK (2009) Economic analysis of bovine mastitis in India and Punjab. Ind J Dairy Sci 62:337–345
5. Hayakawa Y, Akagi M, Hayashi M, Shimango T, Komae H, Funaki O, Kaidoh T, Takeuchi S (2000) Antibody response to toxic shock syndrome toxin-1 of *Staphylococcus aureus* in dairy cows. Vet Microbiol 72:321–327
6. Pyorala S (2003) Indicators of inflammation in the diagnosis of mastitis. Vet Res 34:565–578
7. Sharma N, Singh NK, Bhadwal MS (2011) Relationship of somatic cell count and mastitis: an overview. Asian-Australas J Anim Sci 24:429–438
8. Mitra M, Ghosh D, Ali K, Guha C, Pramanic AK (1995) Prevalence of subclinical mastitis in an organized buffalo farm at Haringhata. Ind Vet J 72:1310–1311
9. Mallikarjunaswamy MC, Krishnamurthy GV (1997) Antibiogram of bacterial pathogens isolated from bovine subclinical mastitis cases. Ind Vet J 74:885–886
10. Datta S, Rangnekar A (2001) Subclinical mastitis in a Jersey herd. Ind Vet J 78:161–162
11. Kumari MP, Ramgupta JB (2002) Diagnosis and therapy of subclinical mastitis in post parturient cows. Ind Vet J 79:89
12. Rajeev NK, Isloor S, Rathnamma D, Shridhar NB (2009) Characterization of *Staphylococcus aureus* and *E. coli* of bovine mastitis. Ind Vet J 86:883–885
13. Collee JG, Miles RS, Watt B (2008) Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A (eds) Mackie and McCartney Practical Medical Microbiology. Churchill Livingstone, London
14. Anonymous (1971) A monograph on bovine mastitis. In: International Dairy Federation Bulletin, Brussels, Belgium
15. Paape MJ, Contreras A (1997) Historical perspective on the evaluation of the milk somatic cell count. Flem Vet J Suppl 66:93–105
16. Sargeant JM, Leslie KE, Shirley JE, Pulkrabek JL, Liim GH (2001) Sensitivity and specificity of somatic cell count and California mastitis test for identifying intramammary infection in early lactation. J Dairy Sci 84:2018–2024
17. Mansell PD, Seguya A (2003) The use of a hand-held conductivity meter for the diagnosis of subclinical mastitis in dairy cows during late lactation. New Zealand Vet J 51:21–25
18. Hamann J, Nipp B, Zecconi A (1998) Evaluation of the electrical conductivity of milk as a mastitis indicator. In: International Dairy Federation Bulletin, Brussels, Belgium
19. Jensen NE, Knudsen K (1991) Inter-quarter comparison of markers of subclinical mastitis: somatic cell count, electrical conductivity, N-acetyl-D-glucosaminidase and antitrypsin. J Dairy Res 58:389–399
20. Botrel MA, Haenni M, Morignat E, Madec JY, Calavas D (2010) Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. Foodborne Pathog Dis 7:479–487
21. Schukken YH, Grommers FJ, van de Geer D, Brand A, van degeer D (1989) Incidence of clinical mastitis on farms with low somatic cell counts in bulk milk. Vet Rec 125:60–63
22. Bhatt VD, Ahir VB, Koringa PG, Jakhesara SJ, Rank DN, Nauriyal DS, Kunjadi AP, Joshi CG (2012) Milk microbiome signatures of subclinical mastitis-affected cattle analysed by shotgun sequencing. J Appl Microbiol 112:639–650
23. Odierno L, Calvinho L, Traverssa P, Lasagno M, Bogni C, Reinoso E (2006) Conventional identification of *Streptococcus uberis i*solated from bovine mastitis in Argentinean dairy herds. J Dairy Sci 89:3886–3890
24. Gonano M, Winter P (2008) Phenotypic and molecular identification of *Streptococcal* species isolated from milk of intra mammary infected Dairy cows in Austria. In: Proceedings of international conference on Mastitis control: from science to practice, September 30th–2nd October 2008, The Hague, Netherlands
25. Khan IU, Hassan AA, Abdulmawjood A, Lammler C, Wolter W, Zschock M (2003) Identification and epidemiological characterization of *Streptococcus uberis* isolated from bovine mastitis using conventional and molecular methods. J Vet Sci 4:213–224
26. King JS (1981) *Streptococcus uberis*: a review of its role as a causative organism of bovine mastitis. Br Vet J 137:160–165
27. Calvinho LF, Vitulich CA, Zurbriggen MA, Canavesio VR, Tarabla HD (1991) Prevalencia de microorganismos patogenos de la ubre en rodeos de la cuenca lechera santafesina. [Prevalence of microorganisms as udder pathogens in the dairy herds of Santa Fe]. Therios 18:189–196
28. Thoerberg BM (2008) Coagulase negative staphylococci in bovine subclinical mastitis. Master's Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden
29. Euzeby JP (2008) List of prokaryotic names with standing in nomenclature. http://www.bacterio.cict.fr/. Accessed 8 July 2012
30. Lim GH, Leslie KE, Kelton DF, Duffield TF, Timms LL, Dingwell RT (2007) Adherence and efficacy of an external sealant to prevent new intramammary infections in the dry period. J Dairy Sci 90:1289–1300
31. Taponen S (2008) Bovine mastitis caused by coagulase negative *staphylococci*. Dissertation, University of Helsinki, Finland
32. Tenhagen BA, Koster G, Wallman J, Henwieser W (2006) Pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J Dairy Sci 89:2542–2551
33. Pitkala A, Haveri M, Pyorala S, Myllys V, Buzalski HT (2004) Bovine mastitis in Finland 2001-prevalence, distribution of bacteria, and antimicrobial resistance. J Dairy Sci 87:2433–2441
34. Trinidad P, Nickerson SC, Adkinson RW (1990) Histopathology of Staphylococcal mastitis in inbred dairy heifers. J Dairy Sci 73:639–647