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Review Article
Coagulase Negative Staphylococcal Species Mastitis: An Overview

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Abstract

Bovine mastitis is one of economically important disease affecting livestock and causing economic losses to dairy farmers in India. Coagulase Negative Staphylococcus (CNS) species also infects the mammary glands of animals and leads to persistent or subclinical mastitis. The prevalence of CNS mastitis in various parts of the world were 6-72 and 6-30% in subclinical and clinical mastitis cases, respectively. Epidemiology of CNS species causing mastitis in animals gives the newer approaches for preventing the occurrence of CNS species mastitis. The CNS species predominantly isolated from milk were *S. chromogenes*, *S. epidermidis*, *S. haemolyticus*, *S. simulans*, *S. warneri*, *S. hominis*, *S. saprophyticus*, *S. xylosus*, *S. hyicus*, *S. sciuri* and *S. intermedius*. The *S. epidermidis* intramammary infections were found in multiparous cow and *S. chromogenes* in primiparous cows. The CNS mastitis shows only mild clinical signs and cause persistent infections, resulting in increased milk SCC, which affects milk quality and may be related to decreased milk production. The clinical signs, diagnosis and treatment of CNS mastitis are necessary for effective control and prevention. There was emergence of CNS species as an important mastitis causing pathogen in subclinical mastitis and persistence infection of udder in dairy animals of India and world. In conclusion, there is need to further identify the CNS at species level and also systematic experimental study of various CNS species in dairy animals is required for better understanding the occurrence of CNS mastitis in livestock of India. Further, comparative study of CNS mastitis with organisms causing severe mastitis in various animal models to know the basic mechanisms of host pathogen interaction using advance molecular biology tools is necessary.

Key words: Coagulase negative staphylococcal species, identification, epidemiology, clinical signs, diagnosis, treatment

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bovine mastitis (mast = breast, itis = inflammation) a major disease affecting dairy cattle worldwide, results from the inflammation of the mammary gland. The severity of the inflammation can be classified into sub-clinical, clinical and chronic forms and its degree is dependent on the nature of the causative pathogen and on the age, breed, immunological health and lactation state of the animal. Sub-clinical mastitis is difficult to detect due to the absence of any visible indications and it has major cost implications. Chronic mastitis is a rare form of the disease but results in persistent inflammation of the mammary gland. The direct effects of mastitis are temporary or permanent loss in milk production, poor milk quality, reduction in price, treatment costs, labour costs, premature culling, etc. In India the total annual economic losses due to mastitis was calculated to be 7165.51 crore rupees (Bansal and Gupta, 2009).

Till date more than 50 *Staphylococcus* species and subspecies have been characterized. The *Staphylococcus* genus has been divided in to coagulase positive and coagulase negative based on their ability to coagulate rabbit plasma. Coagulase Negative Staphylococcus (CNS) species have become more important as bovine mastitis causing agents during recent years. The CNS species are the most frequently isolated microorganisms in bovine intramammary infections (IMI) in many countries. More than 10 different coagulase negative *Staphylococcus* species have been isolated from mastitis bovine milk samples and the species most commonly reported are *Staphylococcus chromogenes* and *Staphylococcus simulans* (Trinidad *et al*., 1990; Matthews *et al*., 1992). *Staphylococcus hyicus* and *Staphylococcus epidermidis* have also frequently been isolated (Myllys, 1995; Thorberg *et al*., 2006). These bacteria are sometimes referred to as environmental staphs and most frequent organisms isolated from milk samples from herds that have controlled the major pathogens (Janus, 2009). In Asia, recent reports indicated the changing trends from *Staphylococcus aureus* to coagulase negative staphylococci as major mastitis causing organism (Sharma *et al*., 2012). The CNS has traditionally been considered to be minor mastitis pathogens, especially in comparison with major pathogens such as *Staphylococcus aureus*, streptococci and coliforms. In India, not much work has been carried out on coagulase negative *Staphylococcus* species causing subclinical and clinical mastitis in bovines. Mouse is a suitable model to study coagulase negative *Staphylococcus* species induced bovine subclinical mastitis (Krishnamoorthy *et al*., 2014b).

IDENTIFICATION

The CNS are normally not identified at species level but are treated as a uniform group. Some CNS species may be more virulent or have different clinical characteristics, but evidence is still lacking. Most of the species are determined based on various phenotypic characteristics, such as colony morphology, haemolysis patterns and various biochemical reactions. Coagulase-negative staphylococci produce large, smooth, entire colonies that are white (un-pigmented), creamy, greyish-white, tan or golden-yellow colour. Colonies of some species may have a rough, irregular appearance and generally have no haemolysis or may produce a narrow (<2 mm) diffuse zone of complete haemolysis at 24 h. A few species will produce a larger, diffuse zone of incomplete haemolysis, not to be confused with the sharp-bordered zones of haemolysis displayed by *S. aureus* isolates. Gram-staining will reveal Gram-positive cocci in pairs, tetrads or irregular clusters (Hogan *et al*., 1999). In addition, difficulties encountered in CNS differentiation to species level and the use of different identification schemes or systems also play a role. This was also the reason why routine veterinary laboratories do not usually identify CNS at the species level or use simple diagnostic tests like DNase activity to differentiate clusters of CNS.

Identification based on these conventional tests was time-consuming and costly; therefore tests like API Staph (bioMerieux, France) and Staph-Zym (Rosco, Denmark), for rapid identification of *Staphylococcus* species were commonly used. Internal transcribed spacer PCR, based on the polymorphism of the 16S-23S rDNA spacer region was an adequate tool for the identification of *Staphylococcus* species from cases of bovine mastitis (Bes *et al*., 2000). It was shown that tDNA-intergenic spacer PCR was very accurate in identifying *S. chromogenes* (Devriese *et al*., 2002). Newer studies have focussed on sequencing of genes like the rpoB gene (Zadoks *et al*., 2006) 16S rRNA gene and/or *tuf* gene (Heikens *et al*., 2005). Zadoks *et al*. (2006) concluded that when DNA-sequence based species identification as gold standard, phenotypic identification was unreliable. For studies aimed at understanding the impact of CNS species on milk quality and udder health, DNA-sequence based speciation was preferable. However, these tests do not identify all *Staphylococcus* species, especially those from veterinary samples (Bes *et al*., 2000). In a study conducted using PCR-Restriction fragment length polymorphism (RFLP) analysis of a partial *groEL* gene sequence showed a reliable and reproducible molecular method of identification of CNS

species responsible for bovine mastitis (Da Silva Santos *et al*., 2008). The $(GTG)_5$-PCR fingerprinting based identification of CNS species achieved a typeability of 94.7% and an accuracy of 94.3% when compared to identifications based on gene sequencing (Braem *et al*., 2011).

The methods based on molecular genetics are developing rapidly and this seems to have created a new problem in that the bacterial phenotypes and genotypes do not necessarily match (Heikens *et al*., 2005). An ideal system would identify the CNS species relevant in mastitis rapidly and accurately directly from milk and perhaps simultaneously detect the *blaZ* gene, indicating resistance to penicillin G. It would be important to know about penicillin resistance of the isolate because it affects the choice of antimicrobial treatment and also prognosis for cure. Some other antimicrobial resistance genes, if such resistance genes were considered important in relation to mastitis treatment, could also be determined. The CNS species responsible for the majority of bovine CNS mastitis cases are probably different from those in human medicine (Jarp, 1991; Waage *et al*., 1999). There was no knowledge about how many of the isolates identified as particular CNS species and do genetically really belong to that species. Most studies concerning CNS species isolated from bovine mastitis were conducted a long time ago, using methods based on phenotypic characteristics of the bacterial isolates. Before any identification method can be adopted into mastitis diagnostics, more knowledge about CNS species involved in bovine mastitis is needed.

EPIDEMIOLOGY OF COAGULASE NEGATIVE STAPHYLOCOCCAL MASTITIS

The epidemiology of CNS mastitis still is unclear, although a number of studies have been conducted to identify the reservoirs of CNS. The CNS species were isolated from different body sites of cows, heifers and calves, from udder secretions, milk and cows environment (Matthews *et al*., 1992). A wide range of CNS species have been isolated and identified most often using methods based on the phenotype. The *S. chromogenes*, *S. epidermidis*, *S. intermedius*, *S. warneri*, *S. haemolyticus*, *S. sciuri* and *S. xylosus* were isolated in several studies from milk samples, teat canals, teat skin or skin on other body sites (Matthews *et al*., 1992; Aarestrup *et al*., 1995; Chaffer *et al*., 1999). The *S. xylosus* and *S. sciuri* were shown to be part of the normal bovine skin microbiota and have been isolated from bedding and the cow's environment. The *S. cohnii* and *S. saprophyticus* were also common in the cow's environment (Matos *et al*., 1991). The percentage prevalence of CNS species in subclinical mastitis and clinical mastitis were presented in Table 1 and 2 respectively. In a study from ovine milk samples using PCR-RFLP of 16S rRNA and *gap* genes showed *S. epidermidis* (57.9%), *S. caprae* (15%) and *S. chromogenes* (13.2%) out of 226 CNS isolates collected from 2201 milk samples from sarda sheep in Italy (Onni *et al*., 2010). Intramammary infections in goat milk of Sweden was studied and found 39 (18%) of the milk samples out of 222 milk samples with udder pathogens. The most frequently isolated bacterial species was CNS species (72%) followed by *Staphylococcus aureus* (23%) (Persson and Olofsson, 2011).

Seasonal differences in occurrence of CNS mastitis have been reported. In Finland, the prevalence of CNS and *S. aureus* mastitis was highest during winter and spring, i.e., during the indoor season (Koivula *et al*., 2007). In Norway, the highest prevalence of CNS mastitis was found during the late indoor season. The prevalence of CNS mastitis is higher in primiparous cows than in older cows (Tenhagen *et al*., 2006). The CNS can colonize the mammary gland of pregnant heifers (Myllys, 1995) and were isolated from the mammary gland

Table 1: Prevalence of CNS in subclinical mastitis around the world

Place	Prevalence (%) in SCM	Reference
Jabalpur, India	16.28	Das and Joseph (2005)
Bangalore, India	16	Sumathi *et al*. (2008)
India	40.47	Ahire *et al*. (2008)
India	72.13	Dutta (2009)
Sikkim, India	23.96	Dubal *et al*. (2010)
Dharwad, India	58.71	Kaliwal *et al*. (2011)
Karnataka, India	47.66	Krithiga *et al*. (2011)
Dutch dairy herds	6	Poelarends *et al*. (2001)
Tennessee, USA	28 (12-41)	Dingwell *et al*. (2004)
US and Canada	15	Dingwell *et al*. (2004)
Finland	50	Pitkala *et al*. (2004)
Norway	16	Pitkala *et al*. (2004)
Germany	35	Tenhagen *et al*. (2006)
Estonia	16	Haltia *et al*. (2006)
Netherlands	10.8 (Quarter level) 34.4 (Cow level)	Sampimon *et al*. (2009)
Three research dairy herds	11.3	Gillespie *et al*. (2009)
China	10-15	Cheng *et al*. (2010)
Korea	40.7	Nam *et al*. (2010)
Sweden	16	Persson *et al*. (2011)
Algeria	25.84	Bakir *et al*. (2011)

Table 2: Prevalence of CNS in clinical mastitis around the world

Place	Prevalence (%) in CM	Reference
India	25	Yathiraj *et al*. (2007)
India	12.63	Ranjan *et al*. (2011)
Israel	9	Shpigel *et al*. (1998)
Switzerland	17	Schallibaum (2001)
Wisconsin, USA	12.7-17.5	Makovec and Ruegg (2003)
Finland	18	Koivula *et al*. (2007)
Canada	6	Riekerink *et al*. (2007)
Central Ethiopia	30.1	Mekibib *et al*. (2010)
Sweden	6	Persson *et al*. (2011)

and teat apices of heifers as young as 10 months old (De Vliegher *et al*., 2003). The prevalence of positive teat orifices from heifers sampled approximately 2 weeks prior to calving was as follows: 28% *S. chromogenes*, 3.5% *S. simulans*, 2.8% *S. hominis* and 2.1% *S. epidermidis* (Matthews *et al*., 1992). The CNS is important pathogens in cattle of all ages, but the predominant CNS species causing infection seems to differ between age groups. The *S. chromogenes* was the major CNS species in pre-calving heifers and primiparous cows (Trinidad *et al*., 1990; Rajala-Schultz *et al*., 2007; Taponen *et al*., 2006) whereas, *S. simulans* was mostly isolated from cows in later lactations (Taponen *et al*., 2006). Multiparous cows generally become infected with CNS during later lactation whereas primiparous cows usually already have the infection at the beginning of lactation (Taponen *et al*., 2007). Prevalence of CNS IMI was higher in heifers compared to older cows (Sampimon *et al*., 2009). In cows with subclinical mastitis, *S. epidermidis* IMI were mainly found in multiparous cows where as *S. chromogenes* IMI were mainly found in primiparous cows (Thorberg *et al*., 2009).

VIRULENCE FACTORS

Various virulence factors, including production of haemolysins, leucocidins, exfoliative toxins, enterotoxins, toxic-shock syndrome toxin, slime and biofilm formation were found in *S. aureus* strains isolated from bovine mastitis (Cucarella *et al*., 2004; Zecconi *et al*., 2006) but only few studies have focused on the search for such virulence factors from CNS isolated from mastitis. Adherence and internalization of CNS into mammary epithelial cells was studied in cell cultures and were able to adhere to bovine mammary cells (Hyvonen *et al*., 2007). The adhesive capacity of various CNS was almost equal to the adhesive capacity of *S. aureus*, but the invasive capacity of *S. aureus* was stronger than that of CNS strains. Kuroishi *et al*. (2003) found that a high percentage of both *S. aureus* and CNS from bovine subclinical, chronic or acute mastitis produced staphylococcal enterotoxins and/or toxic shock syndrome toxin-1. Somewhat surprisingly, production of different staphylococcal enterotoxins and toxic shock syndrome toxin 1 were as common in CNS isolates as in *S. aureus* isolates and as common in isolates originating from subclinical mastitis as in those from chronic or acute mastitis. Biofilm-associated proteins were found among bovine mastitis isolates, including *S. aureus*, *S. epidermidis*, *S. chromogenes*, *S. hyicus* and *S. xylosus* (Cucarella *et al*., 2004). Oliveira *et al*. (2006) found 6 out of 16 *S. aureus* and 6 out of 16 *S. epidermidis* isolates from subclinical mastitis to be phenotypically positive for biofilm production. Biofilm formation in staphylococci isolated from bovine mastitis or humans has been associated at least with gene loci *ica* (intercellular adhesion), *bap* (biofilm-associated protein), *agr* (accessory gene regulator) and *sar* (staphylococcal accessory regulator), but isolates able to form biofilm do not necessarily carry all these genes. The *bap* gene was identified in mastitis causing staphylococci with marked ability to produce biofilm and which belonged to several species, including *S. epidermidis*, *S. chromogenes*, *S. xylosus*, *S. simulans* and *S. hyicus* (Tormo *et al*., 2005). Leitner *et al*. (2003) studied virulence of *S. aureus* and CNS using a mouse model. Seven strains of *S. aureus* and one strain of each of *S. chromogenes* and *S. intermedius* isolated from chronic bovine mastitis were studied. Mice were experimentally infected in one limb and thereafter inspected for morbidity (arthritis and gangrene) and mortality. One *S. aureus* isolate producing α-haemolysin was the most virulent, followed by isolates producing α+β-haemolysin and α-haemolysin. The least virulent isolates were the non-haemolytic *S. aureus* strains, but even they were more virulent than the *S. chromogenes* and *S. intermedius* isolates tested. The two CNS isolates do not caused morbidity or mortality in the mice.

CLINICAL SIGNS

The CNS have been regarded as minor pathogen that mostly infect heifers around calving do not cause clinical signs, cause only a slight increase in the somatic cell count and disappear soon after parturition. It is generally held that in CNS mastitis only mild local signs are usually seen, such as slight swelling and changes in the milk appearance, but studies that have thoroughly investigated clinical characteristics of mastitis caused by CNS are very few. Jarp (1991) reported that clinical signs of CNS mastitis most often were subclinical or mild clinical, although severe clinical signs occasionally were recorded. Bleu *et al*. (2006) reported on three cases of toxic mastitis caused by staphylococci other than *S. aureus,* the status of coagulase production of the isolates was not reported.

Trinidad *et al*. (1990) studied histopathologic changes in 7 mammary glands of unbred heifers experimentally infected with *S. aureus* Newbould 305 (ATCC 27940), one quarter naturally infected with *S. aureus* and three quarters naturally infected with CNS. The quarters infected with *S. aureus* and CNS showed less alveolar, epithelial and luminal areas, more inter alveolar stroma and greater leukocyte infiltration compared with the uninfected quarters. In quarters infected

with CNS, histopathologic changes were not as marked as in quarters infected with *S. aureus*. Benites *et al*. (2002) studied histopathology of lactating dairy cows culled due to mastitis. The histopathologic changes of 99 quarters infected with CNS and 14 quarters infected with *S. aureus* mainly showed chronic inflammatory response or chronic inflammatory response with repair and no differences in the histopathologic changes were observed between *S. aureus* and CNS infected quarters.

EFFECT ON MILK

Compared with infections caused by other common Gram-positive mastitis pathogens, such as *S. aureus* and streptococci, the SCC in quarters infected with CNS was rather low. It is, however, about 10-fold higher than the SCC of healthy quarters, which typically remains under 50,000 cells per milliliter. In a study of Djabri *et al*. (2002), the geometric mean SCC in the CNS infected quarters was 1,38,000 cells per milliliter and in the *S. aureus* infected quarters 3,57,000 cells per milliliter. Although the mean SCC in CNS infected quarters is rather low, CNS infection can occasionally raise SCC markedly. In a study in which dairy cows were followed-up throughout the whole lactation, the geometric mean SCC was over 6,00,000 cells per milliliter in quarters with persistent CNS infection and about 60,000 cells per milliliter in healthy quarters (Taponen *et al*., 2007). In general, increases in milk SCC over 1,00,000 cells per milliliter are associated with reduced milk production. Elevated milk SCC theoretically results in less milk per animal going into the bulk tank. In studies on the effect of CNS IMI on milk production, a slightly decreased milk production has been reported (Grohn *et al*., 2004). Simojoki *et al*. (2009) reported that the mean peak SCC after an experimental challenge with *S. chromogenes* was 20,00,000 cells per milliliter. The *S. chromogenes*, *S. epidermidis* and *S. haemolyticus* showed increased SCC levels at 24, 48, 72 and 96 h after IMI. Coagulase negative staphylococci (C) species can increase the mice milk somatic cell count moderately but two to four fold increase was observed in *S. aureus* which indicated the subclinical nature of CNS infections in animals (Krishnamoorthy *et al*., 2014a). The *S. chromogenes*, *S. epidermidis* and *S. haemolyticus* can increase the mice viable bacterial count moderately but ten to fifteen fold increase was observed in *S. aureus* infected mice mammary gland (Krishnamoorthy *et al*., 2014b). Intramammary infection by CNS causes inflammatory reaction in the infected gland, which can be detected using various indicators for inflammation in the milk. Elevated concentrations of milk N-acetyl- β-glucosaminidase (NAGase) activity, antitrypsin and Serum Amyloid A (SAA) were reported during IMI caused by CNS. The *S. chromogenes*, *S. epidermidis* and *S. haemolyticus* induced haemato-biochemical changes in haemoglobin, WBC, neutrophil, glucose, total protein, AST and LDH, which may be used as indicators for diagnosis of CNS species mastitis in mice (Krishnamoorthy *et al*., 2015).

The normal variation in milk production of different cows is high and therefore large datasets are needed to verify significant difference in the effects on the milk production caused by IMIs with different mastitis pathogens. The CNS is the main cause of mastitis in cows in their first lactation, so it is likely to be responsible for possible production losses. The CNS mastitis seems to be concentrated in the higher producers and comparison of the milk production of cows with or without CNS mastitis may lead to an underestimation of production losses caused by CNS mastitis.

CURRENT APPROACHES IN MASTITIS DIAGNOSIS

California Mastitis Test (CMT): This assay indirectly measures the SCC in milk samples. A bromocresol-purple-containing detergent is used to break down the cell membrane of somatic cells and the subsequent release and aggregation of nucleic acid forms a gel-like matrix with a viscosity that is proportional to the leukocyte number. Advantages: Cost effective, rapid, user friendly and can be used 'On-site' or in the laboratory. Disadvantages: It can be difficult to interpret and has low sensitivity.

Portacheck: This assay uses an esterase-catalysed enzymatic reaction to determine the SCC in milk. Advantages: Cost effective, rapid and user friendly. Disadvantage: Low sensitivity at low SCCs.

Fossomatic SCC: This counter operates on the principle of optical fluorescence. Ethidium bromide penetrates and intercalates with nuclear DNA and the fluoresecent signal generated is used to estimate the SCC in milk. Advantages: Rapid and automated. Disadvantages: The device is expensive and complex to use.

Delaval cell counter: This counter operates on the principle of optical fluorescence, whereby propidium iodide is used to stain nuclear DNA to estimate the SCC in milk. Advantages: Rapid and the device is easily transportable. Disadvantage: Relatively expensive.

Electrical Conductivity (EC) test: This test measures the increase in conductance in milk caused by the elevation in levels of ions such as sodium, potassium, calcium, magnesium and chloride during inflammation. Advantage: Can be used 'On-site'. Disadvantage: Non-mastitis-related variations in EC can present problems in diagnosis.

Culture tests: Laboratory-based tests use selective culture to identify different microorganisms involved in causing mastitis. Advantage: Identifies specific pathogens causing mastitis. Disadvantages: Cannot be used 'on-site' and the waiting time for results can be days.

pH test: The rise in milk pH, due to mastitis is detected using bromothymol blue. Advantages: User friendly, cost effective and rapid. Disadvantage: Not as sensitive as other tests.

Enzymes: Assays are used to detect enzymes, such as N-acetyl β-D-glucosaminidase and lactate dehydrogenase. Advantage: Assays are rapid. Disadvantage: Assays might be laboratory-based.

RECENT APPROACHES

New biomarkers for mastitis and advances in relevant proteomics techniques such as two dimensional gel electrophoresis and mass spectroscopy have led to identification of several new proteins involved in mastitis. Six chaperones with a role in pathogen recognition were identified only in mastitis samples and therefore have potential as new markers for mastitis. Markers like prostaglandin D synthetase and proteins like serotransferrin and bovine serum albumin in milk can be used for diagnosis. Immunoassays such as ELISA can provide a reliable and inexpensive approach provided that suitable antibodies are available against specific inflammation related biomarkers or the causative microorganisms. The genome sequences of many of the major mastitis causing pathogens are now available and can be utilized to develop nucleic acid based testing methods such as PCR. Such tests are generally more expensive than immunoassays. However, they are highly sensitive and specific can be performed rapidly and can overcome the sensitivity and time constraints sometimes encountered with culture based tests and thus could complement or replace them in the long term. Multiplex PCR and real time PCR assay that can simultaneously detect different mastitis causing organisms in milk samples were developed (Viguier *et al*., 2009). A two tube multiplex PCR assay for simultaneous detection of 10 bacterial species like *S. aureus*, *S. chromogenes*, *S. epidermidis*, *S. scuiri*, *S. haemolyticus*, *S. simulans*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Escherichia coli* in milk was developed recently (Shome *et al*., 2011). The proinflammatory mediators showed increased expression of IL-1β, IL-4, IL-6, IL-12, TNF-α and IFN-γ in *S. haemolyticus* and *S. aureus*, IL-1β, IL-4 and IFN-γ in *S. epidermidis*, IL-4, IL-6, IL-12, TNF-α and IFN-γ in *S. chromogenes* infected mice and may be used as probable indicators for CNS species mastitis in animals (Krishnamoorthy, 2013).

TREATMENT

Spontaneous elimination rate of CNS mastitis is generally regarded as high. Some studies have demonstrated spontaneous elimination rates of as high as 60-70% for IMI caused by CNS. There was also evidence that CNS infections may persist for long periods in mammary gland (Chaffer *et al*., 1999). The strategies for treatment of mastitis vary among different countries. In some countries subclinical mastitis was treated during lactation, but in others subclinical and mild clinical mastitis such as CNS mastitis was left untreated or treated using conservative means such as frequent milking-out. Subclinical and mild clinical mastitis caused by CNS are often left untreated, the rationale being that CNS will be eliminated spontaneously. Not many treatment studies that separately report results for quarters infected by CNS have been published. Based on those available, it seems that mastitis caused by CNS responds well to antimicrobial treatment. Bacteriological cure ranges from 80-90% (Taponen *et al*., 2006). Treatment duration varies from 2-4 days in various studies. There was no consensus about the optimum duration of treatment of CNS mastitis. Subclinical mastitis can treated using antibiotics after doing antibiotic susceptibility testing for accurate use of antibiotics sensitive to the mastitis pathogens. The rate of transfer is directly proportional to the concentration gradient across the blood–milk barrier and the lipid solubility of the drug. The milk-to-plasma equilibrium concentration ratio of total (non-ionized plus ionized) drug is determined by (1) The degree of ionization of the drug, which is pKa/pH-dependent, in blood and milk, (2) The charge on the ionized moiety and (3) The extent of binding of plasma proteins and milk macromolecules. It has been shown that only the lipid-soluble, non-ionized moiety of a weak organic acid or base that is free (not bound to proteins) in the plasma can diffuse through the

cellular barriers and more readily reach the mammary gland. Majority of the antibiotics and the antibacterials are weak organic electrolytes, either acids (amoxycillin, ampicillin, cloxacillin, penicillin G, novobiocin and sulfanilamide) or bases (tylosin, lincomycin, spiramycin, erythromycin, trimethoprime, aminoglycosides and polymyxin B) tetracyclines are amphoteric compounds and chloramphenicol is neutral. Fluoroquinolones (enrofloxacin and pefloxacin) are lipophillic and possess low degree of ionization. Accordingly in normal lactating cows (milk pH 6.5-6.8) weak acids attain milk and plasma concentration ratio of less than or equal to 1 because of their ionization at normal blood pH whereas organic bases excluding aminoglycosides (streptomycin, gentamicin, kanamycin and neomycin) and spectinomycin attain milk to plasma ratio of greater than 1. Therefore, lipophilic bases are preferred antibiotics because of these attaining higher concentrations in milk. Although this favoured distribution decreases with increasing pH of mastitic milk, particularly for macrilides, but antibacterial activity of macrolides and aminoglycosides is not interfered. In the presence of mastitis, the pH of milk increases to within the range 6.9-7.2. As a consequence the ion trapping effect on lipophilic organic bases is reduced while the concentrations attained by weak organic acids are somewhat increased. The inflammatory reaction in udder tissues enhances the passage of penicillins into milk. The increased pH of milk does not affect the concentrations attained by amphoteric drugs (fluoroquinolones, tetracyclines and rifampicin), but antimicrobial activity of these drugs is lower in milk than in extracellular fluid or *in vitro* determination would predict. Intramammary route was accepted as the route of choice in the treatment of subclinical, chronic or mild clinical mastitis and as prevention during dry cow therapy. Intramammary administration permits delivery of the antibiotic directly to the mammary gland. However, Intra mammary preparations are used, often in conjunction with parenteral preparations (Sudhan and Sharma, 2010). Mastitis can be controlled by using effective dry cow therapy, consistent application of sanitary procedures during milking, selective culling of chronically infected cows and adequate treatment of clinical cases. Supportive treatment like use of anti-inflammatory drugs, fluid replacement and drugs to counter hypocalcemia and hypokalemia may be used.

Treatment of clinical mastitis due to CNS species sensitive to novobiocin: Penicillins and/or penethamate or cephalosporins (intramammary and/or parenteral route). Treatment of mastitis due to CNS resistant to novobiocin: The treatment is not necessary since spontaneous cures are seen. Antibiotic treatment in drying: Penicillin's and/or Penethamate or cephalosporin's. Sixteen isolates of CNS (*mecA* gene was identified in 15 isolates) were classified as methicillin resistant and 96 isolates as methicillin susceptible by disc diffusion and broth micro dilution for oxacillin susceptibility in 121 CNS isolates tested (Feβler *et al*., 2010). The prevalence of β-lactamase producing isolates varied markedly between CNS species and was significantly higher in *S. epidermidis* and *S. haemolyticus* (~40%), than in *S. simulans* and *S. chromogenes* where none or a few of the isolates produced β-lactamase. Resistance to more than one antimicrobial substance occurred in 9 and 7% of the clinical and subclinical isolates respectively (Waller *et al*., 2011). Persson *et al*. (2011) reported that 35% of CNS isolates were resistant to penicillin G and resistance to other antimicrobials was uncommon. In a study with 180 confirmed CNS species showed lowest susceptibility of 23.23% to penicillin in 310 milk samples screened in Dharwad region in Karnataka (Kaliwal *et al*., 2011).

A single isolation of CNS from a quarter does not economically justify antimicrobial treatment, in particular if only low numbers of bacteria are detected in the milk sample. CNS is common bacteria on the teat skin and can sometimes contaminate the milk sample. Furthermore, the spontaneous elimination rate of CNS infections without any treatment is relatively high. If moderate or severe clinical signs are evident, treatment can be recommended. Intra mammary treatment with antimicrobials can also be recommended for quarters with persistent CNS mastitis. Selection of antimicrobial drugs should be based on susceptibility testing.

CONCLUSION AND FUTURE RECOMMENDATION

The CNS has become the most common mastitis pathogens in many countries. The prevalence of CNS in subclinical and clinical mastitis was 6-72 and 6-30% respectively in various countries. The CNS species predominantly isolated from milk *S. chromogenes*, *S. epidermidis*, *S. haemolyticus*, *S. simulans*, *S. warneri*, *S. hominis*, *S. saprophyticus*, *S. xylosus*, *S. hyicus*, *S. sciuri* and *S. intermedius*, but vary between countries of the world. The CNS species were prevalent in milk samples from heifers and more prominently present in early lactation compared to later in lactation. Persistent intramammary infections were most common in quarter's infected with *S. chromogenes*, *S. epidermidis* and *S. simulans* indicating more relevance for bovine subclinical mastitis than other species. The *S. epidermidis* intramammary infections were found in multiparous cow and *S. chromogenes* in primiparous cows. The CNS mastitis mostly remains subclinical or shows only

mild clinical signs. CNS can cause persistent infections, resulting in increased milk SCC which affects milk quality and may be related to decreased milk production. Treatment choices must be based on sound scientifically based strategies. Infection rates, treatment success and milk quality parameters have to be monitored and reviewed regularly. Public and regulatory concerns for food safety and quality will have a greater influence on animal's disease treatment and drug availability in future. The CNS mastitis responds well to antimicrobial therapy.

Further studies in the field of identification of CNS species from cows are required and not as group identification. The systematic studies on CNS induced subclinical mastitis are necessary to control the bovine subclinical mastitis caused by CNS species. Comparative studies of CNS organisms with that of Staphylococcus aureus in various animal models (mice, rabbit, etc.) or in bovines are necessary to understand the basic mechanisms of host pathogen interaction using advanced molecular biology tools. In future, CNS organisms will be the most important pathogen in sub clinical mastitis which requires more attention in future in areas of diagnosis, treatment and control of mastitis in bovines.

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