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Review Article

# Review on Coagulase Negative Staphylococcus: Newly Emerging Mastitis Causing Pathogens in Ethiopia

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## Abstract

Mastitis is the most frequent and common disease of dairy cattle. Losses due to mastitis can be attributed to both subclinical and clinical disease. The aim of this seminar paper is mainly to review on newly emerging mastitis causing pathogens; CNS. Subclinical mastitis is considered the most economically important type of mastitis because of long term effects of chronic infections. CNS is the main causative agents of subclinical mastitis and they are Gram-positive cocci that inhabit both the outside and inside of infected udders. Often, they are called "opportunistic flora of the skin", because they can be isolated from the skin of the teat, the teat canal, vagina, and the coat and nostrils. The most common species of CNS are isolated from cases of bovine mastitis are *staphylococcus chromogenes, staphylococcus epidermitis, staphylococcus hyicus* and *Staphylococcus simulans*. The highest prevalence of intramammary infections with CNS was reported in Finland and its substantial economic loss has been reported by several authors in different parts of Ethiopian country. But in some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. The virulence factors in coagulase-negative staphylococci are not as clearly established as they are in *Staph. aureus*. Microbiological testing is the most important test for the diagnosis of mastitis control programmes and National policies and strategies for treatment of mastitis are different from country to country. Based on available reports, mastitis caused by CNS seems to respond well to antimicrobial treatment. Managing Environmental factors has been shown to be effective in controlling infections in the short term, but have been limited in controlling the disease long term. To prevent CNS mastitis at herd and cow levels, it is important to know the predisposing factors and Treatment of the animals during dry period and keeping the environment clean is recommended.

## INTRODUCTION

Mastitis is the most frequent and common disease of dairy cattle. Losses due to mastitis can be attributed to both subclinical and clinical disease. Clinical mastitis losses are generally readily apparent and consist of discarded milk, transient reductions in milk yield and premature culling. Subclinical mastitis is considered the most economically important type of mastitis because of long term effects of chronic infections [1].

Organisms such as coagulase negative staphylococci (CNS), environmental streptococci and Mycoplasma spp. are increasingly implicated in mastitis in dairy herds [2]. The genus is divided into coagulase-positive staphylococci and coagulase-negative staphylococci (CNS) based on their ability to coagulate plasma. One of the group of bacteria that cause mastitis is called coagulase negative Staphylococci (CNS). These bacteria are of great interest because they are currently the most commonly isolated microorganisms in cows and heifers in herd, and are currently considered emerging pathogens of bovine mastitis [3]. Coagulase-negative staphylococci have long been regarded as harmless skin commensals and dismissed as culture contaminants. Their important role as pathogens has been recognized only recently, and specific factors involved in pathogenesis are just now being explored [4].

More than ten different CNS species have been isolated from mastitis bovine milk samples, and the species most commonly reported are *Staphylococcus chromogenes* and *Staphylococcus simulans* [5,6]. *Staphylococcus hyicus* and *Staphylococcus epidermidis* have also frequently been isolated [7,8]. In routine mastitis diagnostics, CNS are normally not identified to species level but treated as a uniform group. CNS has traditionally been considered to be minor mastitis pathogens, especially in comparison with major pathogens such as *Staphylococcus aureus*, streptococci and coliforms. The main reason for this is that mastitis caused by CNS is very mild, and usually remains subclinical [9]. The significance of CNS, however, needs to be reconsidered as in many countries they have become the most common mastitis-causing agents [10,11]. Cows and heifers can be infected with CNS before calving [12,5,13].

In lactation, CNS infection is associated with an increased milk somatic cell count (SCC), which can result in economic losses due

to milk price penalties incurred for reduced quality. Increased SCC has also been shown to be associated with decreased milk production [14,15]. Post-milking teat dip is the most effective method of controlling this pathogen. The benefit of pre-dipping to control this organism is unclear. When teat dips are not used during the dry period, or during very cold weather, infections with CNS increase [16]. Coagulase-negative staphylococci have become increasingly resistant to antibiotics, the most recent threat being the emergence of strains with moderate levels of resistance to vancomycin [4]. Therefore, objective of this paper is to review on newly emerging mastitis causing pathogens (CNS).

## LITERATURE REVIEW

### Etiology and Epidemiology

An understanding of the causative agents of disease and their transmission is essential for sustainable disease control at local, national and international levels. Bovine mastitis typically results from infection of the mammary gland and associated tissues and is a consequence of successful colonisation, evasion of host defences and induction of marked and overt inflammatory changes. The infectious agents most associated with bovine mastitis are bacteria. A number of studies have indicated the range of bacteria capable of causing bovine mastitis. Watts described 137 distinct infectious agents linked to mastitis in cattle [17].

CNS are Gram-positive cocci that inhabit both the outside and inside of infected udders. Often they are called "opportunistic flora of the skin", because they can be isolated from the skin of the teat, the teat canal, vagina, and the coat and nostrils. This group of bacteria includes over 50 species and subspecies [3].

The most common species of CNS are isolated from cases of bovine mastitis are *staphylococcus chromogenes*, *staphylococcus epidermitis*, *staphylococcus hyicus* and *staphylococcus simulans*. Species such as *staphylococcus epidermitis*, *staphylococcus saprophyticus*, *staphylococcus simulans* and *staphylococcus warneri* belong to the normal bacterial flora of the teat skin, while other species such as *staphylococcus xylosus* and *staphylococcus scuiri* seem to come from the environment. *Staphylococcus chromogenes* may colonize the skin of the teat and other parts of an animal's body such as hair, the vagina and teat canal. It seems that there are differences in the pathogenicity of different species of CNS that are investigated by techniques of molecular diagnosis [18]. We found species with different antimicrobial susceptibility and diverse virulence factors of CNS isolated from bovine mastitis [3].

The incidence of new infections is highest during the cow's dry period and prior to calving; therefore, the percentage of quarters infected is high at the time of calving. The highest prevalence of CNS is in primiparous animals rather than in mature cows [19,20].

Many producers mistakenly believe that their heifers are healthy, and the presence of mastitis is not observed until calving. Future breeders represent future lactation and care for the udder is basic for ensuring the profitability of dairy farms. Many of the intramammary infections caused by CNS heal spontaneously and the prevalence decreases as lactation progress. Although CNS infections are usually mild or subclinical, it has also been shown that they can cause more severe and persistent processes, causing an increase in the somatic cell counts and a decrease in milk quality and production due to damage to breast tissue [21,3].

### Prevalence of CNS Mastitis

Some decades ago, CNS was seldom reported as a cause of mastitis, or they were classified as "secondary bacteria". In a study from the UK in the late 1970s, 1.7% of clinical mastitis cases were reported to be due to *Staph. Epidermidis* [22]. Gradually, CNS has become the predominant pathogen isolated from subclinical bovine mastitis in many countries [23].

In a study from Germany, 35% of quarters with subclinical mastitis harbored CNS. In Tennessee in the USA, the average proportion of CNS infections in high SCC herds was 28% [24], and herd prevalence ranged from 12% to 41%. In Dutch herds some CNS was isolated from 6% of quarters with bacterial growth in high SCC cows. In a study carried out in the US and Canada, 15% of new intramammary infections post-partum were due to CNS [25]. In an earlier Canadian study, quarter prevalence of CNS infections ranged from 5% to 6% during early lactation and increased from 14% to 17% towards the end of lactation. In a survey from Estonia, 16% of the quarter's positive for bacterial growth harbored CNS. The highest prevalence of intramammary infections with CNS was reported in Finland, where CNS was isolated from 50% of the quarter's positive for bacterial growth in a nationwide survey [10]. In a similar survey in Norway, the prevalence of CNS was 16% [26].

It is difficult to compare results from different countries because the number of colony forming units (CFU) per ml that is used as cut-off to categorize samples as CNS-positive varies between studies. In the Finnish survey with the high prevalence, detection of 500 CFU/ml was used to classify a sample as CNS positive, whereas the cut-off value in the Norwegian survey was 4000 CFU/ml. Use of a high CFU/ml cut-off for diagnosis of CNS infections may contribute to underreporting of CNS mastitis. The proportion of CNS among bacteria isolated from clinical mastitis cases remains very low in many countries. In a recent study from Canada, CNS was isolated from 6% of quarters with clinical mastitis [27]. In a Wisconsin study on milk samples from clinical and subclinical mastitis obtained between 1994 and 2001, the proportion of CNS isolates increased from 12.7% to 17.5%, but separate results were not provided for clinical mastitis [28].

In Sweden, CNS Comprised only 6% of bacteria isolated from clinical mastitis [29]. In Switzerland, the respective figure was 17% [30], and in Israel 9%. Among 77,051 routine mastitis samples submitted to laboratories in Finland during 2004–2006, CNS were the most frequently isolated bacteria in samples from clinical (18%) and subclinical (24%) mastitis cases [31]. In the practice area of the Faculty of Veterinary Medicine, University of

Helsinki, Finland, more than 20% of bacterial isolates from milk samples from clinical mastitis were CNS [32].

In a study on clinical mastitis carried out in the same area about 30 years ago, the proportion of CNS was only 6.5%. Seasonal differences in occurrence of CNS mastitis have been reported. In Finland, the prevalence of CNS and *Staph. aureus* mastitis was highest during winter and spring, i.e. during the indoor season [31]. In Norway, too, the highest prevalence of CNS mastitis was found during the late indoor season the proportion of CNS is generally high in samples collected from animals with subclinical mastitis but low in samples from animals with clinical mastitis. In countries where the biggest udder health problems are caused by major environmental mastitis pathogens, CNS infections may often be ignored [26].

CNS is important pathogens in cattle of all ages, but the predominant CNS species causing infection seems to differ between age groups. *Staph. chromogenes* was the major CNS species in pre-calving heifers and primiparous cows [5,33,9], whereas *Staph. simulans* was mostly isolated from cows in later lactations [9].Multiparous cows generally become infected with CNS during later lactation whereas primiparous cows usually already have the infection at the beginning of lactation [34.35].

**Status of the Disease in Ethiopia:** The disease and its substantial economic loss has been reported by several authors in different parts of Ethiopian country [36]. But in some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects [37,38].

## The Organisms

Until 1975, coagulase-negative staphylococci were grouped together as *Staph. albus* or *Staph.epidermidis*, distinguished from *staph. aureus* by their inability to clot blood plasma. Based on this characteristic and its presumed importance in virulence, coagulase-negative staphylococci were often referred to as a pathogenic staphylococcus. In 1975, Kloos & Schleifer extended the existing classification scheme by adding seven new species to the already known *Staph. epidermidis* and *Staph. saprophyticus* [39]. Today there are 32 coagulase-negative staphylococcal species; about 15 species are indigenous in humans, while the remainder is non-human pathogens [40].

## Pathogenesis

The mammary glands are skin glands, albeit large ones held exterior to the body cavity. Therefore, the mammary tissue forgoes the potential advantage of rigid skeletal support. This brings its own problems. Moreover, the udder of the present-day dairy cow is very large. Because when it is full of milk it is so heavy, damage of the udder and teats is very common. Because of these factors and also because its position exposes it to traumatic influences it is frequently the site of the disease [41].

**Virulence Factors of the CNS:** Virulence factors in coagulase-negative staphylococci are not as clearly established as they are in *Staph. aureus*. None of the major virulence factors or toxins of *Staph. aureus* has been found in coagulase-negative staphylococci, and it seems clear that development and persistence of coagulase-negative staphylococcal infections, which are so often associated with foreign materials, are due to different mechanisms [42].

**Plasmids and Transposons:** Most staphylococci contain a number of plasmids, some of which can be transferred by conjugation between different species (i.e. other coagulase-negative staphylococci or *Staph. Aureus* [43]. This seems to be an important mechanism for the spread of antibiotic resistance determinants, especially for aminoglycoside and beta-lactam resistance. Transposons can move resistance genes among plasmids and from plasmids to chromosomal locations in coagulase-negative staphylococci [42].

**Bacteriophages:** As in *Staph. aureus*, there are bacteriophages specific for coagulase-negative staphylococci. However, attempts to establish a phage typing system similar to that used to classify *Staph. aureus* have not found wide acceptance and have been superseded by modern genetic typing techniques (e.g. pulsed-field gel electrophoresis of chromosomal digests or PCR-based methods) [44].

**Surface Proteins:** Several cell wall proteins of staphylococci have been described, and specific bacterial binding mediated by these proteins to extracellular matrix molecules (i.e. fibrinogen, fibronectin, vitronectin, laminin, and collagen) has been observed [45]. However, the importance of these protein interactions in the pathogenesis of coagulase-negative staphylococcal colonization or infection remains to be demonstrated conclusively. Recently, electron microscopy has revealed a fimbria-like protein structure that may play a role in attachment of coagulase-negative staphylococci to foreign materials in the host.

In contrast to *S. epidermidis*, a number of proteins have been shown to be involved in pathogenesis of *Staph. saprophyticus* infections. A protein-hemagglutinin and surface fibrillar proteins have been associated with attachment to urinary tract epithelium, and invasion of the organism has been attributed to a urease [46].

**Capsular Polysaccharides:** Polysaccharides on the surface of coagulase-negative staphylococci almost certainly are major virulence factors involved in attachment and/or persistence of bacteria on foreign materials, but information still is relatively limited regarding their chemical nature and specific roles in pathogenesis. Recently, other investigators have described a number of polysaccharide components, but their chemical composition, mechanism of action, and relationship to one another remain unclear [47].

## Diagnosis

Diagnosis is necessary, if the quarters with high cell counts or that display clinical mastitis are detected, samples of milk should be taken aseptically and appropriately for subsequent processing

in laboratory. Microbiological testing is the most important test for the diagnosis of mastitis control programmes [18]. The Methodology includes the usual seeding in growth media specific for the major etiological groups. They are incubated at 37 with reading at 24 and 48 hours. Baird Parker Agar is a culture medium specific for staphylococci. It makes it possible to differentiate between CNS and staphylococcus aureus. The identification of the different species of CNS is important to determine their pathogenicity and to develop specific management practices to prevent mastitis [21].

**Isolation and Identification of CNS:** The isolation of coagulase-negative staphylococci from clinical specimens usually is not difficult, since staphylococci grow readily on commonly used media under a broad range of growth conditions. Initial identification is straightforward using conventional as well as automated and semi-automated methods. The thermo nuclease reaction is particularly useful for rapidly differentiating *Staph. aureus* (positive) from other staphylococcal species (negative) and is more accurate than tests based on coagulase production. Demonstration of resistance to novobiocin by disc diffusion testing is adequate for presumptive differentiation of *Staph. saprophyticus*. In general, it is not necessary to identify coagulase-negative staphylococci to the species level. For most of the isolates, the differentiation of *Staph. aureus*, *Staph. epidermidis* and *Staph. saprophyticus* probably is sufficient because no other clear-cut associations between specific clinical syndromes and coagulase-negative staphylococcal species have been confirmed [48].

Among the remaining staphylococcal species, *Staph. haemolyticus*, *Staph. lugdunensis*, and *Staph. schleiferi* have been isolated more often from serious human infections, especially native valve endocarditis. Therefore, it may be useful in certain situations to be able to identify a clinical isolate to the species level because the repeated isolation of the same strain supports its role as an etiologic agent [49,50].

## Clinical Characteristics and Effects on Milk Quality

CNS usually causes subclinical or mild clinical mastitis, but they have also been reported to produce severe local and systemic signs [51]. In a recent study, half of the intramammary infections due to CNS were clinical, but in the majority of the cases the signs were very mild [9]. No significant differences in the severity of clinical signs caused by the two most common CNS species were found in that study, which agrees with the findings of a previous study [51]. CNS infection is generally seen as an increase in the SCC in milk of the infected quarter. Milk SCC usually remains below 500,000 cells/ml [52].

In a study in which dairy cows were followed-up throughout the whole lactation, the geometric mean SCC was over 600,000 cells/ml in quarters with persistent CNS infection, and about 60,000cells/ml in healthy quarters [35]. Even a transient CNS infection caused a temporary increase in milk SCC, which is consistent with the report of. In a study analyzing the relationship between clinical mastitis and SCC patterns, a higher risk for occurrence of CNS mastitis in lactations with high average SCC was found [34].

The direct economic impact of high SCC depends on the violation of limits for poor quality milk or possible quality premiums paid for high quality milk. These differ considerably between countries. The current legal limit in the European Union (EU) is 400,000cells/ml but in the US it is as high as 750,000cells/ml, so increases in bulk milk SCC have a different effect in these regions. Many EU countries pay quality premium for milk with less than 250,000cells/ml [53].

In general, increases in milk SCC over 100,000cells/ml are associated with reduced milk production. Elevated milk SCC theoretically results in less milk per animal going into the bulk tank. The effect of CNS intramammary infections on milk production, a slightly decreased milk production has been reported [15]. Heifers with mastitis had a slightly higher genetic potential for milk production but their recorded milk yield was slightly lower than that of their healthy herd mates showed that multiparous cows with clinical CNS mastitis were originally higher producers than their herd mates without CNS mastitis. Milk production losses due to CNS mastitis could be underestimated if animals were compared with their herd mates rather than with their own pre-infection production levels or genetic potential [7].

CNS are usually mild infections and cause:- subclinical cases of mastitis, increase in SCC and can induce persistent clinical processes that do not respond to antibiotic treatment, milk appearance is normal, but it can induce intramammary infections with alteration in milk, high prevalence in primiparous animal (especially in the time around calving), higher incidence of new infections in cows' dry period, the general state of the animal is not usually affected, nor is severe systemic sign and high spontaneous cure rate [21].

## Treatment

**Anti-Microbial Treatment of CNS Mastitis:** National policies and strategies for treatment of mastitis are different from country to country. In some countries, subclinical mastitis is treated with antimicrobials during lactation. In other countries, subclinical and mild clinical mastitis cases, including most CNS mastitis cases, are left untreated or they are treated using non-antibiotic means such as frequent milking-out. Based on available reports, mastitis caused by CNS seems to respond well to antimicrobial treatment. Bacteriological cure ranges from 80% to 90% [9].

Cows with higher parity have significantly lower tendency to cure [54,55]. Treatment duration varied from 2 to 4 days. There is no consensus about the optimum duration of treatment of CNS mastitis. According to a recent study, extending treatment length to 8 days did not improve cure rates of subclinical CNS mastitis, as compared with treatment of 2 days. The cure rate of CNS mastitis was 44% without treatment. Higher chances of cure were observed in groups treated with pirlimycin but the difference between groups was not statistically significant. CNS mastitis generally responds well to antimicrobial therapy and

that the customary antimicrobial treatment duration of 2–3 days can be used for CNS mastitis [55].

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 [9].

Intramammary treatment with antimicrobials can also be recommended for quarters with persistent CNS mastitis. Selection of antimicrobial drugs should be based on susceptibility testing. If penicillin G is the treatment of first choice, beta-lactamase production can be determined by a rapid nitrocephin test to assess penicillin sensitivity of isolates. Nitrocephin tests were recently shown to be sufficiently reliable to be recommended for routine clinical use to test beta-lactamase production of mastitis staphylococci [56]. For persistent CNS infections, antimicrobial treatment at drying-off remains a good tool, as cure rates of dry cow therapy are generally very high for CNS infections [57].

### Prevention and Control measures

Traditionally mastitis control programs have mainly focused on management. Managing Environmental factors has been shown to be effective in controlling infections in the short term, but have been limited in controlling the disease long term. To the animal breeder, the aim has always been to take care of long-term needs. For CNS mastitis, as for all other types of mastitis, prevention is the key to combating the problem. The 5-point control plan had a major impact on both the rate of new infection and the causative agents of mastitis [58].

They are: -

- The use of antibiotic treatment on all clinical cases (to reduce the duration of infection).
- The use of blanket dry cow therapy (to eliminate any residual unapparent infections present at the end of lactation and to protect the gland from infection during the early dry-period).
- Culling of persistently infected cows (in an attempt to remove chronically infected and highly susceptible animals from the herd).
- Dipping of milked (susceptible) teats in disinfectant (to prevent "invasion" by bacteria deposited on the teat during (or immediately after milking).
- Correct maintenance and use of the milking machine (to reduce the possibility of transferring any milk harbouring infectious agent between dairy cows).

However, more knowledge and experience is needed to find the most effective strategies for prevention of CNS mastitis. CNS has long been regarded as opportunistic skin micro biota that occasionally can cause mastitis [59].

The main focus of mastitis control in the 1970s, 1980s and 1990s was on the contagious major pathogens Streptococcus agalactiae and *Staph. aureus*. In early studies, staphylococci that could be distinguished from *Staph. aureus* on the basis of colony morphology and the coagulase test were generally classified as Staph. epidermidis or "other micrococci" [23].

Control measures against contagious mastitis pathogens such as post-milking teat disinfection reduce CNS infections in the herd [60]. Discontinuation of teat dipping significantly increased prevalence of infections with C. bovis and CNS. In most herds, pregnant heifers are more likely to be infected with CNS than cows. In solving CNS mastitis problems, focus should therefore be on the heifers, i.e., their environment, feeding and management, before calving [61]. Worldwide, farmers have achieved tremendous success in reducing the incidence of contagious mastitis by adopting the 5 basic principles of mastitis control: post milking teat disinfection, universal dry cow antibiotic therapy, appropriate treatment of clinical cases, culling chronically infected cows and regular milking machine maintenance. Control measures must be applied in cows in lactation, in dry cows and also breeder heifers [62].

Rebreeding can be a source of infection on a dairy farm, particularly under the current management system, where heifers are transported and mixed several times before coming to the dairy farm where they will give birth. Generally, not much attention is given to heifers on farms or to cows during the dry period. But if we consider that the heifers are approximately one third of the herd each year, and that together with the dry cows they are the farm's investment for the future, the health of udders and proper functioning of heifers and dry cows should be a number one priority. Control measures should lower the animas' contact with mastitis causing agents before calving [63].

**Handling:** Separate the heifers in individual pens: do not allow them to suckle each other, because this transmits bacteria and causes persistent infections that become established early in the life of the animal [63]. Do not feed lactating heifers with infected milk: avoid transmission of infectious agent from the adult cows to young cows, separate the heifers from the cows before calving and provide clean areas for the cows to calve and for heifers [16].

**Environment:** Control of flies: flies can be vectors of pathogenic agents and also create a lesion on the teat tip, which allows bacteria such as staphylococcus aureus or CNS to become established on the skin of the teat and enter its orifice [64].

## CONCLUSION

CNS has become the most common mastitis pathogens in many countries. 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. The economic impact of the increase in bulk milk SCC depends on the regulatory limits for milk SCC and quality premiums for milk with low SCC in individual countries. CNS mastitis responds well to antimicrobial therapy. Staph. simulans and Staph. chromogenes are probably the predominant CNS species in bovine mastitis. The knowledge on CNS species involved in mastitis is still very limited and benefits would accrue from having more reliable diagnostic methods for species identification. It is important to determine the predisposing factors for CNS mastitis at herd and cow levels. Efficient strategies for prevention of CNS mastitis can then be designed.

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