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Review on Newly Emerging Mastitis Caused by Coagulase Negative Staphylococcus

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Abstract: Mastitis is the most frequent and common disease of dairy cattle. It is caused by coagulase-positive and coagulase-negative staphylococcus. Coagulase-negative staphylococci (CNS) are a frequent cause of bovine intramammary infections (mastitis) in modern dairy herds. They have become the most common bacteria isolated from milk samples in many countries. Mastitis caused by CNS in most cases remains subclinical, or the clinical signs are mild. For some reason, heifers and primiparous cows are most susceptible to CNS mastitis. CNS mastitis increases milk somatic cell count (SCC) in the infected udder quarter. The increase in milk SCC is usually moderate compared with mastitis caused by many other common pathogens, including *Staphylococcus aureus* and streptococci. However, high prevalence of CNS mastitis in a herd can affect the herd bulk milk SCC. CNS comprises almost 50 different species of staphylococci. In mastitis diagnostics they are differentiated from the coagulase-positive mastitis pathogen *S. aureus* using coagulase test. CNS are not identified further by species but are treated as a uniform group. Many different CNS species have been isolated from bovine milk. Many CNS species can also be isolated from cows' hair coat, udder skin and teat canals and are therefore often considered to be opportunistic skin organisms rather than real mastitis pathogens. CNS mastitis is generally expected to be eliminated spontaneously and is commonly left without any antimicrobial treatment.

Key words: CNS · Coagulase Test · Mastitis · Milk · SCC

INTRODUCTION

Livestock represents a major national resource and forms an integral part of agricultural production system. Dairy production is a biologically efficient system that converts feed and roughages to milk [1]. Milk is a very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals. The increase in human population, accessibility to technology input, high demand for animal products and purchasing power in urban center had helped the urban and per urban dairy farms in the country to flourish [2].

FAO [3] estimates that 42% of the total cattle herds, for the private holdings are milking cows. In Ethiopia cows represent the largest population of cattle production of the country. Capital consumption of milk in Ethiopia is as low as 17 kg per head while the average figure for Africa is 26 kg per head [4].

However, milk production often does not satisfy the country's milk requirements due to a multitude of associated factors. The quality and quantity of milk production deteriorate due to biological causes including the low genetic potential of the animals, poor nutrition and prevalence of diseases [5].

Mastitis is among the factors contributing to reduced milk production [6] and it is among the most important diseases in dairy animals with worldwide distribution [7]. Mastitis is an inflammation of the mammary gland characterized by physical, chemical, bacteriological and cytological changes in milk. Pathological changes in glandular tissues of the udder and effects on the quality and quantity of milk have been observed [8-10]. This disease is mainly caused by microorganisms usually bacteria, including Gram-negative and Gram-positive bacteria, mycoplasmas, yeasts and algae [11, 12].

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The three categories of mastitis pathogens are contagious, environmental and opportunistic. The Contagious pathogens, like *Staphylococcus aureus* and *Streptococcus agalactiae,* are normally transmitted from cow to cow, often in the milking parlor [13]. Environmental pathogens, like *Escherichia coli* and *Streptococcus uberis*, infect the mammary gland from the environment, with sources such as soil and bedding [14]. Finally, opportunistic pathogens, coagulase-negative staphylococci (CNS) and *Corynebacterium bovis*, naturally reside on the teat skin and can be transferred into the mammary gland during milking or injury [15].

In mastitis diagnosis, staphylococci are divided into Coagulase positive and coagulase negative *Staphylococcus* (CNS) on the basis of the ability to coagulase rabbit plasma. In diagnostics of bovine mastitis, the clarification has been considered adequate because CNS usual course is subclinical or only mild clinical mastitis. Hence, it is consider as minor pathogen dairy herds in different small holder farms as well as large farms [16-18]. Mastitis is a serious concern to both milk producers since the infection can lead to Considerable economic losses due to reduction in milk yield, decreased milk quality and treatment costs [19, 20].

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 [21]. The costs caused by sub-clinical mastitis are mainly due to loss of milk production, reduced payment caused by lower milk quality and costs for culling and replacement of cows [22-24]. Production losses due to subclinical mastitis on U.S. dairy farms were estimated to cost the dairy industry $1 billion dollars annually [25].

Coagulase Negative Staphylococci (CNS) and *Corynebacterium bovis*, two other highly prevalent pathogens, are historically considered to be of limited importance and are therefore often described as minor pathogens. The impact of CNS is increasing [26], probably because prevalence of major pathogens is decreasing [27].

CNS have become the predominant group of bacteria associated with bovine intra-mammary infections (IMI) worldwide and are regarded as emerging mastitis pathogens [28]. So far more than 15 CNS species have been identified in association with bovine IMI, but the prevalent species are *Staphylococcus haemolyticus*, *S. Chromogenes*, *S. simulans*, *S. epidermidis*, *S. xylosus* and *S. hyicus* [29, 30].

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 [31]. The significance of CNS, however, needs to be reconsidered as in many countries they have become the most common mastitis-causing agents [32, 33]. Cows and heifers can be infected with CNS before calving [34-36].

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 [37-38]. CNS produces few virulence factors; however, they can cause infections in healthy host tissue. They are opportunists and adhere to metal devices to produce a protective biofilm. Production of biofilm reduces the organism's susceptibility to antimicrobials [39, 40]. Widely used antibiotics including penicillins, particularly semi-synthetic penicillins, cephalosporins, macrolides, aminoglycosides and tetracyclines are ineffective to control CNS [41, 42].

Mastitis caused by CNS is an important factor that limits dairy production. These bacteria are of great interest because they are currently the most commonly isolated microorganisms in cows and heifers in herds. These microorganisms are currently considered as emerging pathogens of bovine mastitis and have economic importance [28]. Even though the study was conducted on coagulase negative staphylococcus in lactating dairy cows around the globe, still there is limited knowledge on the pathogenesis, virulence factor and occurrence of CNS in different parts of the world including Ethiopia. Therefore the objective of this review is to review on newly emerging mastitis caused by coagulase negative staphylococcus.

Coagulase Negative Staphylococcus

The Disease: There are different bacterial diseases those responsible for udder inflammation. One of the most important is Coagulase Negative Staphylococci (CNS) is highly prevalent pathogen and historically considered to be of limited importance. Therefore, often described as minor pathogens. The impact of CNS is increasing, probably because prevalence of major pathogens is decreasing [27]. It has become the predominant group of bacteria associated with bovine intra-mammary infections

(IMI) worldwide and are regarded as emerging mastitis pathogens [28].

Etiology Until 1975, coagulase-negative staphylococci were grouped together as *S. albus* or *S.epidermidis*, distinguished from *S. 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 apathogenic staphylococci. In 1975, Kloos and Schleifer [43] extended the existing three classification scheme by adding seven new species to the already known *S. epidermidis* and *S. Saprophyticus*. Today there are 50 coagulase-negative staphylococcal species; about 15 species are indigenous in humans, while the remainder is non human pathogens [44].

It is 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 [28]. This group of bacteria includes over 50 species and subspecies. The most common species of CNS are isolated from cases of bovine mastitis are *Staphylococcus chromogenes, S. epidermitis, S. hyicus* and *S. simulan*s. Species such as *S. epidermitis S. saprophyticus, S. simulans* and *S. warneri* belong to the normal bacterial flora of the teat skin, while other species such as *S. xylosus* and *S. sciuri* 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 [45]

Epidemiology: 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 [11, 33, 46, 47]. Some authors even have found that cows with this form of mastitis had greater milk production than cows with no udder infection [48].

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 [32]. In a similar survey in Norway, the prevalence of CNS was 16% [49].

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 [50].

Clinical Signs: 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. [51] reported that clinical signs of CNS mastitis most often were subclinical or mild clinical, although severe clinical signs occasionally were recorded. One recent study [52] was reported on three cases of toxic mastitis caused by staphylococci other than *S. aureus*. Unfortunately, status of coagulase production of the isolates was not reported. In a pilot study, in which five lactating cows were experimentally infected with *S. choromogenes*, the concentrations of different inflammation parameters in milk were 10 to 100 times lower than in an experimentally induced *Escherichia coli* (*E. coli*) mastitis and the clinical signs were very mild [53].

The quarters infected with CNS showed less alveolar, epithelial and luminal areas, more interalveolar stroma and greater leucocyte infiltration compared with the uninfected quarters. In quarters infected with CNS, histopathologic changes were not as marked as in quarters infected with *S. aureus*. [54] studied histopathology of lactating dairy cows culled due to mastitis.

Virulence Factors: Even though more researchers have realized the importance of CNS intramammary infections, the virulence factors of CNS remain poorly understood. CNS strains also produce several toxins and enzymes that could contribute to virulence, such as haemolysin, leucocidin, lipase, proteases and Dnase [55-57]. Many CNS strains isolated from mastitis samples had higher protease, DNase and lecitinase activity than that of CNS from normal cows [58-60]. However, the roles of these enzymes on the pathogenesis of CNS are unclear.

Pathogenesis: Virulence factors in coagulase-negative staphylococci are not as clearly established as they are in *S. aureus*. None of the major virulence factors or toxins of *S. aureus* has been found in coagulase-negative

Table 1: Interpretation of CMT findings.

No.	Interpretation Visible reaction
0	Negative Milk fluid and normal
T	Trace Slight precipitation
1	Weak positive Distinct precipitation but no gel formation
2	Distinct positive Mixture thickness with gel formation
3	Strong positive Viscosity greatly increased strong gel i.e. cohesive with a convex Surface.

Source: [71]

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 [61].

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 *S. aureus*) [62]. 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 [61].

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. pulsedfield gel electrophoresis of chromosomal digests or PCR-based methods) [63].

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 [64]. 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 four protein structure that may play a role in attachment of coagulase-negative staphylococci to foreign materials in the host [65]. In contrast to *S. epidermidis*, a number of proteins have been shown to be involved in pathogenesis of *S. 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 [66].

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 [67]. Recently, other investigators have described a number of polysaccharide components, but their chemical composition, mechanism of action and relationship to one another remain unclear [68, 69]. Diagnosis.

Mastitis is diagnosed by Physical examination of udder and milk, California mastitis test and Microbiological testing. The udders of the study cows were examined visually and by palpation for presence of clinical mastitis. During examination attention was paid to cardinal signs of inflammation, symmetry, size and consistency of udder quarters. In addition the milk was examined for any abnormalities using strip cup [70]. The California mastitis test (CMT) was conducted to diagnose the presence of subclinical mastitis and it was carried out according to procedures given by [71]. Based on the thickness of the gel formed by CMT reagent and milk mixture, test results were scored as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Milk samples with test result of CMT 1 to 3, were classified as evidence of subclinical mastitis [71, 70].

Microbiological testing is the most important test for the diagnosis of mastitis control programmes [45, 72]. The Methodology includes the usual seeding in growth media specific for the major etiological groups. They are incubated at 37°C 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 *S.aureus*. The identification of the different species of CNS is important to determine their pathogenicity and to develop specific management practices to prevent mastitis [73].

Isolation and Identification of Coagulase-negative Staphylococci: It is not necessary to identify coagulase-negative staphylococci to the species level. For most of the isolates, the differentiation of *S. aureus*, *S. epidermidis* and *S. saprophyticus* probably

Table 2: Differentiation of mastitis causing Staphylococcus

Test	S.aureus	CNS
Catalase	+	+
Oxidase	-	-
Coagulase	+	-
Hemolysis	+	-
Mannitol(A)	+	-

Note: + = positive reaction, =negative reaction, A= acid reaction
Source: [71]

is sufficient because no other clear-cut associations between specific clinical syndromes and coagulase-negative staphylococcal species have been confirmed [74]. Among the remaining staphylococcal species, *S. haemolyticus*, *S. lugdunensis* and *S. 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 [75-76].

Coagulase Test: The tube coagulase test was performed in sterile tubes by adding 0.5 ml of selected isolates of Staphylococcus grown on Tryptone Soya Broth (TSB) at 37°C for 24 hrs to 0.5 ml of citrated rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 ml of sterile TSB and 0.5 ml of rabbit plasma. Clotting was evaluated at 30 min intervals for the first 4 hrs of the test and then after 24 hrs incubation. The reaction was considered positive if any degree of clotting from a loose clot to a solid clot that is immovable when the tube is inverted (tilted) was visible within the tube and no degree of clotting would be taken as negative [78].

Anti-Microbial Treatment of CNS Mastitis: Based on available reports, mastitis caused by CNS seems to respond well to antimicrobial treatment. Bacteriological cure ranges from 80 to 90% [33]. Cows with higher parity have significantly lower tendency to cure [26, 78]. 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 penicillin 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 [78]. Intramammary treatment with antimicrobials can also be recommended for quarters with persistent CNS mastitis. 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 [79, 80].

Prevention and Control Measures: Control measures against contagious mastitis pathogens such as post-milking teat disinfection reduce CNS infections in the herd [81]. Discontinuation of teat dipping significantly increased prevalence of infections with 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 [82]. 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 [83].

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 mast it is causing agents before calving [84].

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 [84]. 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 [85].

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 *S. aureus* or CNS to become established on the skin of the teat and enter its orifice [86].

Teat Disinfection: A post-milking teat dip is effective against CNS IMI and may be the most important step in CNS IMI prevention; pre-milking teat dips have been shown to be ineffective at preventing CNS IMI [87-89]. The combination of pre- and post-milking teat dips can reduce incidence of IMI by 40.9% compared to quarters only post-dipped [90] also demonstrated post-milking teat dips eliminated residual CNS on the teat skin, in addition to other contagious mastitis pathogens.

Status of Coagulase-negative Staphylococci Mastitis in Ethiopia: Ethiopia holds large potential for dairy development due to its large cattle population and favorable climate for improved high yielding animal breeds. However, among many factors mastitis, which incurs serious economic losses to the dairy industry [91]. In Ethiopia, knowledge about the CNS involved in mastitis is limited, but different researchers gave different results. According to several studies conducted in Ethiopia, the highest prevalence of CNS intramammary infection occurs in heifers around parturition [6, 91-94].

[95] and [96] who reported the prevalence of CNS in sub clinical was 28.75 and 12.01% which indicates higher prevalence than CNS from clinical mastitis in which 1.33 and 3.53% in Holeta and in Addis Ababa dairy farms, respectively.

In the study made by [97] which indicated that CNS were not isolated from clinical mastitis, but 51.9% of CNS were isolated mainly from sub-clinical mastitis in Bahir Dar. Also [98] reported 11.93% in Addis Ababa dairy farms. In addition, [99] reported 18.7 and 31.7% of CNS in Adama and Holeta town, respectively. So, there is a serious problem of coagulase-negative staphylococci mastitis in Ethiopia.

CONCLUSIONS

CNS is becoming the most common mastitis pathogens and a major health problem of dairy cows in many countries of the globe. It 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. It is recognized that multiple environmental and managemental factors plays a major role in the occurrence of this pathogen. The economical 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. It responds well to antimicrobial therapy. *S. simulans* and *S. 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. Even though different researches were conducted, a serious problem of coagulase-negative staphylococci mastitis is dormantly circulating in Ethiopia.

Based up on the above conclusion the following recommendations are forwarded: - It is important to determine the predisposing factors for CNS mastitis at herd levels; a serious attention should be given to the mastitis infection caused by CNS; the milkers should wash his/her hands before and after milking; other efficient strategies like separation of heifers in individual pens; limiting to feed lactating heifers with infected milk and cleaning the environment for the cows to calve should be designed for prevention of CNS mastitis; treat the animals during dry period and clean the environment and in Ethiopia, knowledge about the coagulase negative staphylococci should be improved.

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List of Abbreviations:

CFU: Colony forming unit
CNS: Coagulase-negative staphylococcus;
FAO: Food and Agricultural;
PCR: Polymerase chain reaction;
SCC: Somatic cell count;
TSB: Tryptone soya broth

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