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Prevalence and risk factors of bovine mastitis caused by *Staphylococcus aureus*

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Abstract

Mastitis is the greatest prevalent dairy farm animal's disease, imparting vast economic losses to the dairy industry. Mastitis in milking cows is caused by pathogenic bacteria, occasionally yeast, mycoplasma and even algae. The common pathogens comprise contagious bacteria, *Staphylococcus aureus* and *Streptococcus agalactia* e, coli forms and some species of streptococci that are commonly present in the environment and may reach the teat end from that source. The most common pathogenic bacteria that attack mammary gland are Staphylococcus aureus, which is one of the most frequently isolated pathogens in bovine intra mammary infections worldwide and the most common contagious mastitis pathogen isolated from the raw milk. The genus currently comprises 32 species and15 serotypes, many of which are found on humans. Staphylococcus species are prevalent food borne bacterial pathogens that cause food poising in human, when ingested with contaminated foods including dairy products, like cheese and yoghurt. Further transfer of antimicrobial resistant bacteria to humans via the food chain has been reported. In this context, Staphylococcus present in milk may serve as a reservoir for human infections, thus allowing these microorganisms to persist and spread in the community. The exposure to antimicrobials is a risk factor for acquisition and spread of resistance strain of *S.aureus* in humans and most probably also in animals. Different studies conducted in different parts of Ethiopia showed variable prevalence of mastitis depending on the type of farm and managements systems ranging from 34.9 to 71%.

Keywords: Mastitis, Prevalence, Risk factors, *Staphylococcus aureus*

1. Introduction

Mastitis is the greatest prevalent dairy farm animal's disease, imparting vast economic losses to the dairy industry. Mastitis in milking cows is caused by pathogenic bacteria, occasionally yeast, mycoplasma and even algae. The great majority of infections are caused by species belonging to the genera Staphylococci, Streptococci, and several gram-negative bacteria. The common pathogens comprise contagious bacteria (S. aureus and S. agalactiae), coli forms and some species of streptococci that are commonly present in the environment and may reach the teat end from that source (Radostits *et al.*, 2006).When pathogenic microorganisms enter the udder

through the teat canal, induces overcome the cow's defense system, beginning to reproduce in the udder, and produce toxins that are dangerous to the mammary gland. The mammary tissue is being then damaged, which causes increased vascular permeability. Because of this; milk composition is altered, there is leakage of blood constituents; serum proteins, enzymes, and salts into the milk; decreased synthesis of caseins and lactose; and decreased fat quality (Christel, 2009).
The most common pathogenic bacteria that attack mammary gland are Staphylococcus aureus, which is one of the most frequently isolated pathogens in bovine intra mammary infections (IMI) worldwide (Tenhagen *et al*., 2006) and the most common contagious mastitis pathogen isolated from the raw milk (Olde *et al*., 2006).The CDC (Centers for Disease Control) estimates that 240,000 cases of Staphylococcal Food Poisoning (SFP) occur each year in the US, leading to hospitalization in 1000 cases and to six deaths (Scallan *et al*., 2011). Staphylococcus species are initially differentiated by the coagulase test; a positive test is differentiated by clot formation in plasma due to production of enzyme coagulase. Coagulase-producing (coagulase-positive) staphylococci are Staphylococcus aureus, Staphylococcus intermedius, and some strains of Staphylococcus hyicus. Members of these subgroups produce phosphates and are distinguished from Staphylococcus aureus by not forming acid from Mannitol; they are separated by acid production from maltose and acetone from glucose. Various strains of S.aureus have become noted for their resistance to several antimicrobial agents (Frank *et al*., 2008).The genus currently comprises 32 species and15 serotypes, many of which are found on humans.

2. Literature review

2.1. General Characteristics of Staphylococci

2.1.1. General characteristics of *Staphylococcus aureus* organisms

The genus currently comprises 32 species and15 serotypes, many of which are found on humans.

The species most commonly associated with human disease are S. aureus (the most virulent and best-known member of the genus), S. epidermidis, S. saprophyticus, S. capitis, and S. haemolyticus (Murray *et al*., 2002). S. aureus is the only species found in humans that produces the enzyme coagulase, which differentiates it from the other species coagulase-negative staphylococci (Brooks *et al*., 2001).

Staphylococci microorganisms are gram-positive, spherical cells, non-motile, non-spore forming, aerobic and facultative anaerobic, about 1 µm in diameter arranged in irregular clusters. Single cocci, pairs, tetrads and chains are also seen in liquid cultures and clinical material. The Staphylococci were first described by the Scottish surgeon, Sir Alexander Ogston as the cause of a number of pyogenic infections in humans named Staphylococcus (Greek: Staphyle, bunch of grapes; coccus, a grain or berry), after their appearance under the microscope (Adams and Moss, 2000). *S. aureus* causes inflammation of the mammary gland in bovines (Quinn *et. al.*, 2002) and the annual revenue loss on account of mastitis in India works out to be Rs 60532.1 million. *S. aureus* is one of the most frequently isolated pathogens in bovine intra mammary infections (IMI) worldwide (Tenhagen *et. al.*, 2006).

The most important staphylococcal species which affects mammary glands is *S. aureus*,which is named for its yellow-pigmented colonies (aureus = golden) as the result of the carotenoid pigments that form during their growth. Colonies on solid media are round, smooth, raised, and glistening. *S. aureus* usually forms grey to deep golden yellow colonies (Howard and Kloos, 1987). *Staphylococcus epidermidis* colonies usually are grey to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. Various degrees of haemolysis are produced by *S. aureus* and occasionally by other species. Pepto-streptococcus species, which are anaerobic cocci, often resemble staphylococci in morphology (Murray *et al*., 2002).

When compared to other mastitis causing bacteria, Staphylococcus species are initially differentiated by the coagulase test; a positive test is a clot formed in plasma due to production of enzyme coagulase. Coagulase-producing (coagulase-positive) staphylococci are *Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus delphini, Staphylococcus lutrae,* and some strains of Staphylococcus hyicus. Members of these subgroups produce phosphates and are distinguished from Staphylococcus aureus by not forming acid from Mannitol or coagulating plasma; they are separated by acid production from maltose and acetone from glucose. Various strains of S. aureus have become noted for their resistance to several antimicrobial agents (Frank *et al*., 2008).

2.1.2. Food safety indication

Food spoilage through microbial activity is a huge economic problem worldwide, whichis approximately one fourth of the world's food supply, is lost. Raw milk and its derivative consumption are common in Ethiopia (Zelalem, 2003). This is not safe from consumer's health point of view. It may lead to the transmission of various diseases. Besides, poor yield and spoilage of products can cause by bacteria (Jhalka *et al*., 2014) Staphylococcal food-borne disease (SFD) is one of the most common food-borne diseases worldwide resulting from the contamination of food by preformed *S. aureus* entero-toxins. Although several Staphylococcal enterotoxins (SEs) have been identified, SEA, a highly heat-stable SE, is the most common cause of SFD worldwide.

2.1.3. Milk and milk products commonly associated with staphylococcal food poisonings

Staphylococcus species are prevalent food borne bacterial pathogens that cause food poising in human, when ingested with contaminated foods including dairy products, like cheese and yoghurt. The organism can gain access to raw milk and milk products either by direct excretion from udders having clinical and subclinical Staphylococcal mastitis or by contamination from food handlers. Potential transmission of staphylococcal food poisoning to consumers via milk of cows affected by subclinical mastitis, mainly when raw milk is ingested. A two-hundred and eight samples of milk from individual cows showing subclinical mastitis, and 37 samples of bulk tank milk from dairy farms located in São Paulo, Brazil were analyzed and S. aureus strains were detected in 18 (7.3%) milk samples: 14 (6.7%) from samples of individual cows, and 4 (10.8%) from bulk tank milk (Fagundes *et al*., 2010).

(Martin *et al*., 2004) found out that 15% of 157 Staphylococcus isolate from dairy products were enterotoxigenic, whereas Morandi *et al*., (2007) reported that 28.6% of Staphylococci isolated from milk and dairy products were enterotoxin producers. From the 200 Staphylococcus species isolates, 116 originated from American and colonial cheeses and 84 from colonial sausage (Pelisser *et al*., 2009).

2.1.4. Staphylococcal infection

Many different foods can be a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning, including milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings (Bergdoll, 2006). In one case, cheese was involved in an outbreak because it had been made from milk contaminated after pasteurization and before inoculation with lactic starter culture. In this particular case, the starter culture did not grow properly, resulting in a fermentation accident that allowed the *S. aureus* strain to develop and produce SE (Bergdoll, 2006). In 1985, chocolate milk was the origin of a staphylococcal food poisoning in Kentucky, USA. This chocolate milk was contaminated and stored at too high temperature for 4 to 5 h, before pasteurization. Pasteurization killed the staphylococci but had no effect on the SEs. In the case of canned foods that have been correctly processed, bacteria and SEs are usually destroyed. As etiology of a disease Brooks *et al*. (2001) indicated that from five species Staphylococcus considered as potential human pathogens, S. Aureus, S. Epidermidis, S. Saprophiticus, were the most common isolates of food and milk products. S. aureus is often considered to be the most problematic etiology of food poisoning of the three pathogens and is distinguished from the other two species by being the only one able to coagulate plasma (considered as coagulase +). It can produce a range of toxins including enterotoxins (food poisoning), cytotoxins (general systemic toxins), and toxic shocks per-antigens.

2.1.5. Staphylococcal enterotoxins

Staphylococcal enterotoxins are a group of single chain, low-molecular weight (27,000-34,000) proteins produced by some species of Staphylococci, primarily *S. aureus,* but also by *S. intermedius, S. hyicus, S. xylosus* and *S. epidermidis*. To date, 14 distinct enterotoxins have been identified based on their antigenicity and they have sequentially been assigned a letter of the alphabet in order of their discovery. The temperature range for enterotoxin production varies with the medium, but in general the minimum temperature for production is 10°C and the maximum is 45°C(Bergdoll, 2006)

There are variations with the different enterotoxins and with different media. Most strains of Staphylococci will grow at pH values between 4.5 and 9.3, with the optimum being 7.0–7.5; however, the conditions for enterotoxin production are more restricted than for growth. Most experiments have been done by adjusting the medium to a specific pH value with no attempt to control the pH during incubation (Bergdoll and Lee Won, 2006). Staphylococcal enterotoxins (SEs) belong to a large family of staphylococcal and streptococcal pyrogenic exotoxins (PT), sharing common phylogenetic relationships structure, function, and sequence homology. Nine serologically distinct staphylococcal enterotoxins have been identified in the last few decades; Staphylococcal food poisoning has been reported as third cause of food-borne illnesses in the world. Among the foods implicated in Staphylococcal food poisoning, milk, dairy products and meats, particularly handled foods, play a vital role since entero-toxigenic strains of S. aureus have been commonly isolated in them (Ateba *et al*., 2010).

2.1.6. Pathogenesis of *Staphylococcus aureus*

A toxin haemolysin which is encoded on the bacterial chromosome is a 33 KD, a polypeptide that is produced by most strains of *S. aureus.* The toxin disrupts the smooth muscle in blood vessels and is toxic to many types of cells, including erythrocytes, leukocytes, hepatocytes, platelets, and cultivated cells. It becomes integrated in the hydrophobic regions of host cell membrane, leading to formation of 1- and 2-nm ring-shaped pores (Fischetti *et al*., 2000).

The rapid efflux of K+ and influx of Na+, Cat+, and other small molecules leads to osmotic swelling and cell-lyses. A toxin is believed to be an important mediator of tissue damage in staphylococcal disease (Murray*et al.,* 2002).Toxin is also an important cause of damage to the air-blood barrier in S. aureus-induced pneumonia (McElroy *et al*., 1999). Most S. aureus isolates possess the structural gene for alpha-toxin, but are variable in its expression or in the amount of toxin expressed.

2.1.7. Public health and economic importance of *Staphylococcus aureus* infection

Staphylococcus aureus causes a wide range of severe and economically important diseases in human and Animal Health (Leonard and Markey, 2008; Safdar and Bradley, 2008). Antimicrobial resistance is a main public health concern worldwide. Public hazards associated with the consumption of antibiotic contaminated milk could be allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria (Thirapatsakun, 1999).

The expansion of resistance both in human and animal bacterial pathogens has been allied with the widespread remedial use of antimicrobials or with their administration as growth promoters in animals. Further transfer of antimicrobial resistant bacteria to humans via the food chain has been reported (Angulo *et al.*, 2004). In this context, Staphylococcus present in milk may serve as a reservoir for human infections, thus allowing these microorganisms to persist and spread in the community. Up to now, many researchers have focused on the spread of resistant *S. aureus* in clinical setting (De silvaciombra *et al*., 2003; Ateba *et al*., 2010).

2.2. Prevalence and zoonotic effect of mastitis

Different studies conducted in different parts of Ethiopia showed variable prevalence of mastitis depending on the type of farm and managements systems. Getaneh and Gebremedhin, (2017) indicated the pooled prevalence of mastitis was 47% both in clinical and sub-clinical mastitis. Biffa *et al., (*2005) conducted a study on mastitis of 974 lactating dairy cows in Southern Ethiopia as, 34.9% had mastitis; 11.9% clinical, and 23.0% subclinical mastitis respectively. Another study in and around Sebeta, Ethiopia, that looked into the prevalence of mastitis in 180 local and crossbreed dairy cows showed that mastitis (majority of which is sub-clinical in form) can reach a prevalence rate of 52.78% (Hundurra *et al.*, 2005). Mastitis prevalence in dairy farms of Holeta town, Central Ethiopia at cow level was 71.0% (76/107), out of which 22.4% (24/107) and 48.6% (52/107) were clinical and subclinical, respectively. The Holeta study also revealed the quarter level prevalence of mastitis as 44.9% (192/428); from this the clinical form was 10.0% (43/428) and the subclinical was 34.8% (149/428) (Mekibib *et al.,* 2010). A study conducted by Jirata and Indalem (2016) on prevalence of bovine mastitis in Woliata Sodo town, in the southern part of Ethiopia indicated overall a prevalence of 34.7% in cows and 21.94% in quarters.

Debele *et al*., 2021 showed an overall cow level mastitis prevalence of 30.5% (95% confidence interval [CI]:26.0–35.2).Belina *et al*., 2016 indicated the overall mastitis prevalence was 50.3%. Another study shown that *Staphylococcus aureus* prevalence *in*mastitic cows was nearly 40% of (Getahun *et al*., 2008; Abera *et al.*, 2010) .The prevalence of *S. aureus* as a cause of mastitis was the highest of *Staphylococcus* infection comprises 44% *(*Hundurra *et al*. 2005).

Such high prevalence of sub- clinical mastitis in the Ethiopian context indicates that the public health importance of zoonotic pathogens would be of great concern.

2.3. Diagnosis of mastitis

2.3.1. Qualitative examination of milk

According to Quinn.*et al*. (2002), changes in color of milk can be caused by the presence of blood (red or brownish) or pus (yellow). The consistency may be increased, resulting in thicker, "sticky" milk or it may be more than usually watery, flakes and clots are always abnormal. The smell of the secretion may also be altered because of mastitis mostly in the case of clinical mastitis.

2.3.2. California mastitis test

This practical test was developed in the 1950's during a California testing program; it gives a measure of the SCC of the sampled milk. A reagent (4% sodium hydroxide often used) is a detergent, which ruptures somatic cells in the milk, thereby releasing DNA. This forms a precipitate with other serum components, fat particles and the CMT reagent, causing visible gelling of the milk. A pH-indicator (for example bromcresol purple) may be added to the reagent.
The test procedure is simple and straightforward: A squirt of milk from each quarters of the udder is placed in each of four shallow cups in the CMT paddle. Mixture of 2ml of milk with an equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane and a positive gelling reaction occurred in a few second with positive samples. The result was scored from negative without gelling to positive from slight gelling to viscous form based on bacterial load described (Pamela, 2005). All CMT scores of 0 and trace will considered as negative, while CMT scores of 1, 2, and 3 will be considered indicators of sub clinical mastitis. Positive cows will be defined as having at least one quarter with CMT score of (1 +) one plus.

Table 1Interpretation of CMT score

CMT score	Somatic cell range	Interpretation
N(negative)	0-200,000	Healthy quarter
T(trace)	200,000-400,000	Subclinical mastitis
1	400,000-1,000,000	Subclinical mastitis
2	1,000,000-5,000,000	Serious mastitis
3	Over 5,000,000	Serious mastitis

Source: Pamela, (2005)

2.3.3. Culture method

The unquestionable way of diagnosing mastitis is by directly isolating and identifying any pathogenic microorganisms, which may be present in the milk. Cultural methods and a number of additional determinative tests can achieve this. To obtain correct results, avoid contamination, and hence bias, it is important to work as securely and as accurately as possible under the circumstances. Similarly, the procedure of routine mastitis testing should be standardized and work protocols instituted (Quinn *et al.,* 2002).

2.3.4. Confirmatory tests

There are lots of confirmatory tests to confirm whether the isolated microorganism is pathogenic or nonpathogenic with its virulence factor. This may include BIOLOG/Omnilog ID system, PCR and others.

2.4. Risk factors influencing prevalence of mastitis

There are a number of factors that influence the occurrence of mastitis and fewer studies that have investigated cow-level factors associated with the development of clinical mastitis was carried out. The risk of developing Clinical mastitis is greatest in early lactation and increases with parity and level of milk production (Berry and Meaney, 2005). They have also reported that the risk of a cow developing in the subsequent month of lactation is also a function of number of cases of Clinical mastitis in the previous lactation, number of clinical cases in the previous months of the current lactation, and the occurrence of Clinical mastitis in the current month. The risk of contracting Clinical mastitis was 4.8 times greater if the animal experienced Clinical mastitis in the previous stage of lactation. Clinical mastitis occurred in 38% of cows that experienced Clinical mastitis in the previous lactation, as opposed to 23% of those that had not (Berry and Meaney,2005).

The prevalence of infected quarters increases with age, peaking at 7 years (Radostits *et al*., 2006). It may also be a result of a greater cellular response to infection or of a greater amount of permanent udder damage after infection in older cows. Older cows, especially after four lactations were submitted to more lactation, increasing the risk for mastitis and udder tissue damage (Christa, 2008). Most new infections occur during the early part of the dry period and in the first 2 months of lactation, especially with the environmental pathogens (Radostits *et al*., 2006). Christa (2008) reported that the first month of lactation is the most sensitive period for risk of mastitis in the cow, even in well-managed herds. On the other hand, the SCC of cows late in lactation is higher than the average throughout lactation, but this is due to an increased prevalence of sub-clinical infections late in lactation and a reduced milk flow production.
Predisposing factors such as poor management and hygiene, teat injuries and faulty milking machines are known to hasten the entry of infectious agents and the course of the disease (Islam *et al*., 2011). Where predisposing factors are present, the organisms are able to pass along the teat canal and enter udder the set-up infection. These factors may be age (the older the animal the greater the likelihood of the infection), stage of lactation (more likely at the beginning of and the end of lactation), milk yield (high yielder), hereditary factors, trauma, hygiene and unknown factors (ICAR, 2011).

3. Conclusion and Recommendations

Bovine mastitis, with increasing age, number of parity, milk yield, unhygienic milking, late stages of lactation and presence of teat wound were associated factors with the occurrence of *Staphylococcus aureus* implicated. Its unambiguous, silent loss of milk production along with blind teats affects the economies of dairy production. The overall prevalence of mastitis in different studies indicates the ranges from 30.5 to 71% which indicates its high incidence. *Staphylococcus aureus* is mastitis inducing contagious pathogens frequently detected from subclinical mastitis, which causes huge economic loss, denoting that mastitis problem need great concern in the economy of dairy industry. Therefore:

✓ Herders or managers should design and implement strategy of hygienic milking as well as eliminating factors associated with occurrence of mastitis.

✓ Regular CMT screening should be implemented to reduce impact of sub clinical mastitis,

✓ Farmers and workers should be aware of subclinical mastitis infection, its economic loss and antibiotic resistant strain hazard of *S. aureus* to human health.

✓ Culling of chronically infected cows should be made so that potential source of pathogens could be eliminated.

✓ Extra investigation should be conducted, using high-teck ID system, to assess the causative agent and the economic losses of mastitis in general.

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