

A Review on Prevalence of Bovine Mastitis and Antibiotic Residual Effects in Raw Milk and Its Human Health Significance

Mosa Mitiku Asmani
College of Agriculture and Natural Resource, Department of Animal Production and Technology, Wolkite University, Ethiopia

Abstract
This paper has reviewed researches and paper reviews obtained from peer-reviewed literature published between 1950 to 2014 on the prevalence of bovine mastitis and antibiotic residual effects in raw milk and its human health significance in dairy sectors. A dairy is a development tool because it widens and sustains pathways out of poverty through securing assets of the poor, improving smallholder productivity, and increasing market participation by the poor. Hence, the development of the dairy sector in different parts of the countries can contribute significantly to poverty alleviation, improved nutrition, and household income. But, the dairy sector has not been fully exploited and promoted due to inflammation of the mammary gland called mastitis. Mastitis remains one of the most significant causes of morbidity and mortality of adult dairy cows and results in reduced profitability for the dairy industry. Generally, mastitis is one of the most devastating disease conditions leading to significant economic losses globally because of reduced milk production, treatment costs, increased labor, milk withholding following treatment, death, and premature culling. in addition to these, samples of raw milk produced and/or transported to consumers in different parts of the country showed that almost all microbiological counts were above the international accepted standard level (>105cfu/ml and >102cfu/ml for AMBC and Enterobacteriaceae/coliform counts, respectively) and different pathogenic (spoilage) bacteria have identified, it is probably because of problem-related with the health of milking cows, poor production and handling practices, contaminants from milking environment and administration of **antibiotic** drug rule. Therefore, awareness creation about quality milk production and good handling practices produced, transported until consumption is necessary; the concerned body shall control the quality of milk by prevention of the prevalence of mastitis regularly and also set the standard for bacterial quality.
Keywords: Antibiotic; bovine; mastitis; prevalence; quality milk
DOI: 10.7176/JBAH/11-24-01
Publication date: December 31st 2021

Introduction

Dairy provides rural farmers with a way to increase assets, a method to diversify income and nutrition. Dairy is also an important tool to address poverty, enhance agricultural development, and create employment opportunities beyond an immediate household or smallholder dairy operation. Consolidation of dairy herds has resulted in the continued shift of dairy cows from smaller to larger herds. In 2010, dairy herds containing more than 500 cows comprised about 5% of all herds, yet produced 61% of total U.S. milk output (USDA, 2011). Larger dairy farms manage cows in groups, have specialized labor forces, and often have free access to technical services provided by agribusinesses. These demographic shifts have influenced the role of veterinarians in developing and implementing mastitis control programs. On most farms, detection, diagnosis, and administration of treatments for clinical mastitis are the responsibility of farm personnel and veterinarians are often consulted only when a case becomes life-threatening. Several studies have indicated that many dairy veterinarians are only marginally involved in mastitis control programs.

There are ample economic and societal reasons for veterinarians to increase their involvement in mastitis control programs. The occurrence of mastitis reduces milk production, increases the amount of milk discarded, and increases premature culling and production costs (Fetrow, 2000). Additionally, both clinical and subclinical mastitis have been demonstrated to reduce reproductive efficiency (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004). The successful implementation of a milk quality plan as part of the production medicine program will help to meet those objectives and can result in improved economic performance for the dairy farm. It is well-known that mastitis can be controlled by the prevention of new infections and the elimination of existing infections. Historically, milk quality was defined by dairy processors based solely on the characteristics of the raw milk that they purchased. In most regions, quality standards consisted of measurements of somatic cell count (SCC), total bacterial count, and detection of antimicrobial residues (Anonymous, 2009).

Finally, the solution for this problem will be antimicrobials administration. Are routinely used for the treatment of dairy cattle affected with clinical and subclinical infections (Aarestrup, 2005). The use of antimicrobials has, over time, increased the number of antimicrobial-resistant microbes globally, and any use of these agents will to some extent benefit the development of resistant strains and also inappropriate usage of antimicrobials such as wrong dose, drug or duration may contribute to the most to the increase in antimicrobial resistance without improving the outcome of treatment (Williams, 2000). Even if milk produced from the mammary gland of healthy mammals is sterile fluid (Fernandes, 2008), anti-biotic drug residue, contamination of microbes starts from the udder of milking animal, poor milking practice, milking environment (contaminated air, excreta of animals), milking utensils, poor handling practices (lack of treatment like cooling with refrigerator, appropriate heating, and others) and lack of cold chain transportation and storage system until a table for consumption (Robinson, 1990). Even if most of the milk production and handling practice in the dairy sector is poor. Therefore, this paper intends to review research and review results obtained from peer-reviewed literature published between 1950 to 2014 in different parts of the countries on the prevalence of bovine mastitis and antibiotic residual effects in raw milk and its human health significance in dairy sectors.

Definition of mastitis

Mastitis (inflammation of the mammary gland) is one of the most devastating disease conditions leading to significant economic losses globally (Kumar et al., 2010a; Abd Ellah, 2013) because of reduced milk production, treatment costs, increased labor, milk withholding following treatment, death and premature culling (Lightner et al., 1988; Kaneene and Hurd, 1990; Miller et al., 1993; Szweda et al., 2014). Due to multiple etiologies, it always remained a challenge to veterinarians worldwide. Approximately, 140 species of microorganisms have been identified as etiological agents of bovine mastitis. Of these various etiological agents, Staphylococcus aureus is a major pathogen associated with bovine clinical and subclinical mastitis (Wilson et al., 1997; Brito et al., 1999; Tenhagen et al., 2006; Piepers et al., 2007; Bhatt et al., 2011; Cervinkova et al., 2013).

At present, there is a paucity of reports about the occurrence of these virulence factors among aureus isolates from India and about the possible distribution of single S. aureus clones as causative agents of bovine mastitis. Since the introduction of $-lactamase-stable antimicrobial drugs in clinical use, Staphylococcus aureus strains have emerged worldwide as important nosocomial pathogens. Their prevalence in the community is increasing substantially. The indiscriminate use of antibiotics like ampicillin, penicillin, oxacillin, and methicillin may contribute to the increasing occurrence of antibiotic-resistant strains in cows with mastitis. These strains in intramammary dissemination often produce incurable severe intra herd infections (Moon et al., 2007). Resistance of S. aureus to antimicrobial agents can complicate the treatment of its infections (Lowy, 2003).

Mastitis is among the important health problems in dairy cattle. It has been considered as one of the most important threats affecting the dairy industry. Mastitis remains to be the most economically damaging and zoonotic potential disease for the dairy industry and consumers worldwide irrespective of the species of animal (Ojo et al., 2009). It is the major concern of the dairy industry worldwide for several reasons, such as mastitis has deleterious effects on milk composition, yield, and quality of dairy products, it is considered a welfare concern due to the pain cows experience, especially during an episode of acute, severe mastitis (Kemp et al., 2008; Leslie and Petersson-Wolfe, 2012). Mastitis is the most common production limiting and costly disease. Factors that contribute to the economic impacts of mastitis include milk production losses, diagnostic costs, treatment costs, discarded milk, and increased risk of other diseases and culling of dairy animals (Halasa et al., 2007). These all mentioned above are the most important risk factors which contribute to the increase in intramammary infection in dairy farms (Ranjan et al., 2011). Therefore, it was hypothesized that the poor practice in the emerging dairy industry and climatic conditions favor the presence and persistence of mastitis in dairy production.

Prevalence of bovine mastitis

The prevalence of mastitis pathogens varies from herd to herd. However, bacterial pathogens appear to be the most prominent contributor to worldwide mastitis (Wilson et al., 1997). The prevalence of subclinical mastitis in dairy herds is often surprising to producers. Moreover, subclinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high. Cows with subclinical mastitis are those with no visible changes in the appearance of the milk and/or the udder, but milk production decreases by 10 to 20% with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing (Holdway, 1992). Although there are no visible or palpable external changes, the infection is present and inflammation occurs in the udder (Blowey and Edmondson, 1995).

Clinical mastitis is less likely in younger animals. Reduction in clinical mastitis has been a major success over the past 35 years, in countries with a developed dairy industry (Poelarends et al., 2001). Most mastitis occurs as a low-grade infection, a subclinical state, which affects 10-15% of the cows, increasing milk leucocytes content, reducing milk production, and increasing milk bacterial content. These all contribute to reduced milk value as a food and in monetary terms (Barbano, 2004). The prevalence of such infections is a significant risk to uninfected animals in the herd as many mechanisms exist to expose the animals to new infections. Most commonly these include the common lying areas in housing or at pasture, the milking machine, and successive contact of different cows or teats by the milker preparing the teats for milking. The prevalence of Coagulase-negative staphylococci (CNS) mastitis is higher in primiparous cows than in older cows (Tenhagen et al., 2006).

Contagious mastitis is primarily transmitted at milking time and the milking process affects the patency of the teat orifice which can increase the risk of the development of environmental mastitis. Mammary quarter infection prevalence ranges between 28.9-74.6% prepartum and 12.3-45.5% at parturition (Fox, 2009). Coagulase-negative staphylococci (CNS) are the most prevalent cause of subclinical intramammary infections in heifers. Coagulase-positive staphylococci (CPS) in some studies are the second most prevalent pathogens, while in other studies the environmental mastitis pathogens are more prevalent. The risk factors or subclinical mastitis appear to be a season, herd location, and trimester of pregnancy (Fox, 2009). Being aware that especially sub-clinical mastitis is highly spread through herds in developing countries, it is important to identify risk factors and to assess their contribution to the occurrence of the disease. Identification of area-specific and/or farm-specific risk factors is important for the design of control programs for mastitis in cows (Almaw, Molla, and Melaku, 2012).

The occurrence of mastitis is generally higher in high-yielding bovines. Holstein Friesian (HF), Jersey or HF, and Jersey crossbred dairy cows are generally more susceptible to mastitis than indigenous breeds (Moges et al., 2012; Sudhan and Sharma, 2010; Joshi and Gokale, 2006; Dego and Tareke, 2003; Sori, Zerinhum and Abdicho, 2005; Lakew, Tolosa and Tigre, 2009), although Rahman and co-workers found no significant difference between HF crossbreds and zebu (Rahman et al., 2009). Cows with the most pendulous quarters appear to be the most susceptible to mammary infections, the pendulous udder exposes the teat and udder to injury and pathogens easily adhere to the teat and gain access to the gland tissue (Almaw, 2004; Sori, Zerinhum and Abdicho, 2005). Similarly, long teats increase the risk of accidental trauma and such lesions constitute potential sources of micro-organisms, which increases the probability of quarter infection (Almaw, 2004).

The prevalence of SCM increases with age, increasing lactation number and parities (Dego and Tareke, 2003; Joshi and Gokale, 2006; Rahman et al., 2009; Awale et al., 2012; Hameed et al., 2012; Mungube et al., 2004; Girma et al., 2012; Moges et al., 2012; Lakew, Tolosa and Tigre, 2009; Jarassaeng et al., 2012; Islam et al., 2011). It has been shown that the higher prevalence of mastitis in older animals is due to increased potency of teats and increased degree and frequency of previous exposure in multiparous old cows (Girma et al., 2012). Seasonality in the incidence of mastitis has been studied. The occurrence of mastitis varies from season to season because the growth and multiplication of organisms depend on specific temperatures and humidity. Incorrect ventilation, with high temperature and relative humidity, encourages the multiplication of various bacteria. Exposure of animals to high temperatures can increase the stress of the animal and alter immune functions (Sudhan and Sharma, 2010).

Different types of milking methods (e.g. stripping, knuckling, full hand method, machine milking) are practiced by dairy farmers. Faulty milking practices, especially knuckling, cause great harm to tissue and they become prone to infection (Sudhan and Sharma, 2010). A stripping type of hand-milking technique was the predominant method used (90%) in the study conducted by Kivaria and co-workers in Tanzania, and they assumed that this technique causes microscopic trauma of the teat epithelium (Kivaria, Noordhuizen and Kapaga, 2004.). In Pakistan, Hameed et al., (2012) recorded the highest prevalence of mastitis in animals with calf suckling; probably because of the injury inflicted while dragging away during suckle. Moisture, mud, and manure present in the environment of the animals are primary sources of exposure to environmental mastitis pathogens. Milking hygiene reduces the pathogenic organisms and prevents them from inhabiting the immediate environment or skin of the animals and minimizing their spread during the milking process (Sudhan and Sharma, 2010). In fact, in many studies in Ethiopia (such as those conducted by Lakew, Tolosa, and Tigre, 2009; Dego and Tareke, 2003; ungube et al., 2004), a higher prevalence is recorded in cows with poor hygiene in the milking process. Similarly, in India, the practice of regular teat dipping is not common at the small-dairy unit level (Sudhan and Sharma, 2010). Intensively managed cows present a higher risk for the development of mastitis, followed by semi-intensive, with the least risk among extensively managed animals (Sori, Zerinhum, and Abdicho, 2005).

In Ethiopia, the available information indicated that bovine mastitis is one of the most frequently encountered diseases of dairy cows. According to Lemma et al. (2001) of the major diseases of crossbred cows in Addis, Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases, in which 171 cows out of 556 were found to be affected. Generally, the prevalence of clinical and subclinical mastitis in different parts of Ethiopia ranges from 1.2 to 21.5% and 19 to 46.6%, respectively (Kerro and Tareke, 2003). These limited studies showed that bovine mastitis is among the problems hindering dairy productivity in Ethiopia and this requires the development of methodologies of control programs under the prevailing husbandry system. However, according to Hussein et al. (1997) so far efforts have been concentrated only on the treatment of clinical cases. On the other hand, losses from mastitis have been attributed mainly to decreased milk production from subclinical mastitis (DeGraves and Fetrow, 1993). Kassa et al. (1999) carried out a survey of mastitis in dairy herds of the Ethiopian central high lands. Out of 10, 908 quarters examined from 2,681 cows, they found the prevalence of clinical mastitis, non-functional or blocked quarters, and subclinical mastitis to be

1.2%, 3.8%, and 38.9% on a cow basis, respectively. According to Hussein et al. (1997), the prevalence of clinical and subclinical mastitis is found to be 5.3% and 19% on a cow basis and 1.9% and 7.4 % every quarter, respectively, in the central regions of Ethiopia. In a study conducted at Repi and Debre-Zeit dairy farms, out of 186 lactating cows, forty (21.5%) were clinically affected and 71 (38%) subclinically infected (Workineh et al., 2002). The overall prevalence in this study was 59.7%. Bishi (1998) reported mastitis prevalence rates of 34.3% and 5.3% at low levels in the Addis Ababa region, for subclinical and clinical mastitis, respectively.

Subclinical and clinical forms of mastitis

Mastitis in both clinical and subclinical forms is a frustrating, costly, and extremely complex disease that results in a marked reduction in the quality and quantity of milk (Harmon, 1994). Annual losses in the dairy industry due to mastitis were approximately two billion dollars in the USA and 526 million dollars in India, in which subclinical mastitis is responsible for approximately 70% of these dollars losses (Varshney and Naresh, 2004).

The subclinical disease is defined as abnormalities of function that are detectable only by diagnostic or laboratory tests. Subclinical mastitis is the most prevalent mastitis problem on most farms. Detection of subclinical mastitis is based on the use of indirect tests such as enumeration of somatic cells or bacteriological analysis of milk samples. Subclinical mastitis is often undetected and has the greatest economic consequence because of long-term reductions in milk yield. Production losses due to subclinical mastitis have been estimated to cost the U.S. dairy industry 1 billion dollars annually (Ott, 1999). The invisible changes in subclinical mastitis can be recognized indirectly by several diagnostic methods including the California Mastitis Test (CMT), the Modified White Side Test (MWT), SCC, P^H, chlorine, and catalase tests. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfying results (Lesile et al., 2002).

In clinical mastitis (CM), there are visible changes to the normal appearance of milk, which could include a color change, a consistency change, or the presence of flakes, clots, and/or blood. Physical changes to the udder may be present in CM cases ranging from warmth, diffuse swelling, and pain to gangrene in severe cases. Chronic mastitis can result in local fibrosis and atrophy of mammary tissue. When only local signs are evident a case of mastitis is considered mild or moderate. A case of mastitis is considered severe when systemic signs of an inflammatory response are apparent including fever, anorexia, and shock (Erskine, 2011). Signs of clinical mastitis are highly variable and can range from detection of a small amount of garget at the beginning of milking to swollen, red mammary quarters and mortally ill cows.

Pathogenesis of bovine mastitis

Mastitis is a complex and multifactorial disease, the occurrence of which depends on variables related to the animal, environment, and pathogen. Among the pathogens, bacterial agents are the most common ones, the greatest share of which resides widely distributed in the environment of dairy cows, hence a common threat to the mammary. There is evidence that pathogens use various mechanisms to impinge upon cell death pathways (Weinrauch and Zychlinsky, 1999).

Etiology of bovine mastitis

The primary cause of mastitis is a wide spectrum of bacterial strains. However, incidences of viral, algal, and fungal-related mastitis were also reported (Pyorala, 2003). Over 135 different microorganisms (bacterial, algal, or fungal) have been isolated from bovine IMI, but the majority of infections are caused by staphylococci, streptococci, and gram-negative bacteria (Watts, 1988). Microorganisms that because mastitis is generally classified as either contagious or environmental based upon their primary reservoir and mode of transmission. Staphylococcus aureus and Streptococcus agalactiae are contagious pathogens and are commonly transmitted among cows by contact with infected milk. These pathogens are of particular importance because they cause mainly subclinical forms of IMI that are often difficult to detect. Primary environmental pathogens include different types of bacteria: species of streptococci other than Streptococcus agalactiae (Streptococcus species), coliform species (Escherichia coli, Klebsiella species, Enterobacter species), and Pseudomonas species.

Staphylococcus species

Staphylococcus aureus is one of the most prevalent contagious mastitis pathogens that colonize the teats during damage to the skin surface. It produces many enzymes and toxins and penetrates deep into the mammary tissue and resists phagocytosis. Some of the Staphylococcus aureus strains have antibiotic resistance and can cause problems with the treatment (Petersson-Wolfe et al., 2010). Culling, grouping, and dry cow therapy helps fight Staphylococcus aureus infections in a herd (Syensk Mjölk, 2003).

The chronic and subclinical forms predominate and on a herd basis, are the most important. Staphylococcus aureus bacteria produce toxins that destroy cell membranes and can directly damage milk-producing tissue. White blood cells (leukocytes) are attracted to the area of inflammation, where they attempt to fight the infection. Initially, the bacteria damage the tissues lining the teats and gland cisterns within the quarter, which eventually leads to the formation of scar tissue. The bacteria then move up into the duct system and establish deep-seated pockets of infection in the milk-secreting cells (alveoli).

Streptococcus species

Streptococcus dysgalactiae is also one of the contagious pathogens. It can spread throughout a herd from a single infected animal. The infected udder is the most important reservoir for this bacterium. They are transmitted to uninfected quarters mainly at milking time. Contaminated milking machines, udder washcloths, and the hands of the machine operator also transmit these bacteria (NMC, 1996). Breakdowns of contagious mastitis are usually due to the introduction of infected animals to the herd, or the employment of men who carry infection with them. The infections are mainly sub-clinical (NMC, 1996) and there are most frequent in the younger age groups. Streptococcus dysgalactiae is generally characterized as an environmental pathogen, but also may have characteristics of a contagious organism and appears to spread from cow to cow. This pathogen is generally responsive to teat dipping and dry cow therapy, but new infections can occur in a herd when no other udder infections by this organism are present (Harmon, 1996).

Coliforms bacteria

Among coliforms bacteria, E. coli is the most frequently isolated from bovine milk in cows belonging to dairy farms with intensive systems of milk production. E. coli is a member of the Enterobacteriaceae family. Its primary importance is its ability for lactose fermentation. Over 700 antigenic types or serotypes of E. coli have been recognized based on O, H, and K antigens. Two classes of coliforms have to be distinguished: harmless strains (non-pathogenic strains) and strains that cause a wide variety of typical clinical infections pathogenic strains (Tormo et al., 2005).

Public health significance of bovine mastitis

With mastitis, there is a danger that the bacterial contamination of milk from affected cows may render it unsuitable for human consumption by causing food poisoning and provides a mechanism of spread of disease to humans through consumption of raw milk. Many farm families simply consume raw milk because it is a traditional practice and it is less expensive to take milk from the bulk tank than buying pasteurized retail milk. Some believe that raw milk has a higher nutritional value than pasteurized milk. The bacteria that are transmitted through the milk and cause disease problems in man are bacteria causing mastitis in cattle and transmissible to man when a man uses raw milk from the infected udder. Example of such type of bacteria includes Mycobacterium, Brucella, Staphylococcus, streptococcus, Campylobacter, and Listeria species (Heeschen, 1996).

Economic importance of mastitis

Mastitis is one of the most important diseases that cause economic loss in the dairy industry worldwide (Bachaya et al., 2011). The mean annual incidence is 41.6 cases per 100 cows and affected cows suffered a mean of 1.5 cases and 16.4% of quarters suffered at least one repeat case (Bradley and Green, 2001). Bennett et al. (1999) estimated the total economic impact of clinical mastitis to be £119 per cow case in Great Britain.

More than $130 million is lost by the Australian dairy industry ($A200/cow/year) every year due to poor udder health resulting in reduced milk production that is mainly associated with mastitis. A herd without an effective mastitis control program may witness morbidity as high as 40% with infection, on an average of two-quarters of the mammary gland. Of the various clinical manifestations, subclinical mastitis is economically the most important due to its long-term effects on milk yields (Zafalon et al., 2007).

In general, Mastitis is the most economically important disease of dairy cows (Halasa et al., 2007). Mastitis negatively affects the quality of milk, milk production, farm economics, and animal welfare. Calculations of economic losses resulting from mastitis vary among countries. In India, the overall economic loss due to mastitis is estimated to be Rs. 7165.51 crores (Bansal and Gupta, 2009). The average decrease in milk yield due to clinical and subclinical mastitis was estimated to be 50 % and 17.5 %, respectively. The prevalence of bovine mastitis continues to affect dairy herds throughout the world despite continued research activity on the problem over the century (Sadana, 2006).

Prevention of mastitis

The best way to reduce the prevalence of mastitis is to prevent new IMI. However, few intervention studies have been performed and preventive measures are generally suggested based on risk factors associated with mastitis or specific pathogens rather than on the results of intervention studies. Recommended preventive measures are usually based on the proposed Plan (NMC, 2011) and depend on herd management system (i.e. conventional milking or robotic milking, tie stalls or free housing, etc.) and/or on the most prevalent udder pathogens present. Mastitis caused by contagious pathogens are mainly prevented through improvements in milking hygiene, use of post-milking teat disinfectants, blanket dry cow therapy, and treatment, segregation, or culling of infected animals, while environmental pathogens are primarily prevented by improvement in barn or pasture hygiene and general optimization of the cows' immune system.

In Sweden, the most commonly recommended prevention strategies for Staph. aureus and Strep. dysgalactiae comprise post-milking teat disinfection, infectious disease control around calving, well-adjusted milking machines, and good infectious disease control at milking (Växa Sverige, 2015). Primary recommendations for Strepuberis prevention comprise hygiene at milking and improvements in barn and pasture hygiene (Växa Sverige, 2015). However, as presented above, all three pathogens seem to be able to spread both in a contagious and in an environmental fashion complicating prevention and some farms experience mastitis problems despite a perception of well-implemented preventive measures.

Diagnosis of mastitis

Clinical mastitis is recognized by the appearance of abnormal milk, gland swelling, and /or illness. Subclinical mastitis is characterized by normal milk and hence requires indirect tests to detect.

California mastitis test (CMT)

Which remains the only reliable screening test for subclinical mastitis that can be easily used at the cow side? The CMT was developed to test milk from individual quarters but has also been used on composite and bulk milk samples. The CMT involves mixing and swirling equal parts of bromocresol violet reagent and milk in a plastic paddle with a compartment for each quarter (Quinn et al., 1999).

Fresh unrefrigerated milk can be tested using the CMT for up to 12 hours. Reliable readings can be obtained from refrigerated milk for up to 36 hours. If stored milk is used, the milk must be thoroughly mixed before testing because somatic cells tend to segregate with milk fat. The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time. The degree of reaction between the detergent and the DNA of nuclei is a measure of the numbers of somatic cells in milk. The threshold for CMT scores depends on the objective of the study. If it is used to minimize the rate of false negatives, the test should be read as negative versus positive with trace scores regarded as positive. If the CMT is to be used in culling decisions, a threshold with a lower rate of false positives may be desirable (Larsen, 2000).

Culture

The microbiological examination of both individual cow and bulk tank culture are elements of mastitis control. Most mastitis control programs include the use of individual cow cultures to determine which mastitis pathogens are present on the farm. Culturing can be used in a targeted fashion for specific control programs such as segregation plans for contagious mastitis or for surveillance to detect the presence of new or emerging pathogens. Culturing is also used to evaluate treatment efficacy and to establish susceptibility patterns to aid in the development of rational treatment strategies (Larsen, 2000).

Classification of mastitis pathogens

Classically, mastitis pathogens have been classified as contagious pathogens and environmental pathogens (Burvenich et al., 2003). The contagious pathogens are adapted to survive within the host particularly within the mammary gland. They are capable of causing subclinical infections, which are typically manifest as an elevation in the somatic cell count (leukocytes, predominantly neutrophils, and epithelial cells) of milk from the affected quarter; they are typically spread from cow to cow at or around the time of milking (Radostits et al., 1994). In contrast, environmental pathogens are best described as opportunistic invaders of the mammary gland, not adapted to survival within the host; typically, they invade, multiply, engender a host immune response, and are rapidly eliminated. The major contagious pathogens comprise Staphylococcus aureus, Streptococcus dysgalactiae, and Streptococcus agalactiae; the major environmental pathogens comprise the Enterobacteriaceae (particularly E. coli) and Streptococcus uberis (Dogan et al., 2006).

Contagious mastitis (cow-associated)

Contagious mastitis is defined as IMI transmitted directly from cow to cow (Erskine, 2001). The incidence of contagious mastitis depends on the dose and type of microbes to which a cow is exposed as well as physical barriers and the innate and acquired defense mechanisms. Although many different types of bacteria may cause mastitis, those of greatest interest are the pathogens commonly found on dairy farms and those for which the prevalence of IMI is high. The main contagious organisms are Streptococcus agalactia, Staphylococcus aureus, Corynebacterium bovis, and Mycoplasma species. Staphylococcus aureus is generally considered to be the most prevalent cause of IMI.

Environmental pathogens (Mastitis)

The most important change in the epidemiology of bovine mastitis over the past decade has been the rise in the importance of environmental pathogens causing clinical mastitis, relative to contagious pathogens. Environmental mastitis is caused by bacteria that are transferred from the environment to the cow, rather than from other infected quarters (Radostits et al., 2000).

Mastitis caused by environmental pathogens is traditionally considered to occur sporadically without long-lasting effects within the host. The contagious pathogens, on the contrary, can persist within the host for prolonged periods causing the continuous occurrence of mastitis and spreading between quarters and cows

(Passey et al., 2008). More recently, DNA fingerprinting data suggested that some E. coli strains have adapted to survive within the udder and cause recurrent mastitis. There is, however, no apparent single factor that is responsible for the persistence of E. coli infections in the udder (Almeida et al., 2011). The main environmental organisms are gram-negative bacteria, which include coliforms and environmental streptococci. The gram-negative bacteria include Escherichia coli, Klebsiella species, Enterobacter species, Citrobacter species, Serratia, Pseudomonas species, Proteus, and Actinomyces pyogenes. The environmental streptococci include Streptococcus uberis, Streptococcus dysgaladiae, and Streptococcus equinus. The majority of infections caused by coliform pathogens result in acute mastitis when compared to infections caused by contagious pathogens and environmental streptococci, but the infections are generally of shorter duration (less than seven days). The exception to this is Actinomyces pyogenes, which generates large and persistent production losses (Smith and Hogan, 1993).

Treatment and outcome of mastitis

Generally, mastitis treatment choices are made based on bacteriological culture and antimicrobial susceptibility testing when applicable. The choice of treatment is also made from clinical manifestation and prognosis and depends on legislation and available drugs. The prognosis after treatment is defined by a pathogen, antimicrobial susceptibility, chronicity of infection, infection load, age of the cow, breed, and several quarters affected (Sol et al., 1994; Owens et al., 1997; Østerås et al., 1999; Sol et al., 2000; Deluyker et al., 2005; Sandgren et al., 2008).

In Sweden, bacteriological culturing is recommended before the initiation of antimicrobial treatment of CM. All veterinary treatments should be reported by the veterinarian to the Swedish Animal Disease Recording system (SADRS; Swedish board of agriculture, Jönköping, Sweden), and antimicrobials can only be prescribed by a veterinarian. The first choice of antimicrobial treatment of gram-positive pathogens in Sweden is intramuscular administration of benzylpenicillin (The Swedish Society of Veterinary Medicine (SVS), 2011) and mastitis was the reason for 69% of all parenteral treatments with antimicrobials to Swedish dairy cows in 2013/2014 (Växa Sverige, 2014b).

Specific pathogens

Staphylococcus aureus: - Despite the use of antimicrobials chosen according to susceptibility testing, cure rates of Staph. aureus CM are often low (Pyörälä & Pyörälä, 1998; Sol et al., 2000). In addition, as low as 1-30% of Staph. aureus SCM is cured spontaneously (Sandgren et al., 2008), and bacteriological cure after treatment of SCM is variable but generally low (Wilson et al., 1999; Sandgren et al., 2008). The highest cure rates of CM and SCM are associated with lower parity, shorter duration of IMI, a lower number of udder quarters infected, and infections with non-ß-lactamase producing strains (Sol et al., 2000; Taponen et al., 2003b; Deluyker et al., 2005). Breed differences in cure rate have also been reported (Sandgren et al., 2008). After treatment of CM, the SCC can remain high throughout lactation in both primiparous and multiparous cows (de Haas et al., 2002). Intramammary infections with Staph. aureus in early lactation can negatively influence SCC and milk yield throughout lactation (Whist et al., 2009; Paradis et al., 2010), but one study demonstrated no effect on milk yield of Staph. aureus during the follow-up period (Paradis et al., 2010).

Streptococcus dysgalactiae: - Bacteriological cure rate after Strep. dysgalactiae CM treated with benzylpenicillin or related compounds ranges from 65 to 90% in different studies (Taponen et al., 2003a; McDougall et al., 2007a; Kalmus et al., 2014) and spontaneous cure after SCM ranged from 80% in first parity cows of the SR breed to 8% in cows of SH breed in third parity and higher (Sandgren et al., 2008). Data on clinical cure rate after treatment of CM is scarce but has been reported to be 74% for Strep. dysgalactiae in one study (Kalmus et al., 2014). In a study on treatment of heifers for CM caused by Strep. dysgalactiae around calving, 15% of the quarters were non-functional, and another 36% had an increased SCC in the milk and/or an IMI, 30 days after treatment (Waage et al., 2000). Increased SCC after CM has also been reported by De Haas et al. (2002), who described a slow decrease in SCC after VTCM in primiparous cows but an SCC that remained high throughout lactation for multiparous cows. Whist et al. (2007) demonstrated that early lactation IMI with Strep. dysgalactiae was associated with an increase in SCC, VTCM, and culling, and a decrease in milk yield throughout lactation.

Genotype-specific clinical manifestation or outcome has not yet been investigated for Strep. dysgalactiae IMI using molecular methods but in an experimental study of infections with four different Strep. dysgalactiae strains, strain-specific pathogenicity was described (Higgs et al., 1980). Streptococcus uberis. Bacteriological cure rate of Strept. uberis CM treated with benzylpenicillin or related compounds has in field studies been reported to 45 to 92% (Taponen et al., 2003a; McDougall et al., 2007a; Kalmus et al., 2014) and clinical cure rate was 77% in one study (Kalmus et al., 2014). Spontaneous cure of SCM with Strep. uberis is slightly lower than that of Strep. dysgalactiae, with a range of 68% in first parity cows of the SR breed to 4% in cows of SH breed in third parity and higher (Sandgren et al., 2008).

Mastitis and associated risk factors

Many risk factors of mastitis related to the environment, the microflora, and the animal have been investigated despite wide control efforts with few reasonable results. However, bovine mammary gland infection (mastitis) is a frequent and important problem among livestock herds in most countries. The occurrence of mastitis is influenced by management and different environmental factors like the housing of animals, type of milking and milking utensils, and type of feeding, hygienic quality of water, the health of lactating animals, and execution of various preventive procedures. The incidence of mastitis changes with the season, its rate has been reported to be highest during the winter (Nyman et al., 2007). Breen et al. (2009) investigated risk factors that were related to milk leukocyte count in different quarters of cattle. The following individual risk factors, teat end callosity, hyperkeratosis of quarters, body state, udder and leg hygiene, and capacity of milk quality and production were assessed. Significant association with an increased risk of milk leukocyte count was found with increasing lactation number and lactation stage than contamination of skin of quarters and udder. Results suggested that energy status, individual quarter, and different factors at the animal level play an important role to measure intra-mammary infections by measuring milk somatic cells in subsequent lactations. The large number of predisposing factors that contribute to the emergence of mastitis in dairy cattle may be physiological, genetic, pathological, or environmental (Sordillo, 2005) described below: -

Age and parity of the cow

Increasing parity increased the risk of clinical mastitis in cows (Kavitha et al., 2009), although the reason for this association is not clear. Sharma et al. (2007) conducted a study on 500 lactating cows of different ages, parities, and stages of lactation belonging to different organized or un-organized dairy farms. Older cows (>10 years) are at more risk (44.6%), particularly for subclinical mastitis (38.6%) than younger cows (23.6%) in which clinical mastitis was predominant. Cows with many calves (>7) have about 13-times greater risk (62.9%) of developing an udder infection than those with fewer (3) calves (11.3%). It has been demonstrated that the occurrence of mastitis in infected quarters increases with age in cows (Harmon, 1994), being the highest at 7 years of age. This may be due to an increased cellular response to intramammary infection or due to permanent udder tissue damage resulting from the primary infection. Efficient innate host defenses mechanisms of the younger animals are one possibility that makes them less susceptible to infection (Dulin et al., 1988).

Inherited features of the cow

Various genetic traits may also have a considerable impact on the susceptibility of the animal to mastitis. These genetic traits include the natural resistance, teat shape, and conformation, positioning of udders, relative distance between teats, milk yield, and fat content of milk. High milk yielders with higher than average fat content are reported to be more susceptible to mastitis (Grohn et al., 1990). The confirmation of the udder and shape of the teat are inherited characteristics that may also affect susceptibility to mastitis. Cows with elongated teats are more vulnerable to mastitis infection than cows with inverted teat ends (Seykora and Mc Daniel, 1985). Broad udders, lower hindquarters, and teats placed widely help the infectious agent and should be selected against it (Thomas et al., 1984).

Breed and milk yield

The risk of mastitis varies from breed to breed. High-yielding cows are generally considered to be more susceptible to intramammary infection e.g. Holstein Frisian (HF), Jersey or HF and Jersey crossbred dairy cows are more susceptible to mastitis than (Zebu) breeds of cows (Compton et al., 2007). It might be due to more disease resistance and they are low milk producers than crossbred cows. Increased risk of clinical mastitis in Friesian compared with Jersey and Ayrshire heifers (Compton et al., 2007).

Stage of lactation

The incidence of mastitis is reported to be higher immediately after parturition, early lactation and during the dry period, especially the first 2-3 weeks (Fadlelmula et al., 2009) due probably to increased oxidative stress and reduced antioxidant defense mechanisms during early lactation (Sharma et al., 2011). An increase in somatic cell numbers or count (SCC) which is mainly neutrophils is observed immediately after parturition, which remains high for a few weeks irrespective of the presence or absence of infection. This increased SCC is the cow's natural first line of defense to prepare for the onset of the new lactation. Relatively recent studies have revealed that cows in late lactation always show a higher than average SCC than that seen at other stages of the lactation period (Peeler et al., 2000), potentially representing increased subclinical infection, leading to a fall in milk production.

Mammary regression

There are significant functional changes in the udder during the early and late lactation and dry period, which affect the cow's susceptibility to infections. Lactating cows under stress show premature mammary regression. Such a condition compromises udder's natural defense mechanism (Giesecke et al., 1994; Capuco et al., 2003) leading to invasion of the teat canals by potential pathogens. The same condition prevails during the healing process of lesions because the resistance to causal agents remains less effective.

Milking machine
An extraneous factor such as the milking habits of farmers and faulty milking machines favors the pathogens to gain access to the mammary glands and proliferate, potentially leading to mastitis (Mein et al., 2004). In farms where machines are employed for milking, it is important to maintain physiologically optimal pressure [50 kPa for most machines], because pressures over this may lead to injury in the teat. Fluctuations in the pressure due to inadequate vacuum reserve must be avoided to prevent the occurrence of mastitis.

Proper installation, as well as the correct maintenance of milling machines, is important to avoid an inadequate vacuum level, teat and tissue damage, and incomplete milking. The vacuum level created by the vacuum pump is another important factor for complete and high-quality milking. Experiments have shown that a teat subjected to a vacuum level of 10.5-12.5 inches at the time of peak milk flow results in rapid, complete, and high-quality milk yield, and the teat suffers minimum physical pressure (Jones, 2009). Two-chambered teat cups are found to be better than single-chambered teat cups regarding achieving complete milking as well as fewer incidences of teat injuries (Mein and Schuring, 2003). Sanitary milking habits are important to avoid the spreading of bacteria or their proliferation. Faulty milking equipment due to poor installation or maintenance can cause tissue trauma, teat damage, poor milking out, erratic vacuum levels and can also transmit infectious agents at milking time.

Nutrition
The quality and plan of nutrition appear to be an important factor that influences the clinical manifestation of mastitis in heifers and cows (Heinrichs et al., 2009) although no relationship between the incidence of mastitis and either high energy or high protein feed in cows has been reported (Rodenburg, 2012). Vitamin E is one of the important supplements in dairy feed to boost the immune response of cows (Spears and Weiss, 2008) as it has been reported to enhance the neutrophil function as well as the phagocytic properties of neutrophils after parturition. Vitamin E is often combined with selenium, which acts as an antioxidant by preventing oxidative stress. Several investigations have demonstrated that neutrophils of selenium-fed cows are more effective at killing mastitis-causing microorganisms than those not supplemented with selenium (Underwood and Suttle, 1999).

***Weather and climat*e**
The incidence of mastitis is greatly influenced by the weather conditions and prevailing climatic conditions. Heat, humidity, cold, and drought are the important predisposing factors (Reneau, 2012). A higher incidence of mastitis has been reported to occur particularly during summer rainy months (Sentitula et al., 2012). As heat and humidity increase, so does the bacterial multiplication as well as a load of pathogens in the environment. Conversely, an alternative study has reported a higher incidence of coliform mastitis during the cold months of the year when the temperature was reported to be less than 21°C (Ranjan et al., 2011). Housing is also a factor that aggravates the incidence of mastitis, first due to excess numbers of animals in a limited space; and also because of the use of bedding material that easily allows for bacterial survival and growth, which overexposes animals and challenges their immune defense mechanisms (Hogan and Smith, 2003).

Milking hygiene and procedures
Moisture, mud, and manure present in the environment of the cow are the primary sources of exposure for environmental mastitis pathogens, and hygiene scores of cows provide visible evidence of exposure to these potential sources. Milking hygiene reduces the pathogenic organisms from inhabiting the immediate environment or skin of the animals and minimizing their spread during the milking process. The practice of regular teat dipping is not much more common at the household level but washing the udder with clean water and drying with individual cow towels using strip cups, and disinfecting the teat with germicide are some of the milking procedures used to minimize the occurrence of mastitis in the dairy farms. In addition to these, milking of heifer first, healthy cows second, and mastitic cows last are also good practices to minimize intramammary infection. Therefore, the prevalence of mastitis in cows is more at unorganized dairy farms as compared to organized dairy farms. Udder hygiene is significantly associated with the risk of environmental pathogen intramammary infection in cows and milking procedures (Compton et al., 2007).

Measuring and Monitoring of Mastitis and Milk Quality
An effective surveillance system for mastitis includes the following elements: (1) clear case definitions and effective mechanisms to detect both clinical and subclinical mastitis; (2) recording systems that allow for timely evaluation of risk factors; and (3) feedback mechanisms that allow management personnel and veterinarians to manage milk quality. Implementing Effective Practices that Reduce Exposure to Mastitis Pathogens: Mastitis control is based on reducing exposure to mastitis pathogens. In general, high-quality milk is produced when cows are housed in a clean and dry environment and gently milked using practices that favor teat health and minimize exposure to contagious mastitis pathogens (Zdanowicz et al., 2004).

To effectively prevent mastitis, production medicine practitioners should begin by reviewing the following issues: Do cow housing areas (including housing for dry cows) provide clean and dry environments for all stages of the lactation cycle? Are the udders clean enough and are teat ends healthy? Is the milking system properly calibrated and functioning properly? Does the milking routine incorporate currently recommended best management practices? Are milking technicians adequately trained?

Providing a clean and dry environment

On many farms, milking technicians are assumed to have primary responsibility for mastitis control while other farmworkers are responsible for stall maintenance and feeding. Many opportunities for exposure to mastitis pathogens occur outside of the milking facility, and all employees who can influence exposure to pathogens should be aware of the importance of mastitis control and share accountability. Contact with moisture, mud, and manure in cow housing areas can influence the rate of clinical mastitis and the veterinarian should be familiar with environmental hygiene in all stages of the lactation cycle. Rapid movement of animals for handling or milking often results in the splattering of manure onto udders. Overcrowding can concentrate excessive manure in areas designed for fewer animals. Manure handling, type of bedding, and maintenance of cow beds all have a major impact on cows and udder hygiene. Research has demonstrated that herds with greater bulk milk SCC often have less satisfactory hygienic practices as compared to herds with lower bulk milk SCC (Barkema et al., 1998).

Bedding management can be a primary determinant of bacterial numbers on teat ends. The amount of moisture and bacteria that are present in cow bedding are especially important (Hogan et al., 1989; Hutton et al., 1990; Zdanowicz et al., 2004). Organic bedding materials tend to support more bacterial growth as compared to inorganic bedding materials, but significant exposure to Streptococci spp. and Klebsiella spp. may also occur when sand bedding is used. A linear relationship between the rate of clinical mastitis and the number of gram-negative bacteria in bedding has been demonstrated (Hogan et al., 1989). This relationship is especially evident for Klebsiella species.

Evaluating animal hygiene

Feeding high concentrate diets has been associated with looser feces and reductions in cow and facility cleanliness (Ward et al., 2002). Several studies have identified relationships between cow cleanliness and measures of milk quality (Barkema et al., 1998; Reneau et al., 2003; Schreiner & Ruegg, 2003). A scale of 1 (cleanest) to 5 (dirtiest) was used to score five separate areas of cows and was compared to linear somatic cell scores obtained from the same animals (Reneau et al., 2003). Cleanliness of the tail head, flank, and belly was not associated with somatic cell scores (SCS), but SCS of cows with cleaner udders and lower rear legs was less than SCS of cows with dirtier udders and legs, indicating that dirty cows had a greater prevalence of subclinical mastitis (Reneau et al., 2003). A visual scoring system can be used by veterinary practitioners to score udder hygiene (UHS) either during milking or during the performance of other tasks that require the cows to be restrained (such as rectal palpation). No more than 20% of the herd should be categorized as having "dirty udders" (UHS of 3 or 4) (Schreiner & Ruegg, 2003). Cows with dirty udders are more likely to have greater SCC and an increased risk of mastitis (Schreiner & Ruegg, 2003). Hygiene scores of udders should be routinely performed as a quality control measure just as body condition scores are performed to monitor nutritional management.

Managing the milking process

A consistent method of pre-milking sanitation and uniform attachment of properly functioning milking machines are both fundamental processes that help ensure the production of high-quality milk. While most dairy veterinarians are not comfortable assuming primary responsibility for milking parlor design or maintenance of milking equipment, knowledge of basic milking equipment functions is essential. Appropriate testing of milking equipment requires specialized equipment and should follow procedures that have been defined by the NMC (NMC, 2007). Several components of the milking process merit special attention.

Pre-milking teat disinfection. Methods of pre-milking teat preparation have been extensively studied (Galton et al., 1984, 1986; Pankey, 1989; Ruegg & Dohoo, 1997). There is no question that the most effective method to disinfect teats is to pre-dip using an effective disinfectant. Pre-dipping using iodine has been demonstrated to reduce SPCs and coliform counts in raw milk by five - and six-folds, respectively, as compared to other methods of pre-milking udder preparation (Galton et al., 1986). Effective pre-dipping also contributes to improvements in food safety. Pre-dipping has been shown to reduce the risk of isolation of Listeria monocytogenes from milk filters obtained from New York dairy herds by almost fourfold (Hassan et al., 2001). For effective reduction in bacterial numbers, the disinfectant must be in contact with teat skin for sufficient time to adequately kill bacteria. Teat dips must be properly formulated, stored in clean containers, completely applied to debris-free teats, and allowed sufficient time (usually at least 30 s) for action before removal.

Examination of foremilk: the examination of milk before attaching milking units is useful to ensure that abnormal milk is diverted from the human food chain and to identify cases of clinical mastitis at an early stage when the only symptom may be mildly abnormal milk. Fore stripping is adequately performed when two to three streams of milk are expressed and is an effective means to stimulate milk letdown. When both pre-dipping and fore stripping is practiced, there is no data that indicate that the order that the steps are performed will affect milk quality (Rodrigues et al., 2005). Milking technicians should be encouraged to wear disposable nitrile or latex gloves to reduce the potential spread of mastitis pathogens by contaminated hands. Drying of Teats; effective drying of teats is probably the most important step to ensure hygienic teat preparation. Drying of teats has been demonstrated to reduce bacterial counts of teat ends from 35,000 to 40,000 CFU/mL for teats that were cleaned but not dried to 11,000- 14,000 CFU for teats that were dried using a variety of paper towels (Galton et al., 1986).

Attaching the milking unit; one objective of the milking routine is to attach the milking unit to well-stimulated cows that have achieved milk letdown, thus maximizing milk flow. The period between stimulation of the cow and unit attachment is often referred to as the "prep - lag" time. Several studies have been performed to determine the optimal prep - lag time (Rasmussen et al., 1992; Maroney et al., 2004). It is well recognized that the need for stimulation varies depending on yield, stage of lactation, milking interval, and breed (Bruckmaier, 2005). Historically, prep - lag time of 45 – 90 s has been recommended, but negative consequences (reduced milk yield) have not been reported until lag times have exceeded 3 min (Rasmussen et al., 1992; Dzidic et al., 2004; Maroney et al., 2004). The failure to achieve adequate milk letdown will often result in bimodal milk flow and the application of the milking unit without stimulation or immediately after stimulation should be discouraged. It appears that prep - lag times longer than 90 s will not be a uniformly detrimental but premature attachment of the milking unit should be avoided (Dzidic et al., 2004; Maroney et al., 2004).

Managing cows post milking

Post milking teat antisepsis was initially developed to reduce the transmission of contagious mastitis pathogens and is based on killing bacteria that are present in milk that remains on teat skin after milking has been completed. Post milking teat dipping is one of the most highly adopted practices in the dairy industry, and it is the final hygienic defense against infection after milking is completed. While teat dipping is universally recognized as a useful practice, effective implementation of teat dipping is often variable. To maintain excellent hygienic standards and minimize mastitis, continued education of milking technicians about the principles of mastitis control is often necessary. Evaluation of the effectiveness of post milking teat dipping is best performed when milking technicians are not aware of the evaluation. When colored teat dips are used, one effective method of evaluation is to surreptitiously score teats of cows in the return lanes after milking. If possible, teats from at least 20 to 30 cows should be examined, and the goal is to observe complete coverage (75%) of at least 95% of observed teats. Digital photographs of well-covered and inadequately covered teats are an excellent training tool that can be used to demonstrate proper teat dipping.

Training milking technicians

Implementation of standardized milking processes can be difficult because most farms use several milking technicians, and some technicians have little experience or training. Helping to communicate expectations to employees and providing training is a potential role for production medicine veterinarians. Statistics from larger Wisconsin farms (average herd size of 400 cows) indicate that while the use of recommended milking practices was generally high, management of the milking parlor was often neglected (Rodrigues et al., 2005).

Antimicrobial and Antibiotic Residues in Cow milk and its Public Health significance

Antimicrobial and Antibiotic Concepts and Definitions

The terms antimicrobial and anti-antibiotics are often used interchangeably but are not synonymous. In technical terms, "antibiotics" refer only to substances of microbial origin (such as penicillin) that are active against other microbes while "antimicrobial" refers to any substance (including synthetic compounds) which destroys microbes (Guardabasse and Courvalin, 2006). Antimicrobial agents interfere with specific bacterial processes needed for the growth or division of cells.

In modern dairy cattle operations, antimicrobials are administered for both therapeutic and prophylactic purposes. Most antimicrobials are used therapeutically but some antimicrobials are used to prevent disease in healthy animals during periods of increased susceptibility. Mastitis is one of the most frequent infectious diseases in dairy cattle and accounts for most of the doses of antibiotics given to dairy cows (Pol and Ruegg, 2006a). Lactating cows may be treated for clinical mastitis or to pursue a bacteriological cure of a subclinical case. Antimicrobials are also used to treat other infectious diseases of dairy cows, including respiratory and uterine infections and infectious foot disease.

The use of antimicrobials to treat food animals has the potential to affect human health through 2 mechanisms: 1) increasing the risk of antimicrobial residues, and 2) influencing the generation or selection of antimicrobial-resistant food-borne pathogens (Yan and Gilbert, 2004). The risk of antimicrobial residues is well known and has been addressed through the use of appropriate regulatory mechanisms but there is increasing concern about the impact of antimicrobial usage in food animals on the development of antimicrobial resistance. The major use of antibiotics in the livestock sector was for the treatment and prevention of diseases. The history of antimicrobial use for better growth performance of food-producing animals was accidental; it was found that by-products of antibiotic production (dried Streptomyces aureofaciens broth) which contain a high level of vitamin B12 when fed to poultry animals resulted in higher growth (Centre for Science & Environment Study

(CSE, 2014).

Antibiotic residues in milk are of great concern to dairy farmers, milk processors, regulatory agencies, and consumers. The presence of antimicrobial drug residues in milk can provoke allergic reactions in some hypersensitive individuals (Dewdney et al., 1991; Dayan, 1993) and may induce resistant populations of bacteria that do not respond to treatments commonly used for human illnesses (Nijsten et al., 1996; van den Bogaard et al., 2001). Drug residues also alter the processing qualities of raw milk by inhibiting starter cultures used in the preparation of cheese and other fermented dairy products (Brady and Katz, 1988). Pasteurization and other forms of heat treatment eliminate pathogenic microorganisms but have limited or variable effects on drug residues (Moats, 1988).

Antimicrobials are used in dairy cattle production primarily to treat or prevent disease and to a lesser extent to increase milk production or improve feed efficiency. The use of antimicrobial therapy to treat and prevent udder infections in cows is a key component of mastitis control in many countries. Due to the widespread use of antimicrobials for the treatment of mastitis in dairy cows, much effort and concern have been directed towards the proper management and monitoring of antimicrobials used in such treatment to prevent contamination of raw milk. However, widespread use of antimicrobial has created potential residue problems in dairy products (Hillerton., et al 1999).

Antimicrobial residues are a small number of drugs or their active metabolites, which remain in the tissues or products (meat, milk, and eggs) from treated animals. Problems associated with antimicrobial residues in milk include the risk of allergic reactions after consumption by penicillin-sensitized persons and increased resistance of pathogenic bacteria towards antimicrobials. The concerns arise mainly from the possibility that antimicrobial-resistant bacteria may be transferred from livestock to humans, through animal to human contact, through the environment, or in contaminated food products (CAC), 1998). To safeguard human health, the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) have set standards for acceptable daily intake and maximum residue limits in foods (FAO and WHO, 1995). Regulatory limits for antimicrobial residues have been imposed on the dairy industry in many countries (Folly and Machado, 2001). However, Ethiopia has not yet adapted international standards or established specifications for residue limits in the milk.

Role of Antibiotics in the Treatment of Bovine Mastitis

The success of bovine mastitis therapy depends on the etiology, clinical presentation, and antimicrobial susceptibility of the aetiological agent among other factors (Miltenburg et al., 1996). Therapy failure in the management of mastitis could result from pathological changes that occur in the udder, etiology-related factors, pharmacokinetic properties of the antimicrobial drugs, poor animal husbandry, and inadequate veterinary services. However, the control of mastitis has been successfully achieved through the establishment of effective herd health control programs (Erskine et al., 2002).

The fact that the occurrence of antimicrobial resistance varies between countries and a region has the potential to complicate that matter. Furthermore, knowledge of expected resistance is limited by the small proportion of different bacterial pathogens from infected animals that are investigated for their antimicrobial resistance pattern. The evolution of antibiotic resistance in S. aureus strains is a serious cause of concern in dairy animals (Wang et al., 2008). Strains of S. aureus resistant to β-lactam antibiotics are known as methicillin-resistant S. aureus (MRSA). These strains in intra-mammary dissemination often produce incurable severe intra-herd infections (Moon et al., 2007; Kumar et al., 2010). MRSA strains have been observed to be multi-drug resistant, such as aminoglycosides, macrolides, lincosamides, streptogramins, tetracyclines, etc., which are often used in the treatment of mastitis (Wang et al., 2008; Kumar et al., 2010).

Antimicrobials Used in Dairy Production

Intensive livestock farming systems to cater to the need for human consumption of animal proteins are invariably associated with the regular and widespread application of potentially significant antibiotics. So far, no direct data is available regarding the global consumption of antibiotics in both extensive and intensive livestock farming systems. In the latest study conducted by Van Boeckel et al. (2015), they used the Bayesian statistical model to predict the consumption of antibiotics for livestock farming. This method combines maps of livestock densities in various geographic regions, projected demand for meat and meat products along with the current consumption of antimicrobials. Using this model, they succeeded in predicting the global trend of antibiotic use by 2030 following 2010 values. Similarly, some other researchers derived the coefficient of antimicrobial use per kilogram of animal for each class of animal using the data from ESVAC (European Medicines Agency, 2013) and applied it to high-resolution maps of livestock densities to predict the geographic consumption of antibiotics by 2030.

Several antibiotics have been isolated from various sources and are being used to control disease pathogens. These agents are not only used for treating infectious diseases but also used as feed additives such as chemotherapeutic agents (antibiotics, anthelmintics) and growth promoters (Serratosa et al., 2006; Babapour et al., 2012). Antibiotics can be grouped by either their chemical structure or mechanism of action. The most commonly used antibacterial in veterinary medicine includes lactams, tetracyclines, aminoglycosides, macrolides, quinolones, and sulfonamides (Mitchell et al., 1998; Unnikrishnan et al., 2005).

In general, the health of cows and udder also has a profound effect on the excretion of antibiotics in milk. The fibrosis of udder tissue in chronic mastitis leading to poor distribution and absorption of penicillin cause higher concentrations and longer retention of penicillin in the milk of the affected quarters compared to healthy quarters (Edwards, 1964). Intravenous infusion of high dosages of ceftiofur in cows with experimentally induced Escherichia coli mastitis resulted in significantly longer excretion of ceftiofur in milk compared to healthy cows (Erskine et al., 1995).

Beta-lactam antibiotics: - It is interesting to note that the ß-lactam antibiotics, including the penicillins, cephalosporins, carbapenems, and others, make up the largest share of antibiotics used in most countries (Kummerer, 2009). Lactam antibiotics are broad-spectrum antibiotics interfering with cell wall synthesis, used generally to treat Gram-positive and Gram-negative bacterial infections (Droumev, 1983; Sun et al., 2013). lactam antibiotics include penicillin, cephalosporins, carbapenems, and monobactam group of antibiotics. Among the beta-lactam antibiotics, penicillin and cephalosporins form the major category used in veterinary medicine and are frequently used for the treatment of animals all over the globe. Penicillin is the most commonly applied antibiotic for the treatment of bovine mastitis (Haapapuro et al., 1997) which frequently results in their residues in milk. The residues of these antibiotics in milk cause problems in dairy industries and human health hazards (Ghidini et. al., 2002).

Tetracycline: -The tetracyclines are broad-spectrum antibacterial active against Mycoplasma, Chlamydophila, and Rickettsia in addition to bacteria. Tetracyclines are bacteriostatic and acquired resistance is now widespread among bacteria (Fuoco, 2012). Tetracyclines may be administrated parenterally, orally through feed or water, or by intra-mammary infusion. The widely used oxytetracycline and the less often used tetracycline and chlortetracycline have similar properties. Fraction of tetracyclines execrated in bile gets reabsorbed through entero-hepatic circulation and may persist in the body for a long time after administration (Chambers, 2006). The rate of metabolism of tetracyclines in cows has been estimated to be 25-75 % and a significant percentage of the administrated tetracyclines are excreted in bovine milk (Abbasi et al., 2011).

Aminoglycosides*:* - Aminoglycosides were the first antibiotics discovered by systematic screening of natural product sources for antibacterial activity (Hermann, 2007). The laboratory of Waksman reported the discovery and isolation of the streptomycin from soil bacteria Streptomyces griseus in 1944, which was the first antibiotics used effectively against Mycobacterium tuberculosis (Schatz and Waksman, 1944). Aminoglycosides are usually used synergistically with β-lactams for the treatment of serious infections due to Gram-positive and Gram-negative bacteria. They act by binding to the A site of the 30 S small ribosomal subunit, inhibiting the translation process in protein synthesis (Hermann, 2005). The antibiotic, gentamicin in this class is used mainly to treat uterine infections in cattle, particularly dairy animals. However, one of its side effects is its tendency to accumulate and persist in bovine kidney tissue for several months (Elezov et al., 1984). Semi-synthetic derivatives of natural products like amikacin, netilmicin, and tobramycin have been the most fruitful source of new clinically useful antibiotics (Von Nussbaum et al., 2006).

Sulphonamides: - Sulphonamides are among the oldest groups of antibacterials widely used in the treatment of bacterial and coccidial diseases of dairy cattle and as a growth promoter in swine. They have a wide spectrum of bacteriostatic action, effective against both Gram-positive and Gram-negative organisms. These types of antibiotics dissolve quickly, easily distribute in all tissues and body fluids, including the cerebrospinal fluid and fetal circulation (JECFA, 1990). Sulfonamides are also known to produce sensitization and are one of the most common classes of antibacterial that produce allergic reactions at the therapeutic dose (Golembiewski, 2002), but there have been no cases of human allergies that involved exposure to residues in animal foods (Paige et al., 1999).

Chloramphenicol: - Chloramphenicol is a broad-spectrum antibiotic, isolated from the bacterium Streptomyces Venezuela in the year 1947. It is clinically used to treat chronic infection of the respiratory tract, bacterial meningoencephalitis, brain abscesses, and intraocular infections and is active against a variety of pathogens including bacteria, Spirochaetes, and Rickettsiae (Shukla et al., 2011). Chloramphenicol and its metabolites may appear in milk after parenteral administration, however, after oral administration to cattle, it is not excreted in milk (De Corte-Baeten and Debackere, 1976). Chloramphenicol has been associated in a non-dose related manner with aplastic anemia (Shukla et al., 2011) and bone marrow suppression (Ambeker et al., 2000) in a small proportion of humans to whom the drug was exposed. Some of the individuals who survive the bone marrow depression may develop leukemia, which creates concerns about possible carcinogenicity (Doody et al., 1996). Although most countries have banned the use of chloramphenicol in food and dairy animals, nevertheless traces of it have been detected in shrimp and other aquaculture products (Shukla et al., 2011).

Commonly Used Antimicrobials in Dairy Cows are currently several antimicrobials approved for intramammary use in lactating cows (Table 1). Each of these antimicrobials has a prescribed withdrawal period.

After a cow has been milked a full lactation, she is dried off to prepare for calving in the next lactation. Although they are no longer milking, dairy cows are still susceptible to mastitis. For this reason, nearly all cows are treated with long-acting intramammary antimicrobials (Moore et al. 2004).

Table 1. Examples of common antimicrobial agents administered to dairy cattle

Antibiotics Family	Examples
Amino glycosides	Gentamycin
Cephalosporin	Cephaprin
Ionophores	Monensin
Macrolids	Erythromycin, Tylosin
Penicillin	Ampicillin, Penicillin and Cloaxacillun
Tetracycline	Oxytetracycline

Source: Moore et al. (2004).

Antimicrobial administration and residue

Administration of antimicrobials to dairy cattle is usually therapeutics that are in response to the development of symptoms of the disease. These types of chemotherapy shorten the period of antimicrobial administration and usually reduce the number of antimicrobials employed. The use of feed and water grade antimicrobial is prohibited in milking cows, so most antimicrobials are administered orally or given by infusion or injection. Intra mammary treatments are subject to two mechanisms; biological (distribution in udder tissue and transfer into the blood) and physical (mechanical elimination of the milk at each milking). Several antibiotics with very high diffusion rates will rapidly pass through the different membranes (plasma membrane and vessel walls), entering the blood circulation and being excreted in different ways particularly in urea. However, some molecules will stay in the teat and udder. When a milking dairy cow is treated with an antimicrobial, the cow's milk must be withheld for a certain period. The producer must discard this milk and receive no payment for it. All loads of milk are tested for antimicrobial residues to ensure that milk-containing residue does not inadvertently enter the food supply (Wary and Gnanou, 2000).

Antibiotic residues in milk

Unpasteurized milk is an excellent means of transmission of zoonotic and other pathogens to humans. During milking, the milk can easily be contaminated by faces. In addition, milk is also a great growth medium for microorganisms. The number of bacteria in the milk indicates the level of milking hygiene, the storage temperature, as well as the time elapsed since milking. The total amount of bacteria indicates the time elapsed since milking, coliform bacteria mostly indicate the level of fecal contamination. International regulations state that milk shall be delivered and refrigerated within 2 h or 3 h after milking (Addo et al., 2011). Today most people purchasing raw milk boil their milk before drinking it. This is a practice that removes most of the bacteria but many of the chemicals that may be contaminating the milk remains due to their often heat-stable nature.

The most likely Reasons for Antimicrobial Residues in Milk of vocative drug residues are the failure to observe withdrawal times (Paige. 1994). Improper maintenance of treatment records or failure to identify treated animals adequately may lead to their omission. Violative drug residues can also occur as a result of improper use of licensed products through the illegal use of unlicensed substances. Extra-label dosages and the use of drugs that have not been approved for the species in question may lead to violative residues (Kanneene and Miller, 1997). The disease status of an animal and how drugs are administered influence the potential for residues. The disease may affect the pharmacokinetics of the drug, metabolism or the presence of infection and /or inflammation may cause the drug to accumulate in affected tissues (Kanneene and Miller, 1997). The significance of contamination depends on the pharmacodynamics of the compound and the species affected (McEvoy., 2002). The general Significances of Antimicrobial Residues in Milk are: -

Antimicrobial resistance: - the use of antimicrobials in food animals can result in antimicrobial-resistant bacteria reaching the human population through a variety of routes. Antimicrobial-resistant bacteria such as E. coli can colonize the intestine of people. Healthily exposed humans (farmers who use food containing antimicrobials, slaughterhouse workers, cookers, and other food handlers) often have an incidence of resistant E. coli in their feces than the general population (WHO, 2000). While many bacteria are not pathogenic, some bacterial species from the intestine of animals cause zoonotic infection to humans such as Salmonella species, Campylobacter species. The development and spread of antimicrobial resistance represent a serious threat with potential public health implications (WHO, 2000).

Hypersensitivity reaction: - It is an immune-mediated response to a drug agent in a sensitized patient and drug allergy is restricted to reactions mediated by IgE. Drugs are foreign molecules, but their molecular weight is usually too small to be immunogenic, they act as haptens, which must combine with drug-sensitive persons to be immunogenic and elicit antibody formation (Riedl and Cassilas, 2003). Allergic reactions to antimicrobials may include anaphylaxis, serum sickness, cutaneous reaction, and delayed hypersensitivity reactions. These effects are acquired after human beings consume food of animal origin, which contain drug residue that has allergic effects of the antimicrobials employed as food additives or in chemotherapy penicillin and streptomycin appear from clinical use in humans to be more included to produce hypersensitivity or allergenicity than others in present use. About 50% of the human population is considered to be hypersensitive to many substances including penicillin (Booth and McDonald, 1988).

Carcinogenic effect: - Carcinogenic effect refers to an effect produced by a drug having carcinogenic or cancer-producing activity. Among the carcinogenic veterinary drugs in the current use in many countries are nitrofurans, nitroimidazoles, quinoxaline, and griseofulvin. These drugs are acquired through the food of animal origin as antibiotic residues. The potential hazard of carcinogenic residues is related to their interaction or covalently binding with various intracellular components such as proteins, DNA, RNA, glycogen, phospholipids, and glutathione (Also and Mays. 2005).

Mutagenic effects: - The term mutagenic is used to describe chemical agents that damage the genetic component of a cell or organism. Several chemicals including alkylating agents and analogs of DNA bases have been shown to elicit mutagenic activities. There has been an increasing concern that drugs, as well as environmental chemicals, may pose a potential hazard to the human population by the production of gene mutation or chromosome aberration. Either the germinal or somatic cell may be affected. Understandingly; injury to either cell group may lead to serious consequences. However public health standpoint of view, a mutation in germinal cells is of more immediate importance, because of the hazard to further generations (Jerry, 1992).

Dairy industry impact: - The dairy industry is an important segment in the food industry, providing both milk and meat for human consumption. Dairy farmers and all supporting groups (veterinarians, feed supply dealers, milk processors, livestock dealers, etc.) should be concerned and devoted to producing as safe as well nutritious dairy food products. The dairy starter cultures currently used in dairy industries for the primary acidification of milk belong mainly to the genera Lactococcus, Streptococcus, and Lactobacillus. These starter cultures are mainly lactic acid bacteria used in the production of a range of fermented milk products, including cheese, yogurt, and cultured butter. The primary role of starter culture in cheese manufacture is the production of lactic acid from lactose at a consistent and controlled rate (Packham et al., 2001).

Milk is borne intoxication: - Milk may serve not only as a potential vehicle of transmission of disease-causing organisms, but it can also allow these pathogens to grow, multiply and produce certain toxic metabolites, thereby making itself an extremely vulnerable commodity from the public health point of view. (Kluytmans, 1998).

A variety of pathogenic organisms may gain access to milk and milk products from different sources and cause different types of food-borne illnesses. Milk and milk products may carry organisms as such or their toxic metabolites (poisons) called toxins to the susceptible consumers. Ingestion of toxins already synthesized in the food which is, pre-formed brings about poisoning syndromes in the consumers. This is called ‘food intoxication’ and the toxins affecting the gastrointestinal tract are called enterotoxins. The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of Staph. aureus. Milk and other dairy products are frequently infected with S. aureus. Milk of infected animals is the main source of enterotoxigenic S. aureus of animal origin. For example, certain S. aureus strains produce heat-resistant enterotoxins, which cause nausea; vomiting, and abdominal cramps when ingested by humans and are responsible for staphylococcal food poisoning outbreaks (Kluytmans, 1998).

Teratogenic effect: - The term teratogenic applies to a drug or a chemical agent that produces a toxic effect on the embryo of the fetus during a critical phase of gestation. As a consequence, a congenital malformation that affects the structural and functional integrity of the organism is produced. The well-known thalidomide incident involving several children in Europe was a direct testimony to hazard that may occur when such an agent is administered during pregnancy (Joes, 1993).

Disruption of normal intestinal flora: - The bacteria that usually live in the intestine acts as a barrier to prevent the incoming pathogens from getting established and causing diseases. Antibiotics may reduce the total number of bacteria or selectively kill some important species. The broad-spectrum antimicrobials may adversely affect a wide range of intestinal flora and as a consequence cause a gastrointestinal disturbance. For example, da rugs like, flunixin and streptomycin are known for this effect (Jackson, 1980).

Environmental impact: - Animals may excrete metabolites of antimicrobials through urine and feces and reach the soil and water. The most prevalent antimicrobials found in the environment (surfaces of water) belong to the macrolide and sulfonamide groups. Tetracycline or penicillin has only been found in some cases and at low concentrations (Sundlof, S.F., 1990). Some commonly used antimicrobials such as erythromycin, sulfadiazine, and tetracycline are antimicrobials that persist in the soil and remain on the surface of water and soil for over a year (Zuccato et al. 2000).

Safety Evaluations: Regulatory levels have been established for drug residues in food in the form of maximum residue limit (MRL). To assess the safety of ingested antimicrobial residues national and international committees evaluated data on chemical, pharmacological, toxicological, and other properties (Wood ward., 1998).

Acceptable daily intake (ADI): - Acceptable daily intake (ADI) for a given compound is the amount of a substance that can be ingested daily over a lifetime without appreciable health risk. The calculation of ADI is based on an array of toxicological safety evaluation that considers acute and long-term exposure to the drug and its potential impact. This defines a maximum quantity that may be consumed daily by even the most sensitive group in the population without any outward effects. The ADI is determined as a consecutive estimate of a safe ingestion level by the human population based on the lowest no-effect level (NOEL) of toxicological safety studies (EC, 2001).

Maximum residue level (MRL): - The maximum residue level (MRL) is the maximum concentration of residue resulting from the use of a veterinary medicinal product that may be legally permitted or recognized as acceptable in or on a food, allocated to individual food commodities. Substances for which no maximum residue limit can be established because residues of these substances, at whatever limits in foodstuffs of animal origin constitute a hazard to the health of the consumer (EEC, 1990). The maximum residue level of some veterinary drugs in milk is shown in Table 2.

Table 2. Residue limits of some common veterinary drugs (μg/kg) set for Milk

Antimicrobials	MRL (μg/kg)
Procain benzyl penicillin	4
Streptomycin	200
Diaminazine	150
Oxefendazole	100
Neomycin	100
Oxytetracycline	500
Sulfadimidine	25

Source: CAC (1998)

Table 3. Discard time for milk of some antibiotics in dairy cows

Drug	Discard time for milk prior to sale
Ampicillin	48 hours
Erythromycin	72 hours
Procaine benzyl penicillin	72 hours
Sulphadimethoxine	60 hours

Source: Prescott and Baggot (1993).

Withdrawal period: - Use of animal medicines require observance of the withdrawal period. This is the time between the last doses given to the animal and the time when the level of residues in the tissues (muscle, liver, kidney, skin, and fat) and products (milk, eggs, honey) is lower than equal to the MRL. Until the withdrawal period has elapsed, the animal or its products must not be used for human consumption (Jackson., 1980).

Public health hazards associated with antibiotic residues in milk

Milk is one of the most nutritious and complete food. It is rich in high-quality protein providing all ten essential amino acids, fat especially essential fatty acids, most of the minerals and vitamins. Meanwhile, milk as a nutrient has the main role in the human diet, especially for children (Hassan, 2005; Enb et al., 2009). Concerns about food safety, with animal source foods, are increasing in developing countries where urbanization, increasing incomes, and changing of lifestyles are associated with greater dependence on marketed foods by an increasing number of people (Delgado et al., 1999). The safety of food is threatened by various agents including pathogenic microorganisms, aflatoxins, pesticides, and antimicrobial agents. Pathogenic microorganisms constitute the most important food-related to threaten public health. Little information about the level of antimicrobial residues in food is found in developing countries. While pasteurization and other forms of heat treatment eliminate pathogenic microorganisms from animal source food. These procedures have limited or variable effects have on drug residues in animal-originated food (Moa, 1988; Wang et al., 2006).

Dairy cattle are susceptible to many diseases particularly mastitis, in this regard, somatic cell count (SCC) is used as a key indicator of milk quality and reflects the prevalence of subclinical mastitis in a dairy herd. The SCC is an indirect measure of the overall amount of mastitis and herds with high SCC have been reported to have higher rates of clinical mastitis (Rodrigues et al., 2004). Farms experiencing consistently high SCC have considerable motivation to reduce the number of infected quarters. Treatment of infected quarters using antibiotics is one practice used to control mastitis. The use of antibiotics introduces the risk of having an antibiotic residue. Indiscriminate use of antibiotics and mistakes regarding withholding periods causing antibiotic residues in milk and meat. The occurrence of antibiotic residues in milk intended for human consumption is undesirable for several reasons such as to cause allergic reactions, the incidence of bacterial resistance, disrupting of the balance of gut microflora, carcinogenesis, and mutagenesis and malformation risks. In addition, the presence of antibiotic residues in milk be used in the dairy industry can have adverse effects on the production of fermented dairy products such as yogurt and cheese (Suhren, 2002; Tikofsky et al., 2003; Erskine et al., 2004; Mohsenzadeh and Bahrainpour, 2008; Movassagh and Karami, 2010). The objectives of this study were to investigate the occurrence of antibiotic residues in raw cow milk, evaluate SCC value, and survey the relationship between milk SCC and antibiotic residue value in raw milk.

Most of the antibacterial currently used in the control and treatment of farm animal diseases are relatively non-toxic even at higher concentrations, but few antibiotics pose a significant threat to public health when present in sufficiently high concentrations in food (McEwen and McNab, 1997). Antibiotic residues in milk are of great public health concern since milk is being widely consumed by infants, youngsters, and adults throughout the globe (Khaniki, 2007). Considering the issue of public health hazards, milk and milk products contaminated with antibiotics and other chemical contaminants beyond given residue levels, are considered unfit for human consumption (Hillerton et al., 1999; Goffova et al., 2012). Occurrences of veterinary drug residues pose a broad range of health consequences in consumers. The residues of anti-bacterial may present pharmacological, toxicological, microbiological, and immune-pathological health risks for humans (Drackova et al., 2009). In rare situations, the pathogens against which the antibiotics are being used have fewer public health hazards than those posed by the improper use of antibiotics. To enlist one example, mastitis pathogens in milk pose a lower threat to public health if milk is pasteurized. On the other hand, the careless antibiotic therapy to eliminate mastitis pathogens becomes a public health concern due to their residues in milk (Hameed, 2006).

Unscrupulous use of antimicrobials in animal production leads to the development of resistant bacteria and which later transmit to humans through food, environment, or by direct contact. So, it is mandatory to follow withdrawal periods for such compounds when used in animal production to safeguard public health. The withdrawal period is a time between the last dose of antibiotic given to food animals and consumption of food animals or food derived from it. It needs to be mentioned on the antibiotics that are used for animals and if not mentioned properly it is considered to be as 28 days in the Indian context (CSE, 2014).

According to Nisha (2008), the major pathological effects produced by antibiotic residues in food apart from the transfer of antibiotic-resistant strains of bacteria to human beings includes immunological effects, autoimmunity disorders, carcinogenicity especially due to sulfamethazine, oxytetracyclines, and furazolidone, mutagenicity, nephropathy due to gentamycin, hepatotoxicity, reproductive disorders, bone marrow toxicity due to chloramphenicol and allergy due to penicillin.

Antibacterial causes a broad range of health effects, to summarize they can cause development anomalies e.g. bone marrow aplasia (chloramphenicol) and can alter the normal gastrointestinal microflora resulting in GI disturbances (intestinal dysbiosis) and development of resistant strains of bacteria. Therefore, the use of antibacterials may result in the emergence of antibiotic-resistant strains of pathogens, complicating the treatment for both human and animal diseases (Dewdney et al., 1991; Goffova et al., 2012).

These hazards can be categorized into two types as direct-short term hazards and indirect-long term hazards, according to the duration of exposure to residues and the time onset of health effects (Muhammad et al., 2009). The direct health hazards include the health effects caused due to excretion of the rugs in milk, an example, the beta-lactam group of antibiotics regardless of their low concentration in milk causes an allergic hypersensitive reaction in sensitized individuals immediately after consumption (Paige et al., 1997; Sierra et al., 2009).

Several antibiotics are potent antigens or act as happens and occupational exposure daily can lead to allergic reactions. Most of the reported allergic reactions are related to ß-lactam antibiotic residues in milk or meat and the allergic reaction has been associated with exposure to antibiotic residues in foods. Many of the cases refer to people previously treated with antibiotics and hypersensitized to a degree that subsequent oral exposure evoked a response. A hypersensitivity reaction to a drug is either IgE-mediated or Non–IgE-mediated reactions. IgE-mediated reactions occur shortly after drug exposure. Instances of IgE-mediated hypersensitivity reactions include urticaria, anaphylaxis, bronchospasm, and angioedema. Non-IgE-mediated reactions include hemolytic anemia, thrombocytopenia, acute interstitial nephritis, serum sickness, vasculitis, erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis (Granowitz et al., 2008).

Indirect and long-term hazards are the effects caused by long-term exposure of an individual to residues and include carcinogenicity, teratogenicity, and reproductive effects. Long-term exposure to diethyl stilbesterol can cause vaginal clear cell adenocarcinoma and benign structural abnormalities (Offman and Longacre., 2012). The long-term exposure to antibiotic residues in milk may result in an alteration of the drug resistance of intestinal microflora (Ram et al., 2000). The use of the antibiotic avoparcin as a growth promoter in food animals resulted in the development and amplification of vancomycin-resistant enterococci. Subsequent colonization in the human intestine of these resistant strains causes a clinical disease that would be difficult to treat (Tzavaras et al., 2012). Even if the resistant strains of bacteria are not human pathogens, they may still be dangerous because they can transfer their antibiotic resistance genes to other pathogenic bacteria (Hayek, 2013).

Apart from the health hazards, antimicrobial residues in milk are responsible for interference with starter culture activity and hence disrupt the manufacturing process of milk products (Katla et al., 2001). In the fermented dairy products manufacturing plants, such as cheeses and yogurts, the presence of antimicrobial agents can lead to the partial or total inhibition of the lactic bacterial growth. Antibiotic residues can also interfere with the methylene blue test, intended to estimate the total microbial load in milk. The time taken for reduction of the dye will be increased, hence causing underestimation of the microbial load. All of these concerns may result in major economic losses to the dairy industry.

Antimicrobial residue detection methods

At present, the regulatory agencies required methods with high throughput, fast, reliable, and sensitive and which can even process solid samples for antibiotic residue detection. In this context of residue detection, immunoassays and biosensors are of great importance compared to microbial assays (Chafer-Pericas, Maquieira, & Puchades, 2010). Biosensors always provide opportunities for automation, in situ analysis, and the development of a large number of commercial detection kits. Any biosensor system comprises two main components namely, a transducing device and a recognition element.

Screening tests: - Screening tests are used to detect the presence of an analysis or a class of analysis at the level of interest. They are aimed at avoiding false-negative results while false-positive results are tolerable. These tests when used for substances with an established maximum residual limit, the detection should be as low as possible (IDF, 1995). The most common screening methods for antimicrobial drug residues are anti-micro biological tests, based on the growth inhibition of microorganisms (e.g. Bacillus stearothermophilus). There are on-farm screening tests devised for rapid detection of low concentrations of antimicrobial residues in milk (Tyler et al. 1992). The most common assay system monitors inhibition of the growth of a test organism. This type of assay system cannot identify the nature of the compound responsible for the growth inhibition. A well-known essay in this category includes charm inhibition assay and devoted.

The assay systems of some of the newer residue detection tests are based on immunobinding of unique antigenic structures in antimicrobial or inherent antimicrobial receptor interaction (Calderon et al. 1996). Charm inhibition assay uses B. stearothermophlus in tablet form and a specially formulated agar medium. The antimicrobial substances in the milk sample inhibit microbial sporulation and growth, which results in reduced acid production. The pH indicator changes from blue to greenish-yellow if the milk does not contain an inhibitory substance, it remains blue if the milk contains a growth inhibitor (Bishop and White, 1999).

Confirmatory tests: - These methods provide full or complementary information enabling analyzes to be identified unequivocally at the level of interest. The tests are employed to determine the presence or absence of residues in a sample found positive by the routine screening test. These tests are aimed at preventing false-positive results (Heitzmn, 1994).

In general, Measures to reduce antibiotic residue in livestock products include, which were adopted from the CSE report and other published pieces of literature. (1) Reduce antibiotic use in food animal rearing. Many developed countries have banned its use as a growth promoter. (2) Rapid screening methods should be developed for detecting and segregating samples containing above MRL levels of antibiotics. (3) Appropriate MRLs need to be set by regulatory bodies and should enforce it. (4) Appropriate withdrawal periods should be strictly followed and enforced to make the meat rendered safe for human consumption. (5) Improve individual and organizational awareness by enhancing proper knowledge dissemination. (6) Follow best hygiene practices during animal rearing and avoid unwanted use of antibiotics. (7) Alternates to antibiotics like biocontrol measures and Ethno-veterinary practices should be developed and followed. (8) Organic poultry farming may be encouraged by providing appropriate incentives to the farmers in form of subsidies. (9) Use of proper processing techniques to inactivate the antibiotic residue, e.g. refrigeration causes inactivation of penicillin. (10) Use of activated charcoal, resins, and UV irradiation to inactivate residues.

Prevention of antimicrobial residues

A food control system is an official institution set up, at the national and sub-national levels, responsible for ensuring the quality and safety of food supply. It includes the relevant food legislation and regulation, food inspection, food analysis, food import /export inspector, and certification and food control management. It is important to remember that the individuals most likely to come into first contact with antimicrobial-resistant bacteria in the dairy are the dairy producers and their families (Heeschen and Suhern, 1996).

Providing on-farm food safety programs, which address the daily management of the production until concerning animal health and well-being, public health, and environmental health must be a top priority for agriculturalists and veterinarians. Milk quality assurance program will become a valuable tool in maintaining a safe and wholesome product (Wallace, 2007).

The following are some of the prevention strategies of drug residues: practice healthy herd management; establish a valid veterinarian patient relationship; use prescription drugs with a veterinarian's guidance and maintain milk quality and food safety beginning from the dairy products; implement an effective mastitis management program; administer drugs properly and identify treated animals; use drug residue screening test; maintenance and use of proper treatment records in all treated animals; implement employee/family awareness of proper drug use to avoid marketing adulterated milk and complete the milk and residue prevention protocol annually.

Conclusion and recommendations

Dairy management has a role in the complex tasks of producing high-quality milk management and employee must become very knowledgeable about the milk quality factor and tasks that impact each factor training of producers and other holders is a key point to improve quality. Any control strategy should be implemented alongside educational measures to the producer on antimicrobial use and the adoption of sound management practices. The correct identification of the causative agent of the disease and strict adherence to antimicrobial label recommendations is one of the easiest ways of reducing the likelihood that antimicrobial-resistant bacteria will enter the food chain. In conclusion, milk producers should be aware of the risks with antimicrobial residue as a result of failure to respect the withdrawal time.

Mastitis is usually considered the most important disease in dairy cattle and human beings and hence remained a concern of most dairy farmers and veterinarians. The bacteria that are transmitted through the milk and cause disease problems in man are bacteria causing mastitis in cattle. The major bacteria that can be transmitted through the milk and produce disease in man include mycobacterium bovis, Brucella abortus, staphylococcus aureus, Listeria monocytogenes, and Campylobacter jejuni from affected udder due to localization from their septicemic phase. Antibiotic residues following treatment of mastitis can be a potential hazard to humans in allergic reactions and possible transfer of resistance to other organisms. Based on the above facts the following points are recommended: Mastitis control measures should be largely undertaken to reduce bacterial contamination of milk from the udder, Education of the public at large about the hazard of raw milk consumption and the possible control and preventive measures through heat treatment should always be encouraged before milk is consumed and Milk producers should be educated about risks associated with antimicrobial residues as a result of failure to respect the with drawl period. Finally, extensive use of antibiotics as growth promoters in animal feed poses the threat for the development of new resistant strains of bacteria, which may not be susceptible to the most advanced antibiotics available nowadays.

Acknowledgment

I am deeply grateful and glad to all sources of materials used for reviewing this manuscript have been suitably acknowledged.

References

Almeida, R.A., Dogan, B., Klaessing, S., Schukken, Y.H., Oliver, S.P. 2011. Intracellular fate of strains of Escherichia coli isolated from dairy cows with acute or chronic mastitis. Veterinary Research Community, 35: 89-101.

ARESTRUP, F. M. 2005: Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic & Clinical Pharmacology & Toxicology, vol. 96 (4), 2005, p. 271-281.

Babapour, A., Azami, L. and Fartashmehr, J., 2012. Overview of antibiotic residues in beef and mutton in Ardebil, North West of Iran. World Applied Sciences Journal, 19, 1417-1422.

Bachaya, H.A., Raza, M.A., Murtaza, S. & Akbar, I.U.R. 2011. Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan)', Journal of Animal and Plant Sciences, 21: Pp. 16-19.

Barbano, D. 2004. The role of milk quality in addressing dairy food marketing opportunities in a global economy, NC, USA.

Bishop, J.R. and C.H. White, 1999. Antimicrobial residue in milk, a review J. Food Prod., 47: 647-652.

Blowey, R. and Edmondson, P. 1995. Mastitis control in dairy herds, an illustrated and practical guide. Farming press books, Ipswich, UK.

Booth, N.H. and L.E. McDonald, 1988. Veterinary pharmacology and therapeutics, 6th edition, Iowa State University Press. Ames, USA, pp: 1065-1079.

Bradley, A., Green, M.J. 2005. Use and interpretation of somatic cell count data in dairy cows. In Practice, 27: 310-315.

Bradley, A.J. and Green, M.J. 2001. Etiology of clinical mastitis in six Somerset dairy herds. Veterinary Research, 148(22): 683-686.

Bramley, A.J., McKinnon, C.H. 1990. The Microbiology of Raw Milk. In: Dairy Microbiology, I, (Ed.: Robinson, R.K.). London, New York, Elsevier Applied Science, 171.

Breen, J. E., M. J. Green and A. J. Bradley. 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. Journal of Dairy Science, 92:2551-2561.

Bruckmaier, R.M. (2005). Normal and disturbed milk ejection in dairy cows. Domestic Animal Endocrinology, 29: 268 – 273.

Burvenich, C., Van Merris, V., Merhzad, J., Diez-Fraile, A., Duchateau, L. 2003. The severity of E. coli mastitis is mainly determined by cow factors. Veterinary Research, 34: 521-564.

Calderon, V., J. Gonzaleze, P. Dieg and J.A. Berguer, 1996. Evaluation of a multiple bioassay technique of determination of antibiotic residues in meat with a standard solution of antimicrobials. Food adds. Cont., 13: 13-19.

Capuco, A.V., Ellis, S.E., Hale, S.A., Long, E., Erdman, R.A. 2003. Lactation Persistency: insights from mammary cell proliferation studies. Journal of Animal Science, 81: 18-31.

Central Statistical Agency (CSA) (2009): Federal Democratic Republic of Ethiopia Central Statistical Agency (CSA): Agricultural Sample Survey. Livestock and livestock Characteristics vol, 2

Centre for Science and Environment Study. (2014). Antibiotics in chicken meat (36 p.). New Delhi: Pollution Monitoring Laboratory/PR-48/2014.

Chafer-Pericas, C., Maquieira, A., & Puchades, R. (2010). Fast screening methods to detect antibiotic residues in food samples. TrAC Trends in Analytical Chemistry, 29, 1038–1049.

Compton, C.W.R., McDougall, S., Parker, K. and Heuer, C. 2007. Risk factors for peripartum mastitis in pasture-grazed dairy heifers. Journal of Dairy Science, 90: 4171- 4180.

Constable, p. d. – morin, d. e. 2003: Treatment of clinical mastitis. Using antimicrobial susceptibility profiles for treatment decisions. Veterinary Clinics of North America. Food Animal Practice, vol. 19 (1), 2003, p.139-155.

DeGraves, F.J., and Fetrow, J. 1993. Economics of mastitis and mastitis control. Veterinary Clinic North America Food Animal Practice, 9: 421-434.

Dogan, B., Klaessig, S., Rishniw, M., Almeida R.A., Oliver, S.P. 2006. Adherent and invasive Escherichia coli are associated with persistent bovine mastitis. Veterinary Microbiology, 116: 270-282.

Donoghue, D. J. (2003). Antibiotic residues in poultry tissues and eggs: human health concerns? Poultry Science, 82, 618–621.

Drackova, M., Navratilova, P., Hadra, L., Vorlova, L. and Hudcova, L., 2009. Determination residues of penicillin G and cloxacillin in raw cow milk using Fourier transform near-infrared spectroscopy. Acta Veterinaria Brno, 78, 685-690.

Dzidic, J., Macuhova, C.A., Bruckmaier, R.M. (2004). Effects of cleaning duration and water temperature on oxytocin release and milk removal in an automatic milking system. Journal of Dairy Science, 87: 4163 – 4169.

Edwards, S.J., 1964. The diffusion and retention of penicillin after injection into the bovine udder. Veterinary Record, 76, 545-549.

Enb, A., Abou Donia, M.A., Abd-Rabou, N.S., Abou Arab, A.A.K. and El Senaity, M.H., 2009. Chemical composition of raw milk and heavy metals behavior during processing of milk products. Global Veterinaria, 3, 268-275.

Erskine, R. J. 2001. Mastitis control in dairy herds. In: Radostits, O. M. (ed.): Herd Health: Food Animal Production. 3rd ed. Philadelphia: W. B. Saunders Company. 397 – 435.

Erskine, R. J. 2011. Mastitis in cattle. The Merck Veterinary Manual. Merck Sharp and Dohme Corp. Whitehouse Station, N.J.

European Community (EC), 2001. Notice to applicant and note for guidance. Establishment of maximum residue limits for the residue of veterinary medicinal Products in Food Stuffs of Animal Origin, pp: 4-10.

European Economic Community (EEC), 1990. Council regulation 2377/90 of 26 June 1990 laid down a community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Off. J. Eur. Common. L., 224: 1-8.

European Medicines Agency. (2013). Sales of veterinary antimicrobial agents in 25 EU/EEA countries in 2011: Third ESVAC report. London: Author.

Fadlelmula, A. A. l., Dughaym, A.M., Mohamed, G.E., A.l. Deib, M.K., A.l., Zubaidy, A.J. 2009. Bovine mastitis: Epidemiological, clinical and etiological study in a Saudi Arabian large dairy farm. Bulgarian, Journal of Veterinary Medicine, 12: 199-206.

FAO/WHO, 1995. Forty-second report of the joint FAO/WHO Expert Committee on Food Additives, Evaluation of certain veterinary drug residues in food. Technical Report Series No. 851, pp. 19-21. WHO, Geneva.

Folly, M. and S. Machado, 2001. Antimicrobial residue determination using microbial inhibition, protein binding, and immune assay methods, in pasteurized milk commercialized in the northern region of Rio de Janeiro state, Brazil. Cienc. Rural, 31: 95-98.

Food and Agricultural Organization (FAO) and World Health Organization (WHO), 1995. Application of risk analysis to food standards. Report of the Joint FAO/WHO expert consultation Geneva, Switzerland, pp: 13-17.

Food and Agricultural Organization (FAO), 1998. Validation of Analytical Methods for Food Control, pp: 44-56.

Food and Drug Administration. (2010). CVM updates-CVM reports on antimicrobials sold or distributed for food-producing animals. Silver Spring, MD: Author.

FOX, L. K. – GAY, J. M. 1993: Contagious mastitis. The Veterinary clinics of North America. Food animal practice, vol. 9 (3), 1993, p. 475-487.
Fox, L.K. 2009. Prevalence, incidence, and risk factors of heifer mastitis. Veterinary Microbiology, 134: 82-88.
Galton, D.M., Peterson, L.G., Merrill, W.G. (1986). Effects of pre-milking udder preparation practices on bacterial counts in milk and on teats. Journal of Dairy Science, 69: 260 – 266.
Giesecke, W.H., Du Preez, J.H., Petzer, I.M. 1994. Practical Mastitis Control in Dairy Herds. Diagnosis of udder health problems, Butterworth Publishers: Durban, South Africa.
Goffova, Z.S., Kozarova, I., Mate, D., Marcincak, S., Gondova, Z. and Sopkova, D., 2012. Comparison of detection sensitivity of five microbial inhibition tests for the screening of aminoglycoside residues in fortified milk. Czech Journal of Food Sciences, 30, 314-320.
Gracey, J.F., D.S. Collins and R.G. Huey, 1999. Meat Hygiene. 10 ed. Harcourt Brace and Company, pp: 143-174, 328-331.
Granowitz, E.V. and Brown, R.B., 2008. Antibiotic adverse reactions and drug interactions. Critical Care Clinics, 24, 421-442.
Grohn, Y.T., Erb, H.N., McCulloch, C.E., Saloniemei, H.S. 1990. Epidemiology of mammary gland disorders in multiparous Finnish Ayrshire cows. Preventive Veterinary Medicine, 8: 241-252.
Gutierrez, G., Elez, M., Clermont, O., Denamur, E. and Matic, I., 2011. Escherichia coli YafP protein modulates the DNA damaging property of the nitroaromatic compounds. Nucleic Acids Research, 39, 4192–4201.
Harmon, R.J. 1994. Physiology of mastitis and factors affecting somatic cell counts. Journal of Dairy Science, 77: 2103-2112.
Hassan, L., Mohammed, H.O., McDonough, P.L. (2001). Farm - management and milking practices associated with the presence of Listeria monocytogenes in New York state dairy herds. Preventive Veterinary Medicine, 51: 63 – 73.
Hayek, N., 2013. Lateral transfer and GC content of bacterial resistance genes. Frontiers in Microbiology, 4, 41.
Heeschen, W. and G. Suhern, 1996. Principles and practical experience with an integrated system for the Detection of Antimicrobials in Milchwissenschaft, 51: 154-160.
Heinrichs, A.J., Costello, S.S., Jones, C.M. 2009. Control of heifer mastitis by nutrition. Veterinary Microbiology, 134: 172-176.
Hubbert, W.T., Hagstad, H.V., Spangler, E., Hinton, M.H. and Hughes, K.L., 1996. Food Safety and Quality Assurance (Foods of Animal Origin). 2nd Edn. pp: 8, 239-273. Iowa State University Press, United States.
International Dairy Federation (IDF), 1995. Symposium on the residue of antimicrobial drugs and other inhibitors in milk. Proceedings of a joint conference of international and German national committee of IDF, Germany, p: 148-151.
International Livestock Research Institute (ILRI). 2007. Markets That Work: Making a Living from Agriculture. Nairobi, Kenya: International Livestock Research Institute, Annual Report, (2007).
Jackson, B.A. (1980). U.S. Food and Drug Administration, Center for Veterinary Medicine. Hosted at the University of Nebraska Lincoln. Jamai. Vet. Med. Assoc., 176: 1141.
Jackson, G.S., 1980. Safety assessment of drug residue. J. Am. Vet. Med. Assoc., 176: 1141-1144.
Jones, G.M. 2009. The Role of Milking Equipment in Mastitis. Virginia Cooperative Extension.
Kanneene, J.B. and R. Miller, 1997. Problems associated with drug residue in the feed from feeds and therapy. Res. Sci. Tech., 16: 694-708.
Kassa, T., Wirtu, G., Tegegne, A. 1999. Survey of mastitis in dairy herds in the Ethiopian central highlands. Ethiopian Journal of Science, 22: 291-301.
Katla, A. K., Kruse, H., Johnsen, G. and Herikstad, H., 2001. Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. International Journal of Food Microbiology, 67, 147-152.
Katla, A.K., H. Kuse, G. Johansler and H. Helistad, 2001. Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. Int. J. Food Microbial, 67: 147-152.
Kavitha, K.L., Rajesh, K., Suresh, K., Satheesh, K., and Syama Sundar, N.,2009. Buffalo mastitis risk factors. Buffalo Bull., 28(3): 134-137.
Kemp, M. H., A. M. Nolan, P. J. Cripps, and J. L. Fitzpatrick. 2008. Animal-based measurements of the severity of mastitis in dairy cows. Veterinary Research, 163:175-179.
Kerro, O. and Tareke, F. 2003. Bovine Mastitis in Selected Areas of Southern Ethiopia. Tropical Animal Health Production, 35: 197-205.
Khaki, R. J., 2007. Chemical contaminants in milk and public health concerns: a review. International Journal of Dairy Science, 2, 104-115.
Knappstein, K., Suhren, G. and Walte, H., 2003. Influence of milking frequency on withdrawal period after application of beta-lactam antibiotic-based drugs. Analytica Chimica Acta, 483, 241-249.
Kumar, A., Rai, D.C. and Choudhary, K.R., 2011. Prospects and opportunities for exports of dairy products from India. Indian Journal of Animal Science, 81, 188-193.

Kummerer, K., 2009. Antibiotics in the aquatic environment – A review – Part I. Chemosphere, 75, 417-434.
Larsen, D. 2000. Milk quality and mastitis. Veterinary Microbiology, 71: 89- 101.
Mahajan, S., Bhatt, P., Ramakant, Kumar, A., Dabas, Y.P.S. 2011. Risk and occurrence of bovine mastitis in Tarai region of Uttarakhand. Veterinary Practice, 12: 244- 247.
McEvoy, J.D., 2002. Contamination of animal feeding stuffs as a cause of residue in food. A review of regulatory aspects of incidence and control. An. Che. Acta., 473: 3-26.
Mein, G.A., Schuring, N. 2003. Lessons from scrapbooks and scrap heaps of history. Bulletin-International Federation.
Mitchell, J.M., Griffi this, M.W., McEwen, S.A., McNab, W.B., Yee, A. (1998). Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. Journal of Food Protection, 61: 742 – 756.
Moges, N., T. Hailemariam, T. Fentahun, M. Chanieand A. Melaku. 2012. Bovine mastitis and associated risk factors in smallholder lactating dairy farms in Hawassa, Southern Ethiopia. Global Veterinaria, 9: 441-446.
Mohamed, A. M. A., E. Simeon and A. Yemesrach. 2004. Dairy development in Ethiopia. International Food Policy Research Institute, EPTD Discussion Paper No. 123. Washington
Mohsenzadeh, M. and Bahrainpour, A., 2008. The detection limits of antimicrobial agents in cow milk by a simple yogurt test. Pakistan Journal of Biological Sciences, 11, 2282-2285.
Moore, D.A., J.H. Kirk and D.J. Klingborg, 2004. Maximizing quality and profits. J. Dairy Sci.
Muhammad, F., Akhtar, M., Javed, Z. I. and Irfan Anwar, M., 2009. Role of veterinarians in providing residue-free animal food. Pakistan Veterinary Journal, 29, 42-46.
National committee for clinical laboratory standards (nccls), 2002: Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standard. NCCLS Document M31-A2, Wayne, 2002, PA
National Mastitis Council (NMC) (1996). Milk-born intoxication. The importance of milk quality and mastitis control.
Nisha, A. R. (2008). Antibiotic residues-A global health hazard. Veterinary World, 1, 375–377.
NMC. (2009). NMC recommended a mastitis control program. Available online: (accessed 25 August 2009).
Nyman, A. K., T. Ekman, U. Emanuelson, A. H. Gustafsson, K. Holtenius, K. Persson Waller and C. Hallen Sandgren. 2007. Risk factors associated with the incidence of veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and a low prevalence of subclinical mastitis. Preventive Veterinary Medicine, 78:142-160.
Offman, S.L., and Longacre, T.A., 2012. Clear cell carcinoma of the female genital tract (not everything is as clear as it seems). Advances in Anatomic Pathology, 19, 296-312.
Ojo, O.E., Oyekunle, M.A., Ogunleye, A.O., Otesile, E.B. 2009. Escherichia coli, O157:H7
In food animals in part of South-Western Nigeria. Prevalence and in vitro antimicrobial susceptibility. Tropical Veterinary, 26(3):23-30.
Ott, S. (1999). Costs of herd-level production losses associated with subclinical mastitis in US Dairy Cows. In Proceedings: 38th Annual meeting of National Mastitis Council, pp. 152 – 156. Arlington VA.
Packham, W., C. Broome, Y. Limsowtin and H. Regionski, 2001. Limitation of standard antibiotic screening assays when applied to milk for cheese making. Aus. J. Dairy Technol., 56: 15-18.
Paige, J.C., Tollefson, L. and Miller, M.A., 1999. Health implications of residues of veterinary drugs and chemicals in animal tissues. Veterinary Clinics of North America: Food Animal Practice, 15, 31-43.
Passey, S., Bradley, A., Mellor, H. 2008. Escherichia coli isolated from bovine mastitis invade mammary cells by a modified endocytic pathway. Veterinary Microbiology, 130: 151–164.
Peeler, E.J., Green, M.J., Fitzpatrick, J.L., Morgan, K.L., Green, L.E. 2000. Risk factors associated with clinical mastitis in low somatic cell count British dairy herds. Journal of Dairy Science, 83: 2464-2472.
Peter, H. and H. John, 2001. Antibiotics growth promoter. J. Vet. Phamacol. Ther., 24: 5-13.
Petersson-Wolfe, C.S., Mullarky, I.K., Jones, G.M. 2010. Staphylococcus aureus mastitis: cause, detection, and control.
Phillips, E., M. Lovie, R. Knowles, and K. Simor, 2000. A cost-effectiveness analysis of six strategies for cardiovascular surgery prophylaxis inpatient labeled penicillin-allergic residues review. Asian-Australian J. An. Sci., 14: 402-413.
Poelarends, J.J., Hogeveen, H., Sampimon, O.C., Sol, J. 2001. Monitoring subclinical mastitis in Dutch dairy herds. Proceedings of the second international symposium on mastitis and milk quality, Vancouver, British Columbia, 145-149.
Pol, M., Ruegg, P.L. (2007). Treatment practices and quantification of antimicrobial usage in conventional and organic dairy farms in Wisconsin. Journal of Dairy Science, 90: 249 – 261.
Prescott, J.F. and J.D. Baggot, 1993. Antimicrobial therapy inventory medicine, 2nd edition, Iowa University, pp: 564 -565.

Pyorala, S. 2003. Indicators of inflammation in the diagnosis of mastitis. Veterinary Research, 34: 565-578.
Pyorala, S. 2003. Indicators of inflammation in the diagnosis of mastitis. Veterinary Research, 34: 565-578.
Quinn, P. J., M. E. Carter, B. Markey, and G. R. Carter. 1999. Clinical Veterinary Microbiology. Mosby Publishing, London. 327-344.

Radostits, O. M., C. C. Gay, K. W. Hinchcliff and P. D. Constable.2007. Mastitis. In: Veterinary Medicine: A Textbook of disease of cattle, sheep, pigs, goats, and horses 10th edition, Ballier, Tindall, and London, 674-762.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. 2000. Mastitis. In: Veterinary Medicine, 9th ed., W.B. Saunders Company Ltd., London, 603-687.

Ram, C., Bhavadasan, M.K. and Vijaya, G.V., 2000. Antibiotic residues in milk. Indian Journal of Dairy & Bioscience, 11, 151-154.

Ranjan, R., Gupta, M.K., Singh, K.K. 2011. Study of bovine mastitis in different climatic conditions in Jharkhand, India. Veterinary World, 4: 205-208.

Reneau, J. 2012. Gear up for warm-weather mastitis management now. Dairy Star. Research in Ethiopia. 73-81.

Reneau, J.K., Saylor, A.J., Heinz, B.J., Bye, R.F., Farnsworth, R.J. (2003). Relationship of cow hygiene scores and SCC. In Proceedings: 42nd Annual Conference of the National Mastitis Council, 42: 362 – 363.

Reugg, L.P. (2001): Health and production management in dairy herds. In: Radostits, O.M. (ed), herd health, food animal production. 3rd ed. W. B. Saunders Company, Philadelphia, Pennsylvania, pp 211–244

Rich, M.L., Ritterhoff, R.J. and Hoffman, R.J., 1950. A fatal case of aplastic anemia following chloramphenicol (chloromycetin) therapy. Annals of Internal Medicine, 33, 1459-1467.

Riedl, M.A. and A.M. Cassilas, 2003. Adverse drug reactions types and treatment options. Am. Farm. Phys., 68: 1781-1790.

Rodenburg, J. 2012. Mastitis Prevention for Dairy Cattle: Environmental Control. Fact sheet.

Sandholm, M., Kaartinen, L. and Pyorala, S., 2009. Bovine mastitis- why does antibiotics therapy not always work: An overview. Journal of Veterinary Pharmacology and Therapeutics, 13, 248-260. Science, 2, 104–115.

Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tikofsky and R. N. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts. Veterinary Research.

Sentitula, Yadav, B.R., Kumar, R. 2012. Incidence of Staphylococci and Streptococci during winter in mastitic milk of Sahiwal cow and Murrah buffaloes. Ind. Journal of Microbiology, 52: 153-159.

Serratosa, J., Blass, A., Rigau, B., Mongrell, B., Rigau, T., Tortades, M., Tolosa, E., Aguilar C., Ribo, O. and Balague, J., 2006. Residues from veterinary medicinal products, growth promoters, and performance enhancers in food-producing animals: a European Union perspective. Revue Scientifique et Technique, 25, 637-653.

Seykora, A.J., McDaniel, B.T. 1985. Udder and teat morphology related to mastitis resistance: a review. Journal of Dairy Science, 68: 2087-2093.

Sharma, H., Maiti, S.K., Sharma, K.K. 2007. Prevalence, etiology, and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in Drug, Chhattisgarh state. International Journal of Dairy Science, 2: 145-151.

Sharma, N., Singh, N.K., Singh, O.P., Pandey, V., Verma, P.K. 2011. Oxidative stress and antioxidant status during the transition period in dairy cows. Asian-Aust. Journal of Animal Science 24: 479-484.

Sierra, D., Sanchez, A., Contreras, A., Luengo, C., Corrales, J.C., Morales, C.T. and Gonzalo, C., 2009. Detection limits of four antimicrobial residue screening tests for β-lactams in goat‟s milk. Journal of Dairy Science, 92, 3585-3591.

Sordillo, L.M. 2005. Factors affecting mammary gland immunity and mastitis susceptibility. Livestock Production Science, 98: 89-99.

Sori, T., A. Zerihun and S. Abdicho. 2005. Dairy Cattle Mastitis in and around Sebeta, Ethiopia. Int. Journal of Appl. Res. Veterinary Medicine, 3(4): 332-338.

Spears, J.W., Weiss, W.P. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Veterinary Journal, 176: 70-76.

Sundlof, S.F., 1990. Residue detection. A survey of available technology. Bov. Practice, 25-15 17.

Syensk Mjölk. 2003. Seminar on combating mastitis on a heard level. Uppsala.

Tangka D.K., Emerson R.D. and Jabbar M.A. 2002. Food Security effects of intensified dairying: Evidence from the Ethiopian highlands. Socio-economic and policy Research working paper 44, ILRI (International Livestock Research Institute), Nairobi, Kenya, 68.

Tenhagen, B.A., Koster, G., Wallman, J., Heuwieser, W. 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg. Journal of Dairy Science, 89: 2542-2551.

Tormo, M., Knecht, E., Götz, F., Lassa, I., Penadés, J. 2005. Bap-dependent biofilm formation by pathogenic species of Staphylococcus: evidence of horizontal gene transfer. Microbiology, 151: 2465-75.

Tyler, J.W., J.S. Culler, R.J. Emkine, W.L. Smita, J. Dellinger, and K. McClm, 1992. Milk antimicrobial drug residue assay results in cattle with experimental endotoxin-induced mastitis. J. Am. Vet. Med. Assoc., 201: 1378.

Unnikrishnan, V., Bhavadasan, M.K., Surendra Nath, B. and Ram, C., 2005. Chemical residues and contaminants in milk: A review. Indian Journal of Animal Science, 75, 592-598.

Varshney, J.P., and Naresh, R. 2004. Evaluation of homeopathic complex in the clinical management of udder diseases of riverine buffaloes. Homeopathy, 93:17.

Vass, M., Hruska, K. and Franek, M., 2008. Nitrofuran antibiotics: a review on the application, prohibition and residual analysis. Veterinarni Medicina, 53, 469-500.

Ward, W.R., Hughes, H.W., Faull, W.B., Cripps, P.J., Sutherland, J.P., Sutherst, J.E. (2002). Observational study of temperature, moisture, Ph, and bacteria in straw bedding, and fecal consistency, cleanliness, and mastitis in cows in four dairy herds. Veterinary Record, 151: 199 – 206.

Wary, C. and J. Gnanou, 2000. Antibiotic resistance monitoring in bacteria of animal origin analysis of national monitoring programs. Int. J. Antimicrob. Agents, 14: 291-294. Paige, J.C., 1994. Analysis of tissue residue. FDA. Vet., 9: 4-6.

Weinrauch, Y., Zychlinsky, A. 1999. The induction of apoptosis by bacterial pathogens. Annual Revised Microbiology, 53: 155-187.

Wiest, D.B., Cochran, J.B. and Tecklenburg, F.W., 2012. Chloramphenicol toxicity revisited: a 12-year-old patient with a brain abscess. Journal of Pediatric Pharmacology and Therapeutics, 17, 182-188.

Wong, S.H., Silva, F., Acheson, J.F., and Plant, G.T., 2013. An old friend revisited: chloramphenicol optic neuropathy. JRSM Short Reports, 4, 20.

World Health Organization (WHO), 2000. Overcoming antimicrobial resistance, in a report on infectious diseases. Geneva, Switzerland: World Health Organization, pp: 66-67.

Zafalon, L.F., Nader, Filho, A., Oliveira, J.V., Resende, and F.D. 2007. Subclinical mastitis caused by Staphylococcus aureus: cost-benefits analysis of antibiotic therapy in lactating cows. Arq Bras Medical Veterinary Zootec, 59: 577-585.

Zahid Hosen, S.M., Mostafa Kamal, A.T.M., Barua, S., Md Anwar, S., Mazumder, K., Md Hassan, K. and Md. Arifuzzaman, 2010. Detection of residual antibiotics in fresh cow milk, Bangladesh Pharmaceutical Journal, 13, 64-66.

Zdanowicz, M., Shelford, J.A., Tucker, C.B., Weary, D.M., von Keyserlingk, M.A.G. (2004). Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. Journal of Dairy Science, 87: 1694 – 1701.

Zuccato, E., D. Calamari, M. Natangelo and R. Fanelli, 2000. Presence of therapeutic drugs in the environment. The Lancet, 355: 1789-1790.