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Chronic Bovine Mastitis: A Food Safety Issue and Public Health Hazard

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Abstract

Milk is a nutrient dense liquid containing fats, proteins, carbohydrates and vitamins which provides an ideal matrix for the growth of many pathogenic microbial species. The presence of intramammary infections in bovine species has a significant impact on the dairy industry due to losses in the quantity and quality of milk. Causative agents of disease may gain entry into milk-based food items at any stage of food production and is typically an indicator of poor manufacture practices. Species such as S. aureus, S. uberis, S. agalactiae, E. coli and Pseudomonas have been known to induce sub-clinical or clinical mastitis which may persist chronically. In addition to possessing antimicrobial resistance mechanisms, these species are often adapted to the host immune system with virulence factors enabling them to colonize and thrive within the mammary gland. Therefore, while providing an invaluable food source to countless people globally, dairy produce may also act as a reservoir for disease and morbidity to consumers. The World Health Organization has listed Enterobacteriaceae and Pseudomonas as critically important on its watch list of priority pathogens challenging human safety. As such, the bacteriological safety of milk and dairy food represents a serious health hazard which producers must overcome. The association between food and nutrition impact on human and animal health related quality of life and economics is an important aim of the One Health approach. Efficient farm and milk management programs including optimal treatment of clinical mastitis cases, culling of non-responsive cows, cow teat and machine disinfection and maintenance is essential in controlling mastitis outbreaks within herds.

Keywords: Mastitis; Pathogenicity; Hazard; Consumer risk, Resistance; One health

Abbreviations: AMR: Antimicrobial Resistance; BTM: Bulk Tank Milk; CM: Clinical Mastitis; DCCD; Dairy Controls & Certification Division; ESBL: Extended-Spectrum Beta-Lactamase; FEC: Feed Efficiency Ratio; FCR: Feed Conversion Ratio; HACCP: Hazard Analysis Critical Control Points; HGT: Horizontal Gene Transfer; ID: Infectious Dose; IMIs: Intramammary Infections; LPS: Lipopolysaccharide; MDR: Multi-Drug Resistance; MRL: Maximal Residual Level; NMC: National Mastitis Council; PAMPs: Pathogen Associated Molecular Patterns; QAC: Quaternary Ammonia Compounds; SCC: Somatic Cell Count; SCM: Sub-Clinical Mastitis; SE: Staphylococcal enterotoxin; TNF-a - Tumour Necrosis Factor Alpha; WHO: World Health Organization

Introduction

The dairy industry represents one of the most important sources of nutritious and economically viable food options globally as well as providing valuable employment opportunities at all stages of production. Importantly, dairy food provides nutrient dense, low cost nourishment in developing countries which are often dependent on small scale farming for survival at individual and community level. As the increasing global population and climate change remain the forefront of public health concern, the future of animal and human health and sustainability of the planet is unclear. Food producers worldwide must overcome the challenge of supplying more food for the escalating population while navigating issues relating to climate change, increasing production costs, limited natural resources and environmental impacts [1]. The role of food and nutrition in public health of both society and individuals is increasingly being recognized in terms of both quality of life and economics [2]. Studies report that the consumption of dairy foods meets the nutrient recommendations of many countries and may reduce the incidence of noncommunicable chronic diseases including cardiovascular, endometriosis and colon cancers [3] an important point as the incidence of chronic diseases, hunger and malnourishment increases worldwide. Malnourishment is optimally prevented by the consumption of macro and micro nutrient dense foodstuffs consequently reducing rates of morbidity and mortality at individual, national and global levels. The benefits of dairy produce in preventing deficiency diseases associated with vitamin D, calcium and protein are evident [4]. Dairy produce therefore has clear socio-economic, economic and health benefits but is not without its own specific production limiting issues. Infection of the bovine mammary gland with pathogenic species resulting in intramammary infections (IMIs) is one such issue and is one of the biggest contributors to economic losses in the dairy industry. The economic impact at farm and industrial level of mastitis relates to treatment of disease, reduced milk yield and milk quality, reproductive issues and culling of diseased cows. Species of microorganisms associated with IMIs (Table 1) are categorized as environmental coliforms (*Escherichia coli,* Klebsiella), Pseudomonas and Streptococcus uberis or contagious pathogens (*Staphylococcus aureus, Streptococcus dysgalactiae*) resulting in acute or chronic sub-clinical (SCM) and clinical mastitis (CM) in susceptible hosts. The pathogenicity of the species and manifestation of disease relates to the environment, host immunity and the virulence of the infectious organisms. Chronic contagious SCM has a more severe economic impact than CM as it contributes to an increased loss in milk yield even though no visible alteration in the milk quality is evident in most cases. In addition to poor cow health, CM results in pregnancy losses due to the release of lipopolysaccharide (LPS) endotoxins by Escherichia coli (*E. coli*) or inflammatory responses on follicular growth, embryo development, corpus luteum survival [11] and a failure of neonates to survive post-partum. Treatment of infection relies on diagnosis symptoms with CM receiving antibiotic therapy in conjunction with IV fluids, electrolytes and anti-inflammatory agents. Concerns arise however, relating to the presence of antimicrobial resistant (AMR) species particularly in chronic cases of disease. Additional concerns relate to the presence of such AMR species and residual levels of antibiotics in the milk at harvest, potentially resulting in the foodborne transmission of drug resistant pathogens. In 2017 the World Health Organization (WHO) released a list of priority pathogens categorised as critical, high and medium priority; present on this list are several species commonly associated with mastitis, namely carbapenem-resistant, extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae (critical), carbapenem-resistant Pseudomonas (critical) and methicillin and vancomycin resistant *Staphylococcus aureus* (high priority) [12]. Furthermore, WHO endorsed a list of critically important antimicrobials for human medicine; which includes various compounds for the treatment of mastitis namely 3rd & 4th generation cephalosporins, fluoroquinolones and penicillin. Preventative control measures utilising effective disinfection solutions remains key in preventing the spread of both contagious and environmental pathogens, contamination of milking machines and animal housing. The recognition of residual concentrations of such disinfectants in food items exceeding maximal residual level (MRL) legislation and the environmental impact of biocide pollution, however, promotes difficulties for food production relating to biological and chemical food contamination. Furthermore, the expression of chemical resistance by multi-drug resistant (MDR) microbial species impacts on the effectiveness of currently used farm yard disinfectants such as chlorine and quaternary ammonia compounds (QACs) [13]. The contamination of milking equipment and the bulk milk tank at cleaning with unquantified levels of these compounds is also an important risk factor and contributor to dairy chemical pollution. As the dairy industry is such an important source of nutritious food, it is important to understand the impact bovine disease has on food production. In addition, the One Health approach aims to reduce zoonotic disease using preventative measures and practices in food safety, water quality and hygiene in an effort to better protect animal and human health. Consequently, this review aims to outline the varying impacts of sub-clinical and clinical mastitis on the dairy industry and public health safety and to provide insight into consequences of disease in dairy herds.

Table 1: Relative summary of mastitis causing pathogens and risk to consumer safety.

Pathogenic Species	Mode of Transmission	Virulence and Pathogenicity Mechanisms	Consumer Risk	Reference
Environmental				
Escherichia coli	Coliform species, faecal shedding and contamination of bedding surfaces and teat skin, harvest contamination of bulk milk tank	MDR, QAC and chlorine resistance, efflux pump, ESBL production, HGT, intrinsic resistance	Serovar dependent diarrhoea disease, haemorrhagic diarrhoea LPS induced septic shock enteric pathogenicity	Mamat et al., 2015 [5]
Klebsiella pneumonia				Lambertini et al., 2015 [6]
Bacillus cereus	Environmental soil and water, post-pasteurisation contamination of milk, bulk milk tank cleaning with contaminated water	Endospore production, thermoduric, psychrotrophic	Emetic diarrhoea, heat-labile enterotoxin diarrheal syndrome	Gopal et al., 2015 [7]
Pseudomonas aeruginosa		MDR, broad intrinsic antimicrobial resistance, efflux pump, ESBL production, HGT, psychrotrophic	MDR, broad intrinsic antimicrobial resistance, efflux pump, ESBL production, HGT, psychrotrophic	Garedew et al., 2012 [8]

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Streptococcus uberis	Bovine faecal shedding, bedding, uncleaned teat surfaces	AMR, nutritional flexibility, psychrotrophic	Pneumonia – high risk for immunocompromised persons	Domenico et al., 2015 [9]
Contagious				
Staphylococcus aureus	Bovine and human skin, horizontal transmission from milking equipment and personnel	Heat stable staphylococcal enterotoxins	nausea, violent vomiting, abdominal constriction, diarrhoea, SE gastroenteritis	Argudín et al., 2010 [10]
Streptococcus dysgalactiae		AMR, resistance to phagocytosis	Emerging human pathogen	Lambertini et al., 2015 [6]
Mycoplasma spp	Airborne transmission, contaminated water	MDR, gene mutations	Tuberculosis	Bolanos et al., 2017 [11]

Economics of Mastitis

Direct and indirect economic losses resultant from mastitis within herds varies greatly between animals and causative agents of infection. The Feed Conversion Efficiency (FCE) (feed mass into body mass) and Feed Cost Ratio (FCR) (ratio of feed intake to weight gain) are used to calculate the efficiency at which food animals turn feed in to food products for human use and are important concepts for profitability. The FCR also provides insight into the environmental impact of the farming system as it provides an indication of the lost feed nutrients to the environment which may impact on the aquatic ecosystem, biodiversity and eutrophication [14]. Improving FCE in dairy production relates to increasing the amount of milk produced per unit of consumed feed and aims to have a net positive effect economically and environmentally. For bovine species, animal protein is produced via conversion of plant protein, a conversion which is necessary for humans to obtain protein from grass crops. The presence of milk protein in cows is therefore dependent of conversion of feed protein with a ratio of approximately 1:1.8 for milk to feed proteins [15]. Feed which contains low levels of feed protein passes through the gastro intestinal tract of the animal more readily as animal excrement with a lower FCE, resulting in a negative impact on the environment due to nitrogen pollution. The presence of microbial pathogens in the intramammary gland also contributes to a lower FCE in dairy cows as metabolic energy is diverted to support immune function in combating IMIs. Immune cells in milk are measured and used as an indicator of IMIs and mastitis in cattle under the terminology somatic cell counts (SCCs).

Impact of somatic cells on milk production

Bovine mastitis is inflammation of the mammary gland following infection with microbial species which initiates an immune response. The extent of inflammation depends on many factors such as age, parity, lactation and immunity of the host, animal husbandry and the pathogenicity of the infectious species. Numerous species of bacteria, yeast, mycoplasmas and algae may induce IMIs in dairy cows. Following infection and colonization with invasive pathogenic species the immune system of the cow is initiated where anatomical, cellular and soluble factors act in unison to eradicate the invader. These soluble factors and cells include somatic cells (neutrophils) and cytokines, tumor necrosis factor alpha (TNF-a) and interlukin 1 beta. Pathogens are recognised by their pathogen associated molecular patterns (PAMPs) on microbial cell wall structures including lipopolysaccharide (LPS), peptidoglycan and lipoteichoic acid [16]. SCM and CM infections are recognized by an increase in milk somatic cell counts namely neutrophils and milk-secreting epithelial cells (alveoli) that constitute the lining of the mammary gland, which is the recognized international standard measurement of udder health and milk quality. An increase in SCCs indicates the presence of infection in the mammary gland, with high levels of somatic cells having an impact of the quantity and quality of the milk produced containing lower levels of casein and lactose. Mastitis influences the development, activity and productivity of alveolar cells reducing the capacity of the secretory epithelium to synthesis and secret milk [17]. The measurement of SCCs contributes to udder health goals, critical control point 1 of the National Mastitis Council (NMC) recommendations for dairy food production using hazard analysis and critical control points (HACCP) guidelines [18]. Sampling of bulk tank milk (BTM) and quantifying SCCs remains and accurate and effective means of determining milk quality at herd level, with European limits of 400,000fu/ml of BTM for raw milk in place [19]. Studies have shown that IMIs in dairy cows relates to a low FCE as subclinical infected cows had significantly higher SCC than their high FCE counterparts [20] where energy is utilised by the immune cells as opposed to the milk producing alveoli. SCM particularly caused by *S. aureus* has the greatest impact on reducing milk production, as *S. aureus* SCM shows no observable alterations in the organoleptic properties of the milk it is also more difficult to identify. SCM is considered the most economically costly IMI due to its high prevalence within herds and the contagious nature of the causative organisms. Milk yield losses in heifers are greatest after *E. coli* CM and in multiparous animals after Klebsiella induced CM, with both coliforms' species having greater impact on cow fertility than other mastitis related pathogens [21]. Additional economic losses occur from CM due to pregnancy losses in diseased cows due to the release and systemic presence of coliform LPS or inflammatory responses on the developing foetus with post-partum neonates failing to thrive. Such effects have been observed in CM cases resulting from *E. coli* and Streptococcus uberis infections in dairy cows

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[11]. Klebsiella pneumoniae induced CM is more severe as Klebsiella sp display MDR and quickly induce toxic shock and death of the animal. Culling is often the best option for chronic cases of CM where the cow is non responsive to drug therapy, culling may act prophylactically to protect other members of the herd from infection.

Control measures to ensure food safety

Food safety aims to ensure the provision of safe nutritious food to consumers and has become a global concern as the human population continues to increase. Food safety aims to reduce the impact of foodborne disease on individuals, industry, national and international trade subsequently safeguarding economic security. In the dairy industry the Milk Hygiene section and Dairy Controls & Certification Division (DCCD) implement European and national legislation which aims to approve and supervise dairy food production operators. In order to ensure legislation is adhered to guaranteeing the quality and safety of dairy food, controls are implemented and monitored from farm to fork by the DCCD. As part of food safety, the presence of biological food hazards such as microbial pathogens must be prevented and monitored through ongoing safety assessment of products with food production industry adhering to Good Agricultural Practice (GAP), Good Manufacture Practice (GMP) and Good Hygiene Practice (GHP). HACCP has been a key development in improving food safety and security by identifying, assessing and controlling hazards and risks in the food production chain. Additionally, One Health within the dairy industry has encouraged food safety by ensuring herd and cow health at production level reducing foodborne zoonotic disease. Biosecurity measures including biocides, teat dips, teat sealants (dry period) and surface sanitizers which prevent the transmission of pathogens within herds plays a vital role in controlling mastitis outbreaks. A number of active substances have been approved as biocides in food production including chlorine, iodine, sodium hypochlorite, chloramine, hydrogen peroxide (H_2O_2), peracetic acid, QACs, alcohol derivatives, salicylic acid, lactic acid, glutaraldehyde, chlorine dioxide, chlorhexidine and active oxygen [22]. Issues arise however, as these biocides have often proven unsuccessful at preventing microbial foodborne outbreaks as sub-lethal biocide application and AMR allows pathogens their spores and/or toxins to persist post exposure. Food hazard issues also arise with the use of such biocides due to the presence residual concentrations of chemicals in food items where chemical food pollution occurs as a result of surface sanitization [23]. For example, the contamination of dairy food produce with QAC and chlorine residuals has been recognised at concentrations exceeding the FDA regulated MRL of 0.01mg/kg [24]. Furthermore, the use of chemical-based disinfection can contribute to undesirable effects on the food products such as alteration of macro and micro nutrients, textural softening and destruction. The presence of biofilm communities on surfaces and in piping also contributes to the difficulties in maintaining a clean milking environment. Microbial biofilms are typically robust, densely populated and chemically resistant allowing for microbial colonisation and reproduction on surfaces including hard to reach areas. The non-antimicrobial control of dairy pathogens is therefore a multi-faceted approach requiring the effective disinfection and monitoring of milking equipment, animal housing and teat surfaces.

Foodborne disease and consumer risk

It has been established that exposure to antibiotics and subsequent activation of resistance mechanisms particularly the efflux pump contributes to biocide resistance in microbial species. Furthermore, sub-lethal chemical exposure during disinfectant procedures can also increase the resilience of bacterial species due to the activation of stress response proteins and gene alterations enabling virulence factors which also promote pathogenicity [23]. For many mastitis associated species which may gain entry into the food chain the infectious dose (ID) in humans is quite low, with enterohemorrhagic strains of *E. coli* having an ID of 10 cells with no known ID for species of Klebsiella and Pseudomonas. Zoonotic species of major concern include MDR S. aureus and ESBL-producing coliforms including *E. coli* and Klebsiella sp. Numerous serovars of diarrheagenic *E. coli* including enteropathogenic *E. coli* (EPEC), Shiga-toxigenic *E. coli* (STEC), and enterohemorrhagic *E. coli* (EHEC), have been detected in dairy cows, milk and milk food products [6]. Vaccination against *E. coli* (J5-vaccine) has been implemented in the US and some European countries allowing for a reduced severity of mastitis, reduction in milk yield losses and animal culling however, the vaccine has not been shown to reduce the negative impact of CM on reproduction or incidence of CM in herds. Additionally, vaccination will not eliminate *E. coli* or the LPS toxins from the milk which can stimulate a pyrogenic response and eventually trigger septic shock in humans [5], an important public health hazard. Post-harvest food perseveration methods in use for dairy produce include pasteurisation of raw milk to produce a rapid decline in viable microbial species in the treated milk followed by refrigeration. Pasteurisation has proven successful at preventing foodborne outbreaks for decades however, issues relating to the presence of thermoduric Bacillus cereus spores in raw milk surviving the pasteurisation processes and post-pasteurisation contamination are of significant concern. B. cereus spores, the causative agent of cereulide induced emetic and heat-labile enterotoxin diarrheal syndrome have been detected in powdered infant milk formulae and other milk products [7]. Such aerobic spore forming species are typically heat and chemical resistant and pose a serious food safety risk as they produce toxins, spoilage enzymes and negatively impact on the production of many dairy food items. Both human and bovine skin is a reservoir for S. aureus species with transmission to milk by direct contact. The Staphylococcal enterotoxin (SE) produced by this species is heat stable and

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not affected by standard processing mechanisms resulting in foodborne staphylococcal poisoning at a concentration of between 20 to 100ng [25]. The presence of psychrotrophic bacteria such as Pseudomonas, Bacillus and coliforms which are capable of proliferating at refrigeration temperatures remains a serious concern for the dairy industry. Psychrotrophic Pseudomonas causes physical degradation and organoleptic alterations of milk post-harvest by way of thermo-tolerant lipolytic and proteolytic enzymes. Furthermore, Pseudomonas as an MDR species is of critical concern and a priority pathogen due to its nosocomial relevance and pathogenetic nature. Many mastitis pathogens possess broad intrinsic and acquired antimicrobial resistance mechanisms allowing for MDR which is shared via horizontal gene transfer (HGT) amongst species. Avoiding post pasteurization contamination with microbial species is an essential preventative measure which remains a major task for dairy producers to reduce and eliminate dairy food hazards. Sub-standard processing and post processing storage of dairy food items represents a hazard to consumer safety as pathogenic species and the causative agents of diseases including gastroenteritis, emetic diarrhoea and tuberculosis may be present in the food matrix. Tuberculosis is now classified by WHO as a re-emerging disease and major human threat due to the increasing number of disease cases with MDR resulting in 1.5 million deaths in 2015 [10, 26-28].

Conclusion

Milk can become contaminated at numerous stages of production (pre-harvest, harvest and post-harvest processing) with critical control points outlined and monitored by HACCP. The milking environment, farm personnel, cow skin, bedding and piping networks all harbour contagious and/or environmental pathogens which thrive in the nutrient dense milk matrix. The presence of mastitis in food producing animals both negatively impacts on the cow's health and is a significant risk factor for foodborne transmission of pathogenic species, their spores and/or toxins to consumers. Incorrect processing and storage of milk and milk products is associated with the transmission of mastitis pathogens and foodborne outbreaks of disease, particularly in developing countries. The use of food processing techniques including surface sanitization and pasteurisation has decreased the prevalence of dairy foodborne outbreaks however, the presence of bacterial spores and toxins which are heat stable enables sporadic cases of disease to occur. Additionally, as issues emerge relating to the chemical pollution of milk with trace amounts of chemical residues and antibiotics, concern has arisen with increasing the biocide concentrations to defeat chemical resistance in species leading to an inability to meet MRL regulations and food safety laws. Efficient farm and milk management programmes including optimal treatment of clinical mastitis cases, culling of non-responsive cows, cow teat and machine disinfection and maintenance is essential in controlling mastitis outbreaks within herds.

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