

**Transmission of
Staphylococcus aureus in Milk and Milk Products
[A Mini Review]**



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Staphylococcus aureus in Milk and Milk Products
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A Clinical Report Submitted as per Approved Styles and Contents

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List of Abbreviations

Abbreviation	Elaboration
CVASU	Chattogram Veterinary and Animal Sciences University
DVM	Doctor of Veterinary Medicine
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
et al.	et alia (and others)
e.g.	Exempli gratia
CDC	Centers for Disease Control and Prevention
FAO	Food and Agriculture Organization
CFU	Colony Forming Unit
MSc	Master of Science
PhD	Doctor of Philosophy
CP <i>S. aureus</i>	Coagulase-positive <i>Staphylococcus aureus</i>
IMI	Intramammary Infection

ABSTRACT

Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of food borne pathogens among which *Staphylococcus aureus* (*S. aureus*) is one of the most important pathogens over the past several decades found all over the world. *Staphylococcus aureus* (*S. aureus*) is a ubiquitous gram-positive coccus which is a non-motile, non-spore forming, oxidase-negative, hemolytic, catalase-positive, and coagulase-positive and facultative anaerobic bacteria. In dairy cattle, *S. aureus* is frequently associated with subclinical mastitis and may contaminate milk and other dairy products. The major reservoirs of *S. aureus* are infected udders, teat canals, and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils. The most-reported predisposing factors for *S. aureus* transmission in milk and milk products were bovine mastitis (Subclinical & Clinical mastitis): infected animals as asymptomatic carrier; infected heifers; dairy farm workers: “Direct” milkers’ hand-to-milk contact , “Indirect” milkers’ fomite to milk transfer ; farm environment: unhygienic milking, mismanagement of milking equipment; contact with contaminated manageable risk factors; contamination in bulk milk tank, improper heat treatment /inefficient pasteurization of raw milk; poor making plants and value chain hygiene: unhygienic handling, dirty water used for cleaning utensils, absence of sanitary inspection; post-pasteurization contamination (recontamination during or after processing); location of selling points ; lack of education on the aspects of milk and dairy product hygiene and handling practices among the people who are concerned with transportation and sales etc. For writing this review documents including Original research papers from Journals; Review articles; MSc and PhD theses; FAO documents; Project reports etc. were screened and reviewed critically. To reduce the transmission of *S. aureus* in milk and milk products factors discussed here should be checked to maintain food hygiene and health.

Keywords: *Staphylococcus aureus*, Transmission, Milk and Milk Products

Chapter 1: Introduction

1.1 Background of the Study

Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of food borne pathogens (Richter et al., 1992). *Staphylococcus aureus* (*S. aureus*) has emerged as one of the most important pathogens over the past several decades. The prevalence of *S. aureus* in milk and dairy products has been reported from many countries as well as different geographical regions of the same country. It was observed that the prevalence rates varied significantly. Increased prevalence of *S. aureus* has been documented in milk and milk products (e.g., cheese, yogurt, cream, butter and ice-cream etc.) produced from developing countries as compared to the developed countries. The prevalence rates was attributed to the variations in the management system of dairy cows in the respective countries. (Pal et.al, 2020).

Furthermore, milk is produced mainly by individual in small farms that lack proper sanitary measures and may be either consumed fresh, manufactured into dairy products or sell in retail markets that alarming as a major source of Staphylococcal enterotoxin poisoning (El-Sayed et al., 2011) and represent a serious human health problem. Dairy products specially cheese, ice-cream, yogurt etc are liable to be contaminated with *S. aureus* from different sources during their production, processing and handling that make them unfit for human consumption or even a dangerous source of infection among consumers constituting a potential health hazard caused by enterotoxin production. This can occur under certain conditions during production as well as when they are cut and packaged for consumption (Wauschkuhm, 1970).

1.2 Concerning features of Staphylococcus aureus

S. aureus is the most frequent and significant species of organism, because of its potential pathogenicity both in humans and animals.

S. aureus colonizes as the normal flora on the skin, anterior nares, nasopharynx, and mucous membranes of approximately 50% of healthy individuals and animals. It is also found in environmental sites such as soil, water, and air. Nasal passages and skin. *S. aureus* grows at a wide temperature range between 6 to 48°C and therefore posing a serious threat with optimum of 35 to 41°C. It tolerates a pH between 4 to 10 with optimum of 6 to 7, a salt concentration of 0 to 20%, and a water activity (Wa) level of 0.83 to 0.99 with optimum at 0.99 (Cretenet et al., 2011). These growth characteristics enable the bacterium to grow in a wide range of foodstuffs including raw milk (Jorgensen, Mork, Hogasen, & Rovik, 2005), dairy products (Headrick et al., 1998), chicken, pork, beef and salad dishes (Bryan, 1998) and causes food poisoning by secreting enterotoxins appearing around 3 hours after ingestion (range 1–6 hours) up to 24 hours (Balaban and Rasooly, 2000).

1.3. Transmission of S. aureus

The leading cause of raw milk contamination with *S. aureus* is the dairy cows. (Da Silva et al., 2005). In dairy cattle, *S. aureus* is frequently associated with subclinical mastitis and may contaminate milk and other dairy products (Jones et al., 2006). The major reservoirs of *S. aureus* are infected udders, teat canals, and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils. The bacteria are spread to uninfected quarters by teat cup liners, milkers' hands, washcloths, and flies. Heifers are also a reservoir for *S. aureus* transmission (Boddie et al., 1987). The most-reported predisposing factor for *S. aureus* transmission at farm level was the unhygienic environment associated with animals including dust, farm rat and environmental wipes unclean milker's hand has been previously reported (Weese et al., 2004; Friese et al., 2012). Up to 50% of humans may carry this organism in their nasal passages, throats and on their hair and skin.

1.4 The specific objectives of the study

The purpose of this communication is to review literature published on the most likely sources and transmission of *S. aureus* in milk and milk products in the dairy environment.

Chapter 2: Review Methodology

For screening the relevant information, necessary data were collected from peer reviewed journals, MSc and PhD theses, FAO documents, Proceedings of, international conferences, Project reports, University extension/experimental station bulletins, Technical bulletins, Annual reports of Ministry of Agriculture of different countries, Annual reports of Ministry of Dairy food Processors of different countries; Book chapters. The search engines used for this search was Google Scholar and PubMed.

The following search terms were used to find the articles from PubMed and Google Scholar.

1. “Transmission of *Staphylococcus aureus* in milk and milk products” and
2. “Ways of transmission of ‘*Staphylococcus aureus* in milk and milk products” and

The search was further expanded by internet searches using the keywords “SA in milk and milk product”, “transmission”, “dynamics”, and in Google scholar and Google search engine. Results were screened up to the fifth pages returned from the internet search.

Only articles describing the “Transmission of *Staphylococcus aureus* in milk and milk products” or relates with “epidemiological transmission pathways of *Staphylococcus aureus* in milk and milk products” were included in this review. Included articles were summarized in terms of: study aims and transmission pathways.

Author screened the titles and abstracts of articles obtained from the initial search. To finalize articles included in this study, author read the full-text of the shortlisted publications and excluded articles that did not fit into the context for this review.

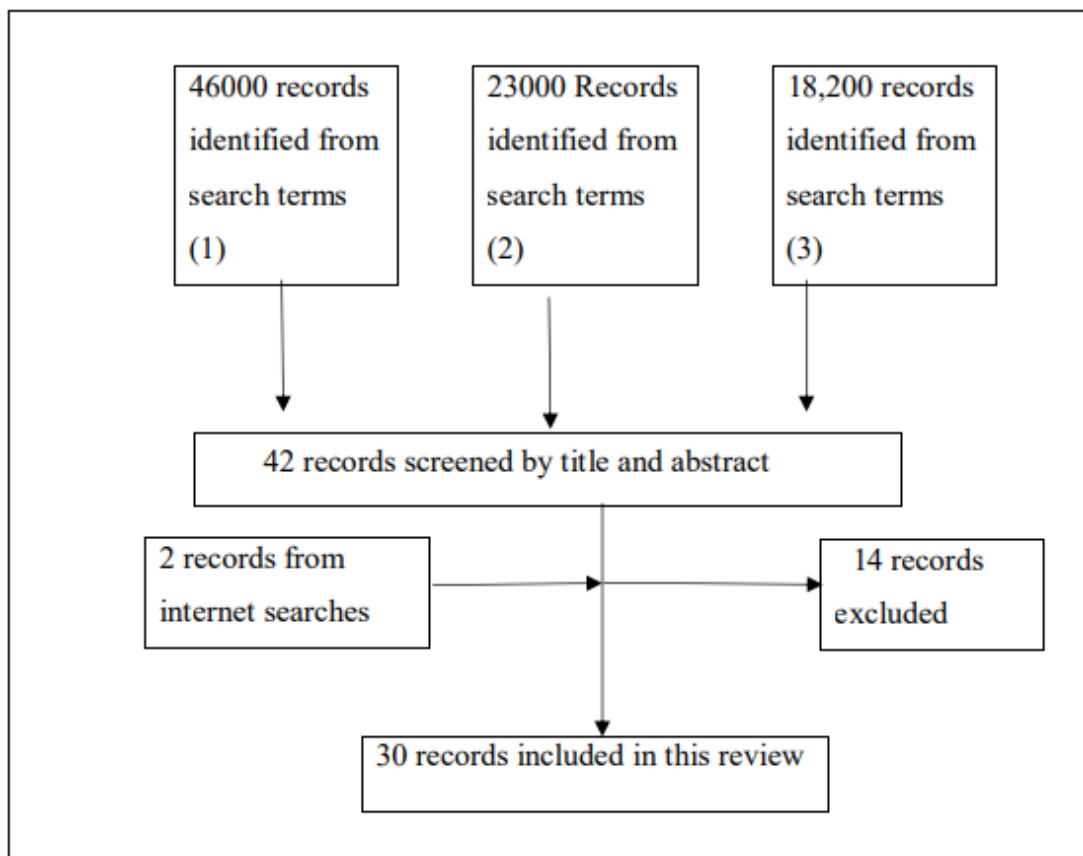


Fig. 1 Flow diagram of Review Methodology

Twenty two articles out of 30 were shortlisted to be included in this study. Eight additional articles were shortlisted by the ad hoc method. After final screening on the shortlisted publications, 30 articles were selected for review.

The relative importance of different transmission pathways of *S. aureus* were not well addressed in all reviewed models. Of the 30 reviewed studies, 22 of them considered for *S. aureus* transmission in milk and milk products. Remaining eight considered for additional transmission pathways of *S. aureus* in milk and milk products.

Chapter 3: Results and Discussion

3.1. Transmission of Staphylococcus aureus in milk and milk products:

A variety of agents predominantly bacteria are considered major causes of milk borne illnesses worldwide. Milk, understandably an important constituent of human diet and raw milk is an ideal growth medium for several microorganisms. Milk and its derivatives are considered vehicles for Staphylococcus aureus infection in humans (Zecconi and Hahn, 2000).

3.1.1. At dairy farm level

In Europe, Asia and USA, *S. aureus* has become one of the most important milk-borne pathogens. *S. aureus* was reported at a prevalence of 62% at dairy farms in Minnesota, USA, (Haran et al., 2011) and at prevalence of 26% from milk-producing herds in Ireland (Murphy et al., 2010). In Italy, 43% of raw milk intended for Caprino cheese making (Foschino et al., 2002) and 68% of raw milk from the Reconcavo area, Brazil (Oliverira et al., 2011) was positive for *S. aureus*. In India, a prevalence ranging from 61.7 to 65.6% was reported in raw cow's milk (Lingathurai and Vellathurai, 2010; Singh et al., 2010). All the above surveys showed that, transmission of *S. aureus* varies in the milk supply chain of the respective areas due to geographical variation as well as management variation at dairy farm level (Tarekgne et al., 2015).

a) Dairy Cow

The first Entrance Point of *S. aureus* into the Dairy Production Chain. The leading cause of raw milk contamination with *S. aureus* is the dairy cows.

i) Bovine mastitis (Subclinical & Clinical mastitis)

S. aureus is frequently found in raw milk and the infections of mammary gland (mastitis) represent a significant reservoir for transmission of toxigenic strains in raw milk. These are mainly the cows that suffer from subclinical mastitis, and therefore had the potential to disseminate this microbe into milk and other dairy products (Da Silva et al., 2005; Jones et al., 2006). The largest ratio of enterotoxin forming *S. aureus* strains has been isolated from milk of mastitis indicating a significant role of *S. aureus* transmission in milk and milk products. *S. aureus* infection frequently persists for a long time in infected mammary glands in cattle (Atalla et al., 2008). Subclinical mastitis is, in general, a more insidious form of the disease because it is invisible to the farmer, leading to delayed diagnosis and also spreads widely among the dairy herds. Moreover, milk from infected cows contaminate whole milk in milking can through transmission of *S. aureus* because of improper milking procedures. This results in reduced milk quality and yield, which in turn leads to a reduction in the farmer's income as well as that of the dairy industry. Once established, *S. aureus* infections in cows do not respond well to antibiotic therapy and infected cows must be segregated or culled from the herd. Recently published work has shown that 3% of all animals are infected with *S. aureus* (Schukken et al., 2009). However, *S. aureus* represents 10 to 12% of all clinical mastitis infections (Tenhagen et al., 2009). If cows develop clinical signs; for preventing *S. aureus* transmission, they must be treated or culled and the milk must be discarded, with milk loss potentially continuing even after recovery (Gröhn et al., 2004; Hertl et al., 2014). Like dairy cows, mastitis in dairy sheep and goats can cause economic losses as a consequence of a decrease in milk production, undervaluation of milk with high somatic cell counts, mortality and treatment costs (Pal et al., 2020).

ii) Infected animals as asymptomatic carrier

Staphylococci do not persist on healthy teat skin but readily colonize damaged skin and teat lesions. The organisms multiply in infected lesions and result in increased chance of teat canal colonization and subsequent udder infection (Pettersson-Wolfe et

al., 2010). The major reservoirs of *S. aureus* are infected udders, teat canals, and teat lesions, which agrees with reports by others (Davidson 1962; Schalm et al., 1971), but these bacteria also have been found on teat skin, muzzles, and nostrils. *S. aureus* was also isolated from wounds and the vaginal mucosa of some animals. Animals with persistently colonized body sites represent a significant primary reservoir of enterotoxigenic strains of *S. aureus* and sources of transmission for other cows and raw milk. The infection is spread at milking time, when *S. aureus* contaminated milk from an infected gland comes in contact with an uninfected gland, and the bacteria penetrate the teat canal. The bacteria are spread to uninfected quarters by teat cup liners, milker's hands, washcloths, and flies (Abebe et al., 2016). Milking technique, milk leakage from infected teat have also been reported as the most significant risk factors of *S. aureus* transmission by different authors. They spread from cow to cow, primarily during milking, and tend to result in chronic subclinical infections with flare-ups of clinical episodes. Animals with subclinical types of inflammatory infections (IMI) are a reservoir of *S. aureus* that serves as a source of transmission in the dairy environment and represents the most common cause of raw milk contamination (Abdel-Hameid Ahmed et al. 2019; Petersson-Wolf et al., 2010)

b) Infected heifers

Heifers are also a reservoir for *S. aureus* transmission in milk and milk products. New infections are commonly found in heifers, either at calving or in early lactation to dam during sucking. Up to one-third of these infections are caused by *S. aureus*. Heifers infected during gestation that carry infections through calving. Often these *S. aureus* infections, if untreated, become clinical and recur throughout the first lactation and into the second lactation and represent an important reservoir from which *S. aureus* can transmit to raw milk uninfected herd mates. In several research trials, 12 to 15 percent of first-lactation cows were found infected with *S. aureus* at calving (Boddie et al. 1987; Trinidad et al., 1990a; Trinidad et al., 1990b).

There is considerable debate surrounding the route of *S. aureus* infection in heifers prior to first calving, but calves fed colostrum from an *S. aureus*-infected dam is a

likely source. Early work suggested *S. aureus*-infected colostrum was not a culprit for first-calf heifers calving with the infection (Barto et al. 1982). However, later work did show a positive correlation between feeding *S. aureus*-infected colostrum to a calf and that calf then calving with *S. aureus* mastitis (Roberson et al. 1998). Though the data is limited, if an *S. aureus* problem exists on a farm, careful colostrum selection, e.g., pasteurization, is certainly one area to consider. Clearly, good mastitis control programs will address the presence of this disease in heifers. (Petersson-Wolfe et al., 2010). Moreover, absence of dry cow therapy regime could possibly be among the major factors contributing to higher prevalence of *S. aureus* at early lactation.

c) Dairy farm workers

Clinically, *S. aureus* is one of the most significant bacteria that cause food poisoning worldwide. Some other studies reported significantly higher levels of contamination ranging from 40% to 61.7 % (Güven et al., 2010; Lingathurai and Vellathurai, 2010; Zakary et al., 2011). Similarly, other studies reported low contamination from 6.2% to 7.3% (Fagundes et al., 2010; Thaker et al., 2013). This is not surprising because milk can be contaminated internally through the production of milk from a diseased animal or externally by an infected person or the surrounding environment (Serraino et al., 2004; Abou-Khadra et al., 2020). *S. aureus* can be transmitted from animal to animal, person to person, as well as from animals to humans and vice-versa. The main source of *S. aureus* in milk at farm level is the udder of infected cows which could be transferred via the milkers' hands, milking utensils, towels, and the environment (Radostits et al., 1994).

i) "Direct" milkers' hand to milk contact

Transmission usually occurs by direct contact, often via the hands, with colonized or infected animals. Previous studies indicated that milkers' hands can be fomites in the spread of *S. aureus* in dairy herds and milk products (Fox et al., 1991). et al., 1991). Cleaned equipment for milking and for cheese production were *S. aureus*-negative, but samples collected from the same equipment post milking and were *S. aureus*-positive.

It is likely that the farmer's hands were contaminated with *S. aureus* during milking (Kümmel et al., 2016). From record of previous study, even though in all the observed herds milkers' used to wash their hands before milking, they do it only before milking the first cow. Hence it is evident that the causative organisms could be transmitted easily from infected to uninfected udder quarters of cows or raw milk through the milkers' hands (Abebe et al., 2016). It is obvious that, failure to milk mastitic cows last would favor spread of mastitis pathogens between cows by milker's hands resulting in contagious mastitis and transmission of *S. aureus* in milk (Belayneh et al., 2016; Abebe et al. 2016)

In addition, hand Swabs of dairy workers and food handlers revealed high frequency of Coagulase-Positive (CP) *S. aureus* colonizing their skins. 80% of hand swab samples were positive for CP *S. aureus*; consequently, they may constitute another sustainable source of CP *S. aureus* contamination of dairy products. As an evidence of the seriousness of hand contamination with CP *S. aureus*, toxic shock syndrome toxin 1 (TSST-1) gene was detected in *S. aureus* isolated from handlers (Rapini et al., 2005) and from milk of clinical and subclinical mastitis cases and from bulk tank milk (Takeuchi et al., 1998). On the other hand, a lower incidence of *S. aureus* isolated from dairy workers' hands also has been mentioned (Sospedra et al., 2012; Kamal et al., 2013)

ii) “Indirect” milkers’ fomite to milk transfer

In view of the wide dissemination of *S. aureus* on the same clothing towel that are used by milkers' to dry teats and scrub teats on more than one cow is possible that teat and milk may acquire the organisms as a result of contact with such fomites. *S. aureus* may transfer into washcloth or towel at the time of udder scrubbing of an infected animal, using of same contaminated towel infect another healthy cow else shaking it transfer pathogen to milk in open container (Pettersson-Wolfe et al., 2010) Previous workers (Green et al., 1945; Bourdillon and Colebrook, 1946; Hare and Mackenzie, 1946, Duguid and Wallace, 1948) have shown that bacteria may be

released into the atmosphere on particles of fluff or dust when contaminated clothing is shaken.

From previous study, two heifers with *S. aureus* IMI at parturition had the same strain as that isolated from the nose of a dairy worker, suggesting that humans may play a role in the transmission of *S. aureus* to cattle as humans may be persistently colonized with *S. aureus* in the anterior nares (Roberson et al., 1998). It can, however, be shown that the organisms may be liberated into the atmosphere by different forms of activity, so that transportation by air currents is an obvious alternative. However, the identification of *S. aureus* from the farmer's nose pre-milking suggests that he was colonized with *S. aureus*, and thus, indicates that he is a potential source of this pathogen transmitted into dairy chain due to zoonosis as nasal egress of infected handlers contaminated air may come in indirect contact with open milk in container along with teat skin and may transmit pathogen to milk and uninfected cow (Kümmel et al., 2016).

d) Farm environment

S. aureus prevalence in raw milk varies due to negligence of farm management practices mainly unhygienic milking, contamination of milking equipments, poor animal management and handling practices without sanitation etc. According to previous studies, In Palestine, 48 (36.9%) milk samples were positive for *S. aureus* from a total of 130 samples (Farhan and Salk, 2007). Park et al. (2007) analyzed 30,019 samples of raw milk in Korea and detected 104 (0.35%) samples contaminated with *S. aureus* and Daka et al. (2012) reported 40.6 % in South Ethiopia. The difference in the prevalence rate was due to variation in the sanitary condition of udder, size of sampling and geographic region (Sadashiva and Kaliwal, 2013 and Shopsin et al., 2000). Also species wise variation in prevalence of *S. aureus* in raw milk may be due to the improper hygiene and poor animal management in dairy farm environments (Ateba et al., 2010).

i) Unhygienic milking

In agreement to the present study, a significant association between *S. aureus* mastitis due to poor udder hygiene and increased risk of *S. aureus* transmission in milk and milk products has also been reported by other studies. The fact that none of the dairy herds implementing routine mastitis prevention practices. The high prevalence of *S. aureus* could be associated with absence of washing of udder before milking, pre and post-milking teat disinfection, pre-squirting of milk, absence of dry cow therapy and the invariable hand milking practice (without prewashing as well as wearing of hand gloves before milking of each time and breakdown of milking sequence from uninfected to infected cows), lack of culling of chronically infected cows among the dairy herds and others. *S. aureus* and other contagious microorganisms are usually found on the udder or teat surface of infected cows and are the primary source of infection between uninfected and infected udder quarters, usually during milking.

According to Belayneh et al. (2016) Based on the udder and leg hygiene score used, all the examined cows had slightly to very dirty udders and legs, and there was a marked increase in the detection of mastitis along *S. aureus* pathogen as the level of dirtiness increases. Unfortunately, none of the dairy farmers have the practice of culling of chronically infected animals, dry cow therapy and post-milking teat disinfection and this has created a good opportunity for the establishment of the *S. aureus* in the dairy herds results in immense transmission in raw milk.(Abebe et al., 2016)

ii) Mismanagement of milking equipment

Indirect transmission usually occurs through contaminated equipment. *S. aureus* infections can occur during milking when organisms penetrate the teat canal. Irregular vacuum fluctuations caused by liner slips, flooded lines, etc., may cause a backflow of

milk against the teat end. With sufficient force, bacteria can be propelled up into the teat canal and teat cistern. Therefore, new transmission in milk may occur owing to mismanagement of milking equipment or else milking equipments don't function properly. Conditions that are associated with high-impact force against the teat end – including liner slips, excessive temporary vacuum losses, low vacuum reserve, inefficient vacuum regulation, and abrupt milking unit removal – should be minimized. (Thaker et al., 2012)

Machine milking unit liners are important fomites for transmission of *S. aureus* in dairy herds. The use of liner backflush reduces contamination with bacterial load. According to Previous study, PFGE (Pulse-Field Gel Electrophoresis) analysis revealed that liners could be contaminated with *S. aureus* from teat skin and with *S. aureus* from milk. This implies that liners are fomites for skin flora and for intramammary infections. Although liners can be contaminated with skin and udder flora, transmission from skin flora to the mammary gland and vice versa seems rare, as indicated by the site-specific pulsotypes. (Oliver et al., 2005)

iii) Contact with contaminated manageable risk factors

Contamination of manageable risk factors acts as a major source of *S. aureus* transmission in farm milk. Presence of foodborne pathogens in bulk tank milk seems to be directly linked to faecal contamination that occurs primarily during raw milk harvesting (i.e., milking, collection, and storage) in dairy farm environments. However, some foodborne pathogens can cause mastitis in which case the organism can be directly excreted into milk such as *S. aureus*. (Arizcun et al., 1998; Roberts and Wiedmann, 2003; Wong, 1998). Epidemiological studies have shown that cattle probably become infected through consumption of water and feedstuffs contaminated with feces and other cattle secretions/excretions due to improper drainage system. Of the total farms investigated, 80% were categorized as poor hygienic due to lack of waste drainage system and accumulation of manure and urine. (Abebe et al., 2016).

S. aureus present in the nose and on the skin is shed into the environment by infected or colonized people and animals, indicating the possibility of airborne transmission in

farm milk as a possible route for infection. In dairy cattle farms, genetically identical *S. aureus* was isolated from milk and their environments in previous studies (Roberson et al., 1998; Hata et al., 2010). *S. aureus* was detected in milking-associated environmental samples such as milk cup, floor, fence, and ventilation instrument of the milking parlor in a *S. aureus*-positive milk farm. Also, all *S. aureus* isolates from the farm environment were genetically identical to those of milk isolates. Although the origin of the *S. aureus* isolates from the environmental samples could not be identified, this result suggests that it may be circulating among cattle and their environment, including the milking parlor (Lim et al., 2013).

Finally, the most common contaminated manageable transmission pathways include the transfer from un-controlled animal traffic between different farms, tonsils and skin of farm pigs, chickens, and turkeys often harbor *S. aureus* and are also potential sources of *S. aureus* contamination in raw milk (Pal et al., 2020). Various environmental samples associated with animals including dust, farm rats, and environmental wipes are responsible for transmission of *S. aureus* in milk has been previously reported (Weese et al., 2004; Friese et al., 2012). Vectors like the housefly (*Musca domestica*) have also been implicated in the transfer of *S. aureus* in farm milk (Tarekgne et al., 2015).

3.1.2. At dairy production level

Milk is regarded as a suitable medium for *S. aureus* growth, where potential contamination occurs during processing stages (Asao et al., 2003; Le Loir et al., 2003; Brasca et al., 2005; Jørgensen et al., 2005). The difference in the prevalence rates of *S. aureus* between milk and milk products may origin from the method of manufacture, storage and handling. (Tyagi & Tyagi, 2019)

Milk is normally sterile in the udder of the cow and buffalo provided if they do not suffer from mastitis in that case negligence of hygienic condition in production level

such as cleaning of bulk tank, storage condition, human, equipments, processing stages and processing environment etc. are greatly responsible for transmission of *S.aureus* in milk product at production level.

a) Bulk milk tank

Staphylococcus aureus is frequently found in bulk milk tank. Enterotoxigenic *S. aureus* was isolated from raw milk samples (Ombui et al., 1992) and in bulk milk collected from dairy farms in Trinidad (Adesiyun et al., 1998). *Staphylococcus* are resistant against drying so unclean vessels could be the additional source of contamination. (Sinell 1980; Halphin-Dohnalek 1989; Miwa 2001). It was inferred that Uncleanliness of milk storage containers/improper cleaning of bulk milk tanks was a potential source of enterotoxigenic *S. aureus* transmission in milk and milk products, and may constitute a health hazard to consumers.

b) Unpasteurized milk / inefficient pasteurization of raw milk

Many farm families consume raw milk simply because it is a traditional practice and it is less expensive to take milk from the bulk tank than buying pasteurized retail milk. Additionally, some believe that raw milk has a higher nutritional value than pasteurized milk (Hegarty et al., 2002). Thus, risk exposure of people in the rural community to potential pathogenic bacteria capable of causing disease in humans via consumption of raw unpasteurized milk can be very high. Major source of *S. aureus* transmission of non-pasteurised milk is the milk coming from the farms with mastitis infected cows (Holeckova et.al, 2002). *S.aureus* shed into the milk which serves as a primary source of infection to individuals who drink unpasteurised milk (Adams & Moss, 2011). Causative factors in cheese-related disease outbreaks were post-pasteurization contamination, faulty pasteurization equipment or procedures, and use of raw unpasteurized milk (Johnson et al., 1990). The high incidence of *S. aureus* in

the examined kareish cheese was due to that the kareish cheese is made by farmers from raw milk that isn't subjected to heat treatment. So, kareish cheese are involved in food poisoning outbreak from public health point of view, as *S. aureus* had been implicated in many cases of food poisoning and gastroenteritis among consumers (Meshref et al., 2019).

Most of the bacterial contaminants must have been eliminated during the process of pasteurization. Pasteurization is achieved by the alkaline phosphatase test which demonstrates that mammalian phosphatase enzyme present in raw milk is inactivated by pasteurization. Large dairies perform the phosphatase test on every batch of milk prior to release but due to the complexity and cost of the phosphatase test it is not used on a daily basis by small on-farm dairies. Although milk pasteurization is regarded as an effective method to eliminate foodborne pathogens including *S. aureus*, some dairy products do not undergo pasteurization (i.e., specialty cheeses) (Oliver et al., 2005).

It should be noted that staphylococcus are resistant against heating at low temperatures (< 55°C) (Adams et.al, 1995). However, the presence of *S. aureus* in pasteurized milk in other cases indicates the process was not satisfactorily done. High levels of bacteria in pasteurized milk primarily stem from failure to heat milk to the proper temperature or hold it at that temperature long enough, which could be caused by operator error or system design, or from poor cleaning of the machine. When pasteurization is incomplete, pathogen that survive can grow rapidly in warm milk. If the heating of the milk in process of preparation of curd was not sufficient to destroy all bacteria and the level of initial contamination was high, the risk of presence of enterotoxin-forming strains is very substantial. If SE once is produced, it will remain structurally stable and biologically active as it is thermo-stable, potent even in very small amount ranging from 20 ng to less than 1 µg resistant to low pH, freezing, and also to the action of different enzymes. Normal pasteurization does not denature the toxin (Bergdoll and Wong, 2006; Hennekinne et al., 2011; Loir et al., 2003; Tarekgne et al., 2015). High levels of bacteria in raw milk have also been suggested as a cause of unacceptable bacteria counts in pasteurized milk (Scott, 2006). However, type of pasteurizer had no

effect on bacteria counts after pasteurization, log reduction, or percentage log reduction ($P > 0.05$) for any of the bacteria studied (Jorgensen et al., 2006).

Moreover, high levels of bacteria in raw milk have also been suggested as a cause of unacceptable bacteria counts in pasteurized milk (Scott, 2006). However, type of pasteurizer had no effect on bacteria counts after pasteurization, log reduction, or percentage log reduction ($P > 0.05$) for any of the bacteria studied (Jorgensen et al., 2006).

Educational efforts should be aimed at making the rural population aware of the health risks associated with consumption of raw unpasteurized milk. Thorough teat sanitization and removal of fore milk might prevent contamination of the raw milk, and this is especially important for unpasteurized milk which will not receive further processing. Although milk sold as pasteurized has a critical control point in the form of heat treatment, producers have no room for complacency as pasteurization failures can occur.

c) Poor making plants and value chain hygiene

High incidence of *S. aureus* is indicative of poor hygienic measures in production chain (Joshi et al., 2004). Farms and cheese-making plants can serve as a reservoir of *S. aureus* and can spread the pathogen into the dairy food product (Pal et al., 2020). It is known that curd and sour cream is the concentrate of milk fats and proteins. The content of *S. aureus* enterotoxins in samples of curd and sour cream is higher in comparison with milk. Another study reported that, fermented milk is characterized by acid production, additives, and cultured bacteria. This environment could have therefore been competitively harsh for *S. aureus* to survive and thrive well and hence the possible cause of few isolates isolated compared to raw milk and pasteurized milk. Low temperatures below 5°C inhibit growth and multiplication of *S. aureus* and this could be the reason why its presence in ice-cream was notably very low compared to all other products. This might be attributed to the effect of heating then freezing during their manufacturing which inhibit the multiplication of the microorganism and kill the

microorganism (Zakary et al., 2011).The difference in prevalence rates of *S. aureus* among products may originate from the method of manufacture, storage and handling.

The viability of *S. aureus* during the manufacturing of dairy products as cheese, yoghurt and ice-cream, depend on the addition of milk production techniques to control water content, salt concentration, lactic acid starter culture, drop of pH and temperature are cardinal factors during processing and ripening of raw milk products to prevent growth of *S. aureus* and production of staphylococcal enterotoxins. It should be noted that enterotoxin-forming *S. aureus* strains are described as one of the main causes of food infection, especially strains of *S. aureus* forming enterotoxin A (Evenson et.al, 1988; Yamashita et.al, 2003; Ikeda et.al, 2005). Some publications emphasize the prevalence of SEB, SEC and SED serotypes (Tollersrud et.al, 2000; Stephan et.al, 2001; Nagase et.al, 2002) but the regional difference strongly influences staphylococcus populations as indicated by different researchers (Annemüller et.al, 1999; Stephan et.al, 2001; Joo et.al, 2001).

It's worth in mention that, due to poor hygienic handling, inadequate personnel hygiene and inefficient pasteurization the presence of *S. aureus* in milk and dairy products even in low numbers must be regarded a public health hazard; because it has been established that the *S. aureus* may lose it's viability in food, but enterotoxins still exist (Meshref et al., 2019).It should be noted that aggregate milk from the farm contains milk from different cows and hygienic conditions and occurrence of sub-clinical mastitis should be controlled carefully. Commercial production commonly is connected with separation of bacterial cells with separators and centrifuge. Small farms usually are not applying these technologies. (Abebe et al., 2016)

3.1.3. Both production and marketing level

The traditional methods of milk products manufacturing, handling and sale is could provide a favourable environment for *S. aureus* transmission in food. Moreover, unclean hands of workers, poor quality of milk, unhygienic conditions of the manufacturing unit and water supplied for washing the utensils could be the source for accelerating *S. aureus* transmission in milk and milk products beside the post manufacturing contamination (Tariq Masud et al., 1988; Johnson, 1961; Elmahmood et al., 2007; Jhonson et al.,2008; Singh &Prakash, 2008)

a) Unhygienic handling

The dairy chain opens various entrance points for the human pathogen *S. aureus*, including the primary production environment and people involved in the dairy production and processing due to zoonosis of *S. aureus*. (Haveri et al., 2008; Johler et al., 2016b). This is of public health significance since it is a commonly recovered pathogen in outbreaks of food poisoning attributed to dairy products (Oliver et al., 2005).The naturally sour/fermented milk and buttermilk are made from raw bulked milk. Butter and cottage cheese are made by churning sour milk and heating of sour buttermilk, respectively. The traditional milk preparation activities have been shown to be unhygienic and consequently exposed to microbial contamination (Yilma et al., 2007). Importance of personal hygiene is high especially for the cases when hand work is involved during the production of the curd (Abebe et al., 2016). The microbial counts were significantly lower in farmer's raw milk and highest in fermented milk. An implication that farmers' milk was of better quality but the quality deteriorated along the supply chain due to the proliferation of the microorganisms initially present in milk or/and due to cross-contamination. Ice-cream has been incriminated as a vehicle of staphylococcal enterotoxin in several food poisoning outbreaks as ice-cream mix was usually contaminated at the time of preparation and during the long period of cooling as well as before the mix freezing (APHA, 1992). The high contamination rates of

examined ice-cream samples are mainly due to use poor quality of milk and materials used in ice-cream manufacture, environmental contamination as unclean hands of worker either suffering from diseased or apparent healthy carriers, unsanitary production or marketing practices (Araujo et al., 2002; Meshraf et al., 2019). So, difference of *S. aureus* transmission in between production and marketing level may be attributed to differences in the milk hygiene situation during processing, handling, distribution and marketing or storage .

b) Dirty water used for cleaning utensils

It should be noted that the use of water of unsatisfactory microbiological quality for milking and manufacturing of milk products is associated with the risk of contamination of milk with *S. aureus* (Kivaria et al., 2006). Most (71.43%) of the containers used as ‘bulk tanks’ at the Fulani settlements were probably of unsatisfactory cleanliness with dirty water which could have resulted in milk contamination *S. aureus*. In addition, a significant association was found between of the pathogen in Nigerian fermented milk (nono) samples collected and the kind of water used for cleaning utensils at the product’s selling points. Similarly, a significant association was found between the occurrence of *Staphylococcus aureus* in locally-pasteurized milk (kindirmo) and the kind of water used for cleaning utensils at the product’s selling points (Yakubu et al., 2020)

Cross contamination can be avoided at production and selling level if the hands of workers and production utensils are washed adequately with detergent and clean water in between using the utensils. Hand washing is a basic component of transmission control at production and marketing level (Larson et al., 2003).

c) Absence of sanitary inspection

S. aureus transmission in milk and dairy products is greatly responsible due to absence of sanitary inspection both in production and marketing level. *S. aureus* usually finds

its way to bulk milk and in turn contaminates other raw milk products (Kamal et al., 2013). To avoid contamination of dairy products with *S. aureus* and its enterotoxins, strict preventive measures and sanitary inspection are required. In farms where bulk milk is intended for raw milk products, bulk milk quality should be monitored by frequent bacteriological analyses and of quarter samples from individual animals. (Jørgensen et al., 2005)

It is therefore recommended that since compliance with basic hygiene requirements is not guaranteed, during dairy processing, additionally, good manufacturing practices (GMPs) and food safety management system based on the principles of hazard analysis and critical control points (HACCP) concepts should be applied as mandatory requirements in manufacturing units under control of the regulatory authorities (A. Abdel-Hameid Ahmed et al., 2019). Finally, the responsible authorities under the control of the milk commission (the commission must have a sanitary inspector and veterinarian) should impose severe inspection measures for monitoring an effective total hygiene concept of the informal sale of dairy foods.

d) Post-pasteurization contamination (recontamination during or after processing)

Dairy products are one of the major vehicles for the transmission of *S. aureus* to man (Jahan et al., 2015). Previous report documented by Umoh (1989) that fermented foods are not good media for the survival and growth of *S. aureus*. The occurrence of the organism in these processed foods implies recontamination during and/or after processing (Yakubu et al., 2020).

In addition, *S. aureus* could multiply in wide range of growth limiting determinants such as temperature, a_w , NaCl and gaseous atmosphere (Cretenet et al., 2011; Kadariya et al., 2014) and hence samples that contain *S. aureus* in unpasteurized dairy products as well as dairy products that are re-contaminated in the post-pasteurization processing environment $< 5 \log_{10}$ CFU/ml could impose a public health hazard. Lack of cold chain facilities or refrigeration can increase the chances of recontamination with *S. aureus* (Kivaria et al., 2006). Moreover, an increase in time or temperature before consumption could lead to further proliferation of the pathogen and the

production of toxins by enterotoxigenic strains if any present and causes SFP (Hennekinne et al., 2011; Seo and Bohach, 2007). This amount of toxin is produced when the enterotoxigenic *S. aureus* population is greater than 5 log₁₀ CFU/ml (Food and Drug Administration, 2012; IESR, 2001) (Tarekgne et al., 2015). So, it is of great importance to use a well-controlled cold-chain system to avoid fluctuating temperature in the milk supply chain in order to prevent further recontamination of the final product (A. Abdel-Hameid Ahmed et al., 2019). Additional measures to reduce the incidence of post-pasteurization contamination would be separation of the raw milk from the finished dairy products together with an adequate environmental sanitation (A. Abdel-Hameid Ahmed et al., 2019).

3.1.4. At the product's selling / marketing level

a) Location of selling points

The unsanitary conditions of the places where the products are marketed might have contributed to their contamination. Such as, street vendors put kareish cheese in pans exposed to dust and flies (Zakary et al., 2011). So, kareish cheese are involved in food poisoning outbreak from public health point. A significant association was found between of the results of the risk factors associated with the occurrence of *Staphylococcus aureus* in fresh and fermented milk and locations of samples collected. Higher occurrence of the bacterium was found in a Nigerian fermented milk (nono) and locally-pasteurized milk (kindirmo) samples collected from selling points with high street activities (mainly vehicular movement) compared to samples collected from selling points with low street activities. The isolation of *Staphylococcus aureus* from fresh and fermented milk is a cause for public health concern because many people in the area consume the products. (Yakubu et al., 2020).

Furthermore, the prevalence of *S. aureus* was significantly higher in cafeteria and restaurant samples than from other selling points. This situation may occur because the initial *S. aureus* load from the farm may multiply during transportation, as cold chain facilities are not available in all selling points, or more *S. aureus*, from human sources, may contaminate the milk because of poor personal and/or equipment hygiene during the value chain. Although a molecular epidemiological study is required to verify the source, it indicates that there is need to improving food handlers and equipment hygiene as well as application of cold chain facilities in the milk value chain in order to protect the consumer from milk-borne hazards. (Tarekgne et al.2015).It is also reported that, many of the selling points were located in or close to motor parks, shops, and other non-dairy activities which put the products at a high risk of contamination (Yakubu et al., 2020).

The findings of this work lend credence to the assertion that, dairy products locations of selling point are one of the major vehicles for the transmission of *S. aureus* to dairy food products. (Jahan et al., 2015; Yakubu et al., 2020)

b) Lack of education on the aspects of milk and dairy product hygiene and handling practices among the people who are concerned with transportation and sales

The use of traditional method of production also exposes the products to bacteria found on the hands and clothes of the people that are concerned with the production and also in the containers used. The occurrence of *Staphylococcus aureus* in the study area is an indication of defective or absence of public health measures (Akram et al., 2013). The unsanitary conditions of the places where the products are marketed might have also contributed to their contamination (Yakubu et al., 2020). Moreover, a significant association was found between the occurrences of *Staphylococcus aureus* in water used for cleaning utensils at the product's selling points. Direct contact, often via the hands, with colonized or infected animals or people and contaminated equipment and surfaces are also responsible for *S. aureus* transmission in products at selling level.

These are the indication of defective or absence of public health measures and poor sanitary habits among the people that are concerned with, milk and milk product handling, transportation and sales as these have been documented to be factors that predisposes *S. aureus* transmission in milk and milk product and contamination with pathogens (Akram et al., 2013).

So, there is a need for frequent education of the sellers on the aspects of milk hygiene and handling practices, which will help in no small measure in improving the quality standards of the products at the selling points. There is need for relevant authorities to educate the sellers and public on the dangers of Staphylococcal food intoxication .This can be achieved through teaching and training programmes using participatory approach method (Yakubu et al., 2020)

In order to, preventing *S. aureus* transmission in milk and milk products it is required to check transmission factors as well as improvement of management practises.

For minimising transmission at farm level, milkers' should always wear gloves and change them frequently, especially when dirty or after stripping an infected animal. It is recommended to forestrip five squirts of milk from each quarter and check for abnormal milk or flakes. Dirt should be brushed off teats with the use of a dry, single-use towel. Water should not be used as part of any milking procedures, even if a sanitizing solution is added. Commonly used dairy sanitizers, namely those containing chlorine and iodine, acid anionic compounds, and quaternary ammonium-based sanitizers should be used regularly. A commercially available predip should be applied with a dipper or cup and given 30 seconds of contact time. Sprayers can be used, but proper coverage is difficult to attain, especially on the teats furthest from the milkers'. Foamers are also commonly used, but caution must be taken to ensure that adequate parts per million of the active ingredient reach the teat end and teat skin. A separate paper or cloth towel should be used to dry teats and scrub teats five times or for 20 seconds (Rasmussen et al. 1992). Towels must not be used on more than one cow. An

effective post-milking teat dip should be applied after milking, ensuring that the entire barrel of each teat is covered. At the end of each milking any teat dip left in the dip cup should be discarded and cups should be rinsed with water and allowed to dry.

In case of production and marketing level, emphasis is placed on keeping pathogens out of the bulk tank, storage container, processing environment plant, eliminating them through proper processing, preventing post-processing contamination, and sanitation. Increased emphasis is focused on proper maintenance of equipment, particularly the plates in the HTST pasteurizer; use of clarifiers; use of footbaths; and control of the plant environment. The Hazard Analysis and Critical Control Point Program, specifically designed to minimize the threat of *S. aureus*, is also being reemphasized.

Conclusion and recommendations

Milk and milk based products possess a high nutritional profile which helps to transmit *S. aureus* vary easily at different stages from farm milk harvesting to final consumers intake. Consumption of *S. aureus* contaminated milk and dairy products considered as a potential source of public health hazard. Hence, there is an urgent need to check *S. aureus* transmission factors elucidated in this paper e.g., milk collection from infected animals as asymptomatic carrier; infected heifers; unhygienic activities of farm workers; poor management of farm environment: unhygienic milking, mismanagement of milking equipment; contact with contaminated manageable risk factors; contamination in bulk milk tank, inefficient pasteurization of raw milk; poor making plants and value chain hygiene: unhygienic handling, dirty water used for cleaning utensils, absence of sanitary inspection; post-pasteurization contamination; location hygiene of selling points for preventing transmission of *S. aureus* in milk and milk products from the point of production to the point of consumption of milk is necessary for consumer safety.

In addition, milk must be produced, distributed, handled and marketed under the control of milk commission and the commission must have a sanitary inspector and veterinarian to enforce its methods and standards. From a food safety point of view, food handlers at different points in the milk value chain should be educated on how to reduce transmission of milk and milk products with *Staphylococcus aureus* pathogen through personal and equipment hygiene as well as through provision of cold-chain equipment in the milk supply chain. On the other hand, farmers should also be educated on methods of reducing raw milk contamination from the environment and from the cow (mastitis) itself for putting a stop to *S. aureus* born food poisoning and securing consumers good health.

Limitations

The study is limited for transmission of *S. aureus* in milk and milk products in few aspects among which variation of geographical area, farm hygiene and environment management, pasteurization, production industry and selling point etc are most noteworthy.

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Biography

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