



Microbial analysis and health risk of *Listeria spp* and *S. aureus* isolated from cheese and raw milk marketed in Lahore, Pakistan

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Abstract: Objective: The study was conducted to evaluate microbial quality and associated health risk of raw milk and cheese in Lahore region, Pakistan. **Methods:** Microbial assessment of raw milk and cheese was undertaken by evaluating 30 samples of raw milk collected from sheds and by bicycle boys and 17 sample different type of cheese from hyper store during August 2017. Samples were cultured on respective media i.e. Nutrient agar, Brilliance listeria agar and Mannitol salt agar. Growth was confirmed by performing Gram staining and different biochemical tests. **Results:** The bacterial count of *S. aureus* was high in Semi hard cheese and raw cow milk as compared to goat milk and other types of cheese. The frequency of *Listeria spp* was found higher in soft cheese and raw goat milk. **Conclusion:** The study concluded that raw milk and cheese in the study is of poor bacteriological quality and hazardous for human consumption. This highlights the need to implement good hygiene practices and effective monitoring from production through the delivery chain to the consumer. Further studies are needed for detection of *Listeria spp* at molecular level and other harmful microorganisms.

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Keywords: microbial association, risk factor, raw milk, *Listeria*, *S. aureus*, cheese

Introduction

Species of *Listeria* are present everywhere in the food industrial location and farm and they often spoil foods including a wide variety of products made from milk, vegetables, products made from milk and fish (Pak *et al.*, 2002). Between the genus of listeria, which causes diseases in humans and animals, *L.monocytogenes* is a main disease causing microorganism, and the infrequently happening bacteria *L. ivanovii* is disease causing for human beings (Khalil *et al.*, 2020a, b; McLauchlin *et al.*, 2004). Disease caused by listeria is mostly due to spread of microorganism through contamination of food (McLauchlin *et al.*, 2004) Minor symptoms in most of cases are condition in which feces are discharged from the bowels frequently and in a liquid form, temperature, continuous pain in the head, and pain in muscles (FAO/WHO, 2004). Severe symptoms of Listeriosis in humans and animals include blood poisoning, inflammation of the membranes of the brain and the adjoining cerebral tissue and miscarriage. Immunocompromised people, expecting

women and new borns are mostly at risk (Meyer-Broseta *et al.*, 2003). Listeriosis linked with the intake of extensive range of foods that include products made from fish and meat and food that you don't need to cook food that has already been cooked (Rana *et al.*, 2020; Khan *et al.*, 2020; McLauchlin *et al.*, 2004). *S. aureus* ranks third important source of disease in the whole world between the reported illnesses related to food borne (Ali *et al.*, 2020; Danish *et al.*, 2020; Zhang *et al.*, 1998). Normal micro flora of *S. aureus* is mucous membranes and skin of animals and human beings and it is also present in the environment (Jay, 1997). Food products might be initially contaminated through or when treated. *S. aureus* has been separated from different food products like chicken, milk and meat products, vegetables, fish products, meat and meat products (Ahmad *et al.*, 2020; Nazir *et al.*, 2020; Tamarapu *et al.*, 2001). Food poisoning caused by *S. aureus* is due to intake of food that contains enterotoxins (SE). Symptoms include a feeling of sickness with an inclination to vomit, vomiting and condition in which faeces are discharged in liquid

form (Jablonski and Bohach, 1997). Eighteen types of staphylococcal enterotoxins are labeled SEA-SEE, SEG-SER and SEU (Omoe et al., 2003). Milk producing animals are major cause of contamination with *S. aureus* (Vautor et al., 2003). Animals having inflammation of the mammary gland in the breast or udder due to *S. aureus* shed great amount of *S. aureus* into milk. Impurity may be possible from environment, through manufacturing and handling from human beings (Genigeorgis, 1989). The process of calculating that how much risk is involved showed that *S. aureus* in new and short-time matured raw milk cheese might cause risk of health as large portion of cheese might compromise inadequate levels of *S. aureus* at the time of ingesting (Lindqvist et al., 2002). This study was conducted to evaluate the frequency of microbial contaminants in milk and cheese.

Material and methods

Sample collection

Cheese was collected from super market and raw milk was collected from hyper market in Lahore.

Isolation and identification of bacteria

Sample of milk and cheese were used. Different types of cheese were mixed by turning the falcon upside down 5 or more times, and 10 fold dilutions were made using sterile saline solution. Samples were then labeled and dilutions were run on brilliance listeria agar. Inoculated dishes were protected at 37°C for 24 hours. For isolation, purification and identification of bacteria different media were prepared that include blood agar and manitol salt agar and brilliance listeria agar. Bacterial colonies were characterized on the basis of colony morphology, gram staining and biochemical tests. Gram staining was performed to observe the morphology of bacteria. Confirmation of bacteria was done by performing biochemical tests according to James Cappuccino Natalie Sherman like catalase test, citrate test, coagulase test, methyl red test, urease test, triple sugar iron test, nitrate reduction test and oxidase test.

Results and discussion

The biggest perceived *Listeriosis* flare up in Germany, connected to a profoundly polluted acid curd cheese produced from sanitized milk. Proof originated by applying molecular technique to *listeria* samples taken from sick people and from cheese. The greater part of cases with accessible particular epidemiological or microbiological data was connected to the flare up. This flare up usually for the disease caused by *listeria* was widely spread and continued for few weeks showing long incubation time and long timeframe of realistic usability (Gerner-Smidt et al., 2006). The present study revealed the prevalence of *Listeria* in raw milk and cheese. The disease causing organisms are present everywhere

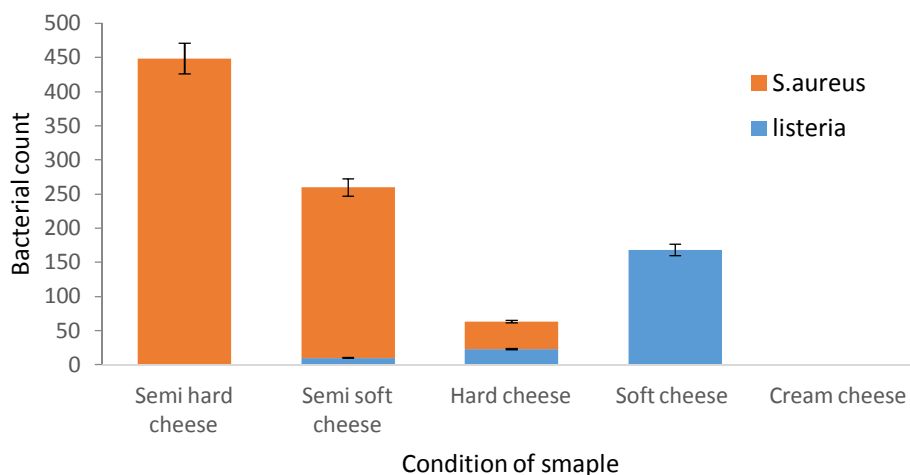
that's why impurity can occur ensuing handling by heat. Soft cheeses are especially inclined to bacterial development. Specie of listeria i.e. *L.monocytogenes* has been distinguished in cheeses produced from treated milk during food checking (Rudolf and Scherer, 2001). In our study *Listeria* was more prevalent in soft cheese and in raw goat milk. Disease from *listeria* might be spread from diseased creatures or through ingestion of polluted vegetables or meat and by dairy products. In shahrekord drinking of untreated milk and cheese made in unlicensed plants from untreated milk is extensively rehearsed. The result from this study have exposed that untreated milk that is used in cheese development in Iran can help as a path to spread *listeria* causing disease in human beings. Subsequently all untreated milk should be thought to be polluted with listeria and sufficient steps are needed by dairy makers to confirm proper pasteurization and to stop impurity of treated milk products and to confirm the production of safe items (Moshtaghi and Mohamadpour 2007). Bulks of cheese made by dairy farms are lesser related to whole manufacturing of cheese in Sweden. The majority of dairy farms were little ventures which recommend that people were occupied with errands both on the homestead and in the dairy. This circumstance focuses on the requirement for successful cleanliness hindrances since control of microbial impurity before treating e.g. from animals having inflammation of udder tissues, the nature or water, together with process control have been distinguished as safe elements for creating safe cheese (Jorgensen et al., 2005). *E.coli*, *S.aureus* and specie of *listeria* known as *L.monocytogenes* mostly found in dairy products like yogurt and cottage cheese (Oranusi et al., 2007). A lethal chemical known as enterotoxin is released by *S. aureus*. It causes illness even if present in small amount of 1.0µg in food. This amount of the poison has been found at 105cells/g of food (Ananthanarayana et al., 2001). Another study was conducted to check the prevalence of *L.monocytogenes* and *S.aureus* in bulk tank milk. *S.aureus* was found in 39.8% and *L. monocytogenes* was present in 2.7% of farms. For improving the milk quality good hygiene practices should be needed (Mehmeti et al., 2017). In the present study indicated high frequency of *S.aureus* in semi hard cheese and in raw cow milk. This study was conducted to check the presence of *listeria* and *S.aureus* in cheese, raw goat milk and raw cow milk. Milk plays an important part in our life and is mostly used in dairy products. The bacterial count of *S.aureus* is more in semi hard cheese and *listeria* in soft cheese. Raw goat milk is more contaminated of listeria than of *S. aureus*. However raw cow milk is more contaminated of *S. aureus*.

Table 1. No. of organism isolated from sample

Types of cheese	No. of samples	<i>listeria</i>	<i>S.aureus</i>
Semi hard	5	0(5)	3(5)
Semi soft	4	1(4)	2(4)
hard	3	1(3)	1(3)
Soft cheese	2	2(2)	0(2)
Cream cheese	3	0(3)	0(3)

Table 2. Bacterial count in samples of cheese

bacteria	Semi hard cheese	Semi soft cheese	Hard cheese	Soft cheese	Cream cheese
<i>listeria</i>	0	10	23	168	0
<i>S.aureus</i>	449	250	40	0	0

**Table 3. No. of organisms isolated from sample**

Types of milk	No. of samples	<i>listeria</i>	<i>S.aureus</i>
Raw goat milk	15	7(15)	4(15)
Raw cow milk	25	5(25)	10(25)

Table 4. Bacterial count in samples of milk

Bacteria	Raw goat milk	Raw cow milk
<i>listeria</i>	53	32
<i>S.aureus</i>	41	665

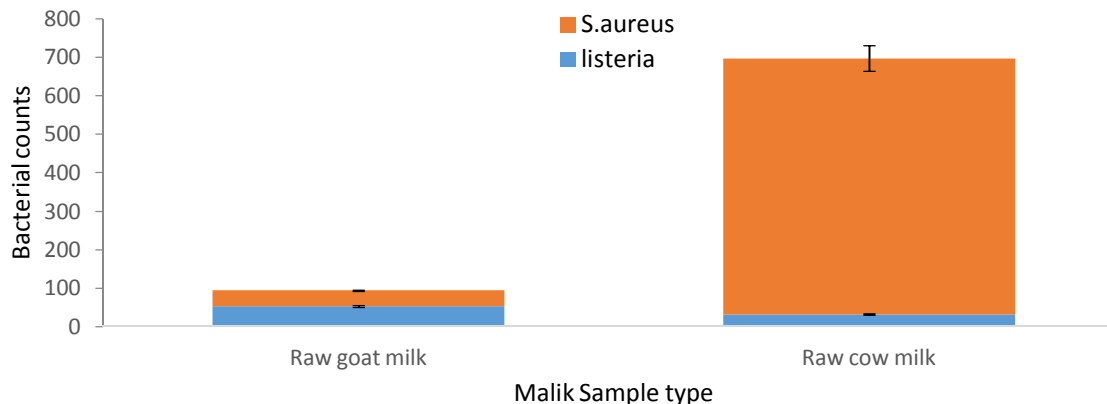


Table 5. Biochemical results of isolated bacteria

Biochemical tests	<i>listeria</i>	<i>S.aureus</i>
Catalase test	+	+
Citrate test	-	+
Indole test	-	-
Methyl red test	+	+
Coagulase test	-	+
Hemolysis	+	+
H ₂ S	-	-
Motility test	+	-

Conclusion

In conclusion, for lowering the contamination of milk and dairy products we strictly need to develop preventative measures to avoid the spread of pathogens during manufacturing or at the sites of packaging. This study was conducted to check the presence of *listeria* and *S.aureus* in cheese, raw goat milk and raw cow milk. Milk plays an important part in our life and is mostly used in dairy products. The bacterial count of *S.aureus* is more in semi hard cheese and *listeria* in soft cheese. Raw goat milk is more contaminated of *listeria* than of *S.aureus*. And raw cow milk is more contaminated of *S.aureus*.

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