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ISOLATION AND IDENTIFICATION OF ZOONOTIC IMPORTANCE BACTERIA FROM MILK, MILK PRODUCTS AND HUMAN IN DIYALA, IRAQ

Rofeida Muhammad Hussein and AL-Khafaji Nazar Jabbar

Department of Medicine, College of Veterinary Medicine, University of Diyala, Iraq.

Abstract

To study bacteria of zoonotic importance, contaminated milk and milk products, a total 186 samples; represent 76 samples raw milk; 35 milk products , in addition to 75 swabs from workers in shops of milk products, were collected, from August, 2019 to April, 2020. The samples were submitted to laboratory examinations, for isolations and identifications. The results showed, that from 186 samples: 26 (13.98%) were free from bacteria, while from others, 160 samples (86.02%), 288 isolates were isolated, either in single form, 75 (40.3%) or mixed in two isolates in a sample 47 (25.3%), or there 33 (17.7%), or four 5 (2.7%) in a sample. The highest numbers of isolates was Staph.58/288 (20.1%); Sal 39/288 (13.5%); Lact.35/288 (12.2%); *E. coli* 30/288 (10.4%); Pseud. 30/288 (10.4%); Kleb. 29/288 (10.1%); Ent. 24/288 (8.3%); Prot.17/288 (5.9%); Cit.17/288 (5.9%); Strept. 5/288 (1.7%); List 4/288 (1.4%). From a total 76 samples of raw milk from udder and bulk tank from cow, buffalo, sheep and goat. 142 isolates were isolated: from which *Sal.* sp. 27/142 (19.0%); Staph. sp. 24/142(16.8%); Lact. 22/142 (15.5%); *Ent.* sp. 17/142 (12.0%); *E. coli* 15/142 (10.6%); *Cit.* sp. and *Prot.* sp., each 12/142 (8.5%); Kleb.8/142 (5.6%) and *Pseud.* sp., 5/142(3.5%). While from a total 35 samples of milk products (Cheese and Yoghurt), 62 isolates were isolated: Lactobacillus 14/62 (22.6%); *Salmonella* 12/62 (19.4%); *E. coli*., 10/62 (16.1%); Staph. 7/62 (11.3%); Pseudomonas 6/62 (9.7%); Proteus 5/62 (8.1%); Klebsiella 4/62 (6.5%); Enterobacter 3/62 (4.8%); Citrobacter 1/62 (1.6%).The highest isolates were from raw milk 141/186 (75.8%) then, workers 85/186 (45.7%), and milk products 62/186 (33.3%).

Key words: isolation; identification, zoonotic agents; milk, milk products

Introduction

Milk may serve as an ideal substrate for the growth and survival of an array of bacteria and fungi, thereby leading to the public health threat (Zucali *et al*., 2019).. Milk in healthy udder cells is considered sterile, but thereafter contamination can originate from different sources, as teat apex, milking utensils, feed, grass, soil, surrounding air, feces, water or moisture content, and other environments (Verdier-Metz *et al.,* 2012; Vacheyrou, *et al.,* 2011). The aims of study were isolate the most common zoonotic pathogens from milk and milk products from, cow, buffalo, sheep and goats. Furthermore, identify these isolates.

Materials and Methods

All culture media, were prepared according to instruction of Manufactures Corporation and sterilization were, according to (Quinn *et al.,* 2004).

Samples

The study was conducted in Department of Medicine, College of Veterinary Medicine, University of Diyala, Iraq, from August, 2019 - April, 2020.

Collection of raw milk samples

A total 186 samples, represent; (76) raw milk*, from cow, buffalo, sheep and goat*, collected either from udder or from bulk tanks; (35) cheese and yoghurt samples *were* purchased from shop, sales shops*,* in addition to (75) swabs obtained from workers and their equipment, utilized in shops or in preparation and transportation of milk and milk products *in Diyala Province*. *Collected aseptically* in clean, dry and sterile tubes, in a cool places, bring to laboratory, within 24 h. and examined upon arrival to the laboratory, for bacteriological analysis as described by (Fawole MO, Oso BA. Laboratory manual of Microbiology, 2001; *Islam et al., 2016*).

Bacterial examination

Initially, 25ml of each raw milk sample dispended into a sterile flask containing 225 ml of 0.1% peptone water and mixed thoroughly. Subsequent serial decimal dilutions of each sample were prepared in 0.1% peptone water according to (APHA, 2001).

Swabs culturing

The swabs were submitted to culture by inoculation into nutrient broth and incubated at 37°C for 5 hr. Loop full from the incubated broth was distributed onto surface of MacConkey agar then incubated at 37°C for 24 hr. according to (Stromberg, 2015).

Characterization and identification of the colony

Characterization and identification of the colony isolates was achieved by initial, morphological examination of the colonies on plate. Gram staining and the biochemical tests ,with standard reference organisms with those of known taxa, as described by Bergey's manual for determinative Bacteriology(Syed *et al.,* 2014; Bharathy *et al.,* 2015).

Results

Workers

From a total 75 swabs, 85 isolates were isolated. Staph., 27/85 (31.7%); Pseud. 19/85 (22.4%); Kleb.17/85 (20.0%); *E. coli*. and Strept each 5/85 (5.9%); List., Ent. and Cit. each 4/85(4.7%) table 1.

Cow

Total 45 samples obtained from cow, from which 81 isolates were isolated: Staph.15/81 (18.5%); Sal.13/81 (16.0%); Lact.12/81 (14.8%); Ent. 10/81 (12.3%); *E. coli* 9/81 (11.1%); Prot., 8/81 (9.9%); Citro. 6/81 (7.4%); Kl. 5/81 (6.3%); Pseud.3/81 (3.7%) table 1.

Buffalo

A total of 15 raw milk obtained from buffalo from udder and bulk tank, from which, 30 isolates were isolated. Sal. 6/30 (20.0%); Staph. and *E. coli*, 5/30 (16.6%); Lact. and Ent. each 3/30 (10.0%); Kleb.; Pseud.; Prot. and Citro. each 2/30 (6.7%) table 1.

Sheep

From 10 raw milk obtained from sheep, 17 isolates were isolated : Sal. and Lact. each 4/17 (23.5%); Ent. 3/17 (17.6%); Cit. 2/17 (11.8%); Kleb.; Staph.; *E. coli* and Prot. each 1/17 (5.9%) table 1)

Goat

From 6 raw milk samples obtained from goat, 13 isolates were isolated: Sal. 4/13 (30.7%); Staph. 3/13 (23.1%); Citro. and Lact., 2/13 (15.4%); Prot. and Ent. 1/13 (7.7%) table 1.

From a total samples of raw milk from udder and bulk tank from cow, buffalo, sheep and goat 76 samples. 142 isolates were isolated: From which *Sal.* sp. 27/142 (19.0%); *Staph.* sp. 24/142(16.8%); Lact. 22/142 (15.5%); *Ent.* sp. 17/142 (12.0%); *E. coli* 15/142 (10.6%); *Cit.* sp. and *Prot.* sp., each 12/142 (8.5%); Kleb. 8/142 (5.6%) and *Pseud.* sp., 5/142(3.5%) table 1.

Milk products: Cheese

A total 25 samples of cheese local and kala were collected from which, 48 isolates were isolated. Sal. and Lact. 9/48 (18.8%); *E. coli*, 8/48 (16.7%) ; Pseud. 6/48 (12.5%); Staph, 5/48 (10.4%); Kl. and Prot. 4/48 (8.3%); Ent. 2/48 (4.2%); Cit. 1/48 (2.1%) table 1.

From 10 samples of Yoghurt ; Sulaimania, Kanoon, 14 isolates were isolated 14: Lact 5/14 (35.7%) ; Sal 3/14 (21.4%); Staph. and *E. coli* 2/14 (14.3%); Ent. and Prot. 1/14 (7.1%) table 1.

From a total 35 samples of milk products, 62 isolates : Lact.14/62 (22.6%).; Sal. 12/62 (19.4%); *E. coli*., 10/62 (16.1%); Staph. 7/62 (11.3%); Pseud. 6/62 (9.7%); Prot. 5/62 (8.1%); Kleb. 4/62 (6.5%); Ent. 3/62 (4.8%); Citr. 1/62 (1.6%) table 1.

From a total 186 samples; represent 76 samples raw milk; 35 milk products and 75 swabs from workers in

Table 1: Total numbers of isolates in current study.

Origin	No.	Kl.	Ps.	St.	E..	Sal.	Lact.	Cit	Ent.	Prot.	Stre.	List.	Total
Work.	75	17	19	27	5	0	0	4	4	0	5	4	85
Cow teat	4	0	0	2	1	2	0	1	1	1	0	0	8
udder	30	4	1	10	6	8	10	4	7	5	0	0	55
Tank	11	1	2	3	2	3	2	1	2	2	0	0	18
Buff. Udder	10	1	1	4	2	3	2	1	3	1	0	0	18
Tank	5	1	1	1	3	3	2	1		1	0	0	13
Sheep	10	1	0	1	1	4	4	2	3	1	0	0	17
Goat	6	0	0	3	0	4	2	2	1	1	0	0	13
Cheese	25	4	6	5	8	9	9	1	2	4	0	0	48
Yoghurt	10	0	0	2	2	3	5	0	1	1	0	0	14
Total	186	29	30	58	30	39	35	17	24	17	5	4	288

shops of milk products: 288 isolates were isolated. The highest numbers of isolates was Staph.58/288 (20.1%); Sal 39/288 (13.5%); Lact.35/288 (12.2%); *E. coli* 30/288 (10.4%); Pseud. 30/288 (10.4%); Kleb. 29/288 (10.1%); Ent. 24/288 (8.3%); Prot.17/288 (5.9%); Cit.17/288 (5.9%); Strept. 5/288 (1.7%); List 4/288 (1.4%) table 1.

Total numbers of samples in current study were 186. From which, 26 (13.98%) were free from bacteria, while from others, 160 samples (86.02%), a total of 288 isolates were isolated; from which 75 (26.0%) as single isolate, other as mixed with one or more isolates: in two isolates (47) :47×2 = 94 (32.6%); or there isolates, 33 (33×3) = 99 (34.5%); or four isolates 5 (4×5) = 20 (6.9%).

Discussion

In current study from 186 total samples, 26 (13.98%) did not yield isolates, while 160 (86.02%) yield isolates: 288 isolates were isolated. Among these isolates the Staphylococcus sp. was the most prevalent 58/288 (20.1%); followed by Sal 39/288 (13.5%); then Lact. 35/288 (12.2%); *E. coli* 30/288 (10.4%); Pseud. 30/288 (10.4%); Kleb. 29/288 (10.1%); Enterobacter 24/288 (8.3%); Proteus 17/288 (5.9%); Citrobacter 17/288 (5.9%); Strept. 5/288 (1.7%); List 4/288 (1.4%).

From 190 samples, 52 (27.37%) did not yield any isolates. Microorganisms were isolated from 138 samples. Among these, *Staphylococcus aureus* was with 52 (27.37%), followed by coagulase negative *Staphylococcus* spp. 24 (12.63%), *E. coli* 17(8.95%), *Pseudomonas* spp. 15 (7.89%), *Streptococcus* spp. 11(5.79%), mixed bacterial infection 9 (4.74%). *Klebsiella* spp. 3(1.57%) and Bacillus spp. 1(0.52%) isolates (Mohini *et al*., 2002; Grewal *et al.,* (2005).

Workers and equipment

In current study, from 75 swabs obtained from worker, only four of them did not yield isolates. While from others 71 samples, 85 isolates were isolated either in single form, [Staph. 19, Cit. 3, Pseud.13, Kleb.13, Strep. 5, List.1, Ent. 2 , *E. coli* 1] , or in two isolates in a sample (14). The highest numbers of isolates was Staph 27/85 (31.7%); followed by Pseud 19/85 (22.4%); then Kleb.17/85 (20.0%); *E. coli*. and Strept each 5/85 (5.9%); List., Ent. and Cit. each 4/85(4.7%).

The milk can be contaminated directly by farm machinery and storage facilities. The microflora of the bacteria in the machinery varies greatly (Michel *et al.*, 2006). Poor quality of milk, the use of unclean milking and transport equipment, poor hygienic quality, unhygienic conditions of manufacturing units, machinery, farm staff, unclean hands of workers, inferior quality of material used and water supplied for washing the utensils, the environment including bedding, air, grass, collection vessels, could be the source of accelerating the bacterial contamination of milk product and the post manufacturing contamination [Verdier-Metz *et al.,* 2009; Quigley *et al*., 2013].

In current study 4 swabs were obtained from external surface of teat in cows, all were positive to bacterial contamination. 8 isolates were isolated, two isolates in each sample; Staph. and Sal. each of 2/8(25.0%), Enterobacter, Citrobacter and Proteus each1/8 (12.5%).

Initial microbial colonization of raw milk comes from the teat surface, as the organisms present in unclean teats. These have been demonstrated to harbor a diversity of species , including members of the phylia Firmicutes, Actinobacteria, Proteobacteria and Bacteroides. [Verdier-Metz *et al.,* 2012; Bream *et al.,* 2012; Monallier *et al*., 2012]. The teat microbiota was shown to vary greatly, from cow to cow, and farm to farm, due to the manner in which it was acquired from, bedding, machinery, farm staff during milking and the environment [Verdier-Metz *et al.,* 2012; Braem *et al.,* 2013]. Braem *et al.,* 2013, identified coagulase- negative Staphylococci as the dominant residents.

In current study, from 6 raw milk samples obtained from goat, 13 isolates were isolated , one in single form [Staph. 1], other in mixed, three isolates in a sample (4).: The highest one is Sal. 4/13 (30.7%); Staph. 3/13 (23.1%); Citro. and Lact. each 2/13 (15.4%); Prot. and Ent. each 1/13 (7.7%).

In current study, from 10 raw milk samples obtained from sheep, two were free from contamination and 17 isolates were isolated from other samples, either in a single form (2) [Ent. 1; Sal.; 1], or in mixed forms, two isolates (4) , three isolates (1): Sal. and Lact. each 4/17 (23.5%); Ent. 3/17 (17.6%); Cit. 2/17 (11.8%); Kleb.; Staph.; *E. coli* and Prot. each 1/17 (5.9%).

The predominant isolated coliform strains in the examined raw goat's and ewe's milk samples were *E. coli*, *Citrobacter amalonaticus, C. freundii, Escherichia adecarboxylata, Enterobacter aerogenes, Ent. agglomerans, Ent. cloacae, Ent. gergoviae, Klebsiella oxytoca, K. pneumonia* sub spp. ozaaenae, *K. pneumonia* sub spp. pneumoniae and *Hafnia alvei* at percentages varied between 0 to 17.14. The incidence of coliforms was 68.57% and 60% for raw goat's and ewe's milk respectively (Ombarak and Elbagory, 2017). Comparatively lower counts of coliforms were recorded by (Salem, 2003) for raw goat's milk and

(Bahout 1995) for raw ewe's milk.

In current study, coliform strains occupied 156/186 (83.87%). Sal.; *E. coli*.; Entero.; Kleb; Prot.; Citro account for (39; 30; 24; 29; 17 and 17) respectively.

In current study, *E. coli* occupied the fourth position (30/186(16.1%), post Staph., Sal., Lact., from raw milk 15/76919.7%), From milk product 10/35 (28.6%), from workers 5/75 (6.7%).

Sheep and goats may act as a reservoir of pathogenic *E. coli* and their milk may serve as vehicle for the pathogen transmission to humans (Abd El-Ael and Awad, 2008). Relatively lower incidence was reported by (Foschino *et al*., 2002 and Dontorou *et al*., 2003), but higher incidence was reported by (Bahout, 1995; Abd El-Ael and Awad, 2008). The incidence of *E. coli* (14.29 and 11.43%) in raw goat's and ewe's milk, respectively (Ombarak and Elbagory, 2017). This to some extent also recorded by (Foschino *et al*., 2002). *Soomro et al.,* 2002 isolated *E. coli* in 57% of the 100 raw milk samples.

Staph. occupied the first position in current study, (58/186 (31.18%), from raw milk 24/76 (31.6%), from milk product 7/35(20.0%), from workers 27/75 (36.6%).

Alisarli and Solmaz (2003), detected *S. aureus* 38% of the 100 raw milk samples. Coliform organisms and *S. aureus* are good indicators of the standard of hygiene and handling.

S. aureus was isolated from 3 cows' milk samples, 6 sheep's milk samples and 3 goats' milk samples. While *E. coli* was not detected in cows' milk samples; it was detected in 1 sheep's milk sample and 5 goats' milk samples. *Salmonella* spp. was not detected in all samples analyzed (Ekici *et al*., 2004).

Out of 118 raw milk samples processed, 12 (10.16%) samples yielded the growth of *S. aureus*this occuer by Paterl *et al.,* (2018). The prevalence rates were differing according to regions of India, at Navsari district region showed prevalence of 10.16%; While Thaker *et al.,* (2013), reported 6.215% in Anand (Gujarat) region of India; Meanwhile, Kumar and Prasad, (2010). reported 26% prevalence in milk samples collected from local vendors of Pantnagar, India. Sarkar *et al.,* (2013) documented 74.5% (149/200) of the milk samples were positive for *S. aureus* from the Karnal, North India and Lingathurai and Vellathurai, (2010) reported 61.7% of prevalence of *S. aureus* from 60 raw milk samples from Madurai region of South India.

Farhan and Salk, (2007) studied on 130 milk samples in Palestine and found 48(36.9%) samples were containing *S. aurus* . Ekici *et al.,* (2004) found (18.18%) of the milk samples positive for *S. aureus* while studying 66 samples in Turkey. In Morocco, Bendahou *et al.,* (2008) studied 27 samples and found 40% of the milk samples were containing *S. aureus*; while in India, 61.7% of the raw milk samples were found positive out of 60 samples studied (Lingathurai and Vellathurai, 2010).

Prevalence rate from Morocco, Palestine and Bangladesh reported by Bendahon *et al.,* 2008, Farhan and Salk (2007) and Jahan *et al.,* (2014) as (40%, 36.9%), and 25.53% respectively, which were higher to Patel *et al.,* (2018) study. However, similar prevalence has been previously reported by Fagundes *et al.,* (2010). From Sao Paulo State, Brazil, The ratio was (10.8%) by Ayano *et al.,* (2013). (13.8%) from Holeta, Ethiopias. From all these study results of above mentioned indicates prevalence of *S. aureus* is varied from place to place and regions to regions around the world and it highlights that hygienic practice of milking and selling influence the prevalence of *S. aureus* in milk.

Thaker *et al.,* (2013) showed that out of total 160 samples, (100) milk and (60) milk products *i.e.* curd (30), and pedha (30). *S. aureus* was isolated in 10 isolates from 160 samples (6.25%); as 6 (6.00%), of 100 milk samples, 3 (10.0%) from 30 pedha and 1 (3.33%) from 30 curd samples. Fagundes *et al.,* (2010) recorded (7.3%) and Kumar and Prasad (2010) (6.6%).

Higher level of incidence of *S. aureus* have been reported by Singh and Prakash, (2008); Ekici *et al.,* (2004); Santana *et al.,* (2010); Zakary *et al.,* (2010) and Lingathurai and Vellathurai (2010), who found 17.39%, 18.18%, 18.80%, 40% and 61.7% incidence respectively.

The finding of Thaker *et al.,* (2013) study are in accordance with the findings; Ekici, *et al.,* (2004); (9.5 %) Normanno *et al.,* (2007) (12.8%); Singh and Prakash (2008) (10.34%); Kumar and Prasad, 2010 (6.6%) and Addis *et al.,* (2011), (10%).

A total of 47 raw milk samples were tested and *S. aureus* was isolated from 12 (25.53%) samples (Jahan *et al*., 2015). The prevalence of *S. aureus* in raw milk and dairy product was found to be 56% in Turkey by Gundogan and Avci (2014) which were significantly higher than study of Jahan *et al.,* (2015).

Jahan *et al.,* (2015) from raw milk samples from dairy cattle.12 samples were positive for S. aureus (25.53% (12/47). Zafolon *et al.,* (2008) studied at Nova Odesa, Sao Paulo; showed that the prevalence of *S. aureus* was 54.4% . the results of Jahan *et al.,* (2015) are higher when compared to those of (Shitandi and Sternesjo, 2004; Gundogan *et al.,* 2006).

The high incidence of *S. aurus* is indicative of poor hygienic measures during production, handling and distribution, stated in the findings of Zakary *et al.,* (2011).

Salmonella sp. occupied the second position, post Staph. in current study (39/186 (21.0%), from raw milk 27/76 (35.5%), from milk product 12/35 (34.3%).

Salmonella spp. has been detected in raw sheep milk (Fotou *et al*., 2011). However, this pathogen is one of the main microbiological hazards in raw cow milk (Claeys *et a.,* 2013).

In current study , Pseudomonas occupied the eighth position, 30/186 (16.13%), from raw milk 5/76 (6.6%); from milk product 6/35 (17.1%0; from workers 19/75 (25.33%).

In current study, *Listeria* sp. was last position 4 out of 186 total samples in study, (2.15%), as it isolated from workers and equipment only 4/75(5.33%).

In Asmaa *et al.,* (2017) milking equipment had the highest isolation rate of *Listeria* spp., followed by raw milk and hands swabs. There was no significant difference between *Listeria* spp. isolation rates between the three sources.

Listeria monocytogenes is ranked as the third major pathogen that is transmitted by food (Scallan *et al*., 2012) and its occurrence in milk and dairy products has a negative impact on dairy industry and public health (Usman *et al*., 2016).

The occurrence of *Listeria* spp. isolated from raw milk concurred with findings, 26% in Colombia (Vanegas *et al.,* 2009) and 23% in Iran (Rahimi *et al.,* 2010). A higher isolation rate of *Listeria* spp. in Asmaa *et al.,* (2017) study compared to previous studies in Egypt and other countries has been observed (Ismiel *et al.,* 2014 and Jamali *et al.,* 2013) and lower incidence (53%) than recently reported in Nigeria by Usman *et al.,* (2016). On the other hand, previous studies reported the occurrence of *Listeria* spp. in 1.5% of tested milking equipment [59]. Isolated from milking equipment and hand swabs in Asmaa *et al.,* (2017) study. It is known that the main sources of *Listeria* spp. contamination in dairy farms are infected animals, poor silage quality, and environment (Pantoja *et al.,* 2012). The infected animals and insufficient hygienic measures during milking and milk storage are likely the most common sources of *Listeria* spp. contamination. (Asmaa *et al.,* 2017).

El Marnissi *et al.,* (2013) exhibited 5.90% as an overall prevalence of *L. monocytogenes* in raw milk. Boubendir *et al*., (2016) observed a comparable occurrence in bovine raw milk from the Northen Eastern Algaria, Jami *et al.,* (2011) reported a lower contamination rate for milk samples in Mashhad, Iran.

It has been shown that lactic acid bacteria (LAB) are the predominant microorganism in most fermented foods (Gulitz *et al.,* 2013; Guetouache *et al.,* 2015). They ferment lactose to lactate and are the dominant population in bovine, goat, sheep and buffalo milk prior to pasteurization. Raw milk contains about 30% of undesirable micro-organisms in total microbial count, therefore, this problem suggests inflexible hygienic measures must be followed in cheese making (Melkamsew *et al*., 2012; Pazakova *et al*., 2001).

In current study, from 25 samples of cheese, only 4 were free from contamination and 48 isolates were isolated, either in single form 4 [Psed.1, *E. coli*, 2, Lact.1], or in two isolates 8, three isolates 8, or 4 isolates 1. The highest number was, Sal. and Lact. Each (36.0%), followed by *E. coli* (32.0%), Pseud. (24.0%), Staph. (20.0%), Kleb. and Prot. each (16.0%), Cit. (4.0%). While from 10 samples of Yoghurt, submitted for examination, 14 isolates were isolated, 5 in single form, [Staph. 1, Sal. 1, lact. 3], other in mixed forms, three isolates (3) in a sample. The highest one was Lact. (50.0%), Sal. (30.0%), Staph. and *E. coli* each (20.0%), Ent. and Prot. each (10.0%).

Cremonesi *et al.,* (2007) tested 33 samples of raw milk cheese and found all the samples were positive for *Staphylococcus aureus* contamination. *E. coli* was isolated from 76 samples out of 77 random samples, and 19.48% of isolates were belonged to EPEC serogroup in Kerman, Iran (Melkamsew *et al*., 2012; Pazakova *et al*., 2001).

Coliform bacteria, *Escherichia coli*, *Staphylococcus aureus* and mold-yeast counts were detected in some dairy products in Kirklareli, Turkey (Çetin *et al*., 2015).

The final microbiota of raw milk cheese varies depending on the type of cheese and ripening process used, as well as the location within the cheese that is sampled (Montel *et al.,* 2014; Irlinger *et al*., 2015).

Microbiota of raw milk cheese comes not only from the raw milk, but also from added starters and the environment (Gatti *et al*., 2014; Neviani *et al*., 2013).

Traditional cheese making processes often involve the use of a wooden surface, either in the form of a storage vat or a ripening shelf. Wood is a natural reservoir for microbes and hence transfers this microbiota to the cheese (Settanni *et al.,* 2012). LAB species abundance and diversity increases after exposure to wooden vats (Licitra *et al.,* 2007; Lortal *et al.,* 2009), while ripening on wooden shelves leads to transference of coryneform

bacteria, moulds, and yeasts (Mariani *et al.,* 2007).

Despite improvements in dairy processing, domestic soft cheeses are still very popular. This type of cheese is usually made from raw milk with insufficient hygienic quality in rural regions. Hence, raw milk can be primarily considered the main source of microbial contamination (García and Díaz, 2011). In addition, worker's hand, packaging, transportation and marketing can be the secondary cause in poor conditions of the soft cheese. Also, non-hygienic water rather than tap water mostly used in the cleaning of the utensils used in cheese making as well as general daily uses Hill *et al*., 2012; Uyttendaele *et al*., 2015).

Temelli *et al*., (2006) investigated the possible sources of the cheese contamination and found that starter culture was a possible contamination source for coagulase positive staphylococci, enterococci and psychrophilic bacteria, while floor and packaging material were as the contamination source of psychrophilic bacteria. Although soft cheese is a nutritious food, it may act as a good means for pathogenic microorganisms (Araújo *et al*., 2002). *E. coli* was isolated from milk products like Mawa, Khoa, Cream, Dahi, Cheese, Butter, Gulabjamum (Vernoz- Rozand *et al.,* 2005).

The prevalence of *E. coli* in Ras cheese was 21.7%, while that of raw milk and Karish cheese was 76.4 and 74.5% respectively, out of 187 dairy products including raw milk samples, 55 Karish cheese and 60 Ras cheese, 222 *E. coli* isolates including 111; 89 and 22 were obtained from 55 raw milk samples (76.4%), 41 Karish cheese (74.5%) and 13 Ras cheese (21.7%) respectively). The higher *E. coli* contamination rate of Karish cheese, compared to Ras cheese, may be due to the differences in cheese making process and the characteristics of final product between these two cheese (Ombarak *et al.,* (2016).

Higher prevalence of *E. coli* in cheese has been reported from Brazil (96 to 97.7%) Araújo *et al*., 2002; Araújo *et al*., 2002; Zimbabwe (66.6%) (Gran *et al.,* 2003); South Africa (23.3%) (Lues *et al.,* 2003); India (31.6 to 57%) (Nanu *et al*., 2007; Singh and Prakash, 2008).

Listeria monocytogenes has been found in a high percentage of hard cheese made from raw or low heat treated sheep milk in EU countries, including Italy (Ministry of Work, Health and Social Policies, (EFSA, 2015).

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