



Original Article

Microbiological Assessment of Raw Milk Available in the Metropolitan City of Sindh, Karachi, Pakistan

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ABSTRACT

Currently, milk quality is judge on its health and hygienic parameters equally as its composition. Raw milk is a rich medium for the growth and proliferation of different pathogenic microorganisms such food-safety hazard may incorporate into the dairy chain, not only affects its safety and quality but also causing various milk borne diseases. **Objective:** To evaluate the microbiological assessment of raw milk accessible in Karachi with the intention to develop risk tracking strategies and its mitigation plan in the future. **Methods:** Total 270 raw milk samples were collected from different sites throughout the dairy supply chains in each district of the city. Microbiological assessment of raw milk was carried out by following the bacteriological analytical manual (BAM, FDA) protocol. **Results:** The raw milk safety and quality at the dairy farms were satisfied, except raw milk storage tanks samples. The bacterial count boosted in almost all the samples collected during its transportation, higher aerobic and pathogenic load was observed in the retail shop's samples however, maximum counts were noticed in the selling point milk samples. None of the milk samples contained other pathogens like *Listeria* or *Salmonella*. **Conclusions:** The milk quality of the supply chain, retail shops and storage tanks at dairy farms were un-satisfied based on different microbiological parameters.

INTRODUCTION

Currently, the milk safety and hygienic parameters are equally judged as its composition due, to the present trends in consumer preferences to use naturally pure or minimally processed raw milk [1, 2]. Normally, raw milk gets contaminated by direct transfer from the blood to the milk through an udder infection called mastitis and post milking contamination occurs during its handling. According to literature review, enterotoxin-producing *Staphylococcus aureus*, human pathogenic verocytotoxin-producing

Escherichia coli (VTEC), human pathogenic, *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Leptospira spp.* *Salmonella spp.* etc. are deemed the main microbiological hazards related to raw milk consumption [1]. Milk is a significant source of *staphylococcal* food poisoning which leads to septicemia, pneumonia, gastroenteritis and dermatitis in humans [3]. *Salmonella* causes illness in human through fecal contamination of food or water [4]. The main source of

human illness is milk and milk products contaminated with *Salmonella* species, that have been considered responsible for causing typhoid fever, enteritis and bacteremia [5, 6]. *E. coli*, a fecal coliform, is an indicator bacterium of unhygienic handling of food. *E. coli* is responsible for a wide range of infections like mild diarrhea, gastroenteritis, septicemia and typhoid fever [7]. *Listeria monocytogenes* is usually present in the dairy farm's soil, manure, plants, water and food chain environment [8]. It can thrive in harsh environment and even in raw milk that has been refrigerated. It can cause mastitis in cow and can be shed in milk of healthy cows [4, 9]. Additionally, improper milk handling, storage and transportation from the farm to the table increases the risk of pathogenic microbial contamination, which has a negative impact on the milk's quality and makes it unhealthy for human consumption [10]. Contaminated or open raw milk is responsible for many zoonotic diseases like hepatitis, typhoid, animal contact disease, influenza, and aerosolized dust illnesses are common in Pakistan due to absence of suitable food standards, unhygienic conditions, poor sanitation, poverty and illiteracy are the main factors contributing to the rise of food-borne illnesses [11, 12]. The inadequate monitoring and ineffective law enforcement, is substantially worse in developing and underdeveloped nations [13]. Therefore, maintaining and control milk quality is one of the big challenges in the dairy sector. Implementing Good Veterinary Practices (GVP), Good Management Practices (GMP), and Good Hygienic Practices (GHP) is crucial to reducing or eliminating the risks [14]. Therefore, this study was designed to evaluate the microbiological assessment of raw milk available in Karachi city with the intentions to develop risk tracking strategies and its mitigation plan in future.

METHODS

Raw milk and their complete supply chain within the city were studied to assesses their quality and safety attributes according to different Microbiological Standards as indicated in table 1.

Food	Microorganisms	Standards*		
		1	2	3
Milk	Aerobic Plate Count	10 ⁵ CFU/mL	2.5x10 ⁵ CFU/mL	5x10 ⁴ CFU/mL
	Coliforms	-	10 CFU/mL	5 CFU/mL
	<i>Escherichia coli</i>	-	3/CFU/mL	2 CFU/mL
	<i>Staph. aureus</i>	-	0/mL	0/mL
	<i>Salmonella</i>	0/25mL	0/mL	0/mL
	<i>Listeria monocytogens</i>	0/25mL	0/25mL	0/25mL

Table 1: Microbiological parameters of milk assessment

1. The Punjab Pure Food Rules, 2007
2. Australia/New Zealand; Microbiological Limits for Food Standard 1.6.1, issue 78
3. European Commission - Microbiological Criteria for Dairy Products

For the evaluation of the microbiological assessment the dairy supply chains were studied in the five districts of Karachi as shown in figure 1. Total 9 sampling spots were selected throughout the milk supply chain, as specified in the table 2.

Sampling Spots								
Dairy Farm				Transportation		Shop		
1	2	3	4	5	6	7	8	9
Direct from Animal	Milking Jug	Milk Collecting Bucket	Storage Tank	Milk Dispensing Cans	Milk Receiving Cans	Storage Tank	Temporary Storage Tub	Selling Point Tub

Table 2: Raw milk sampling collecting points

Four sampling points were chosen from dairy farms cites, 2 spots were selected during supply chain while, 3 venues were selected for sampling from retail shops.

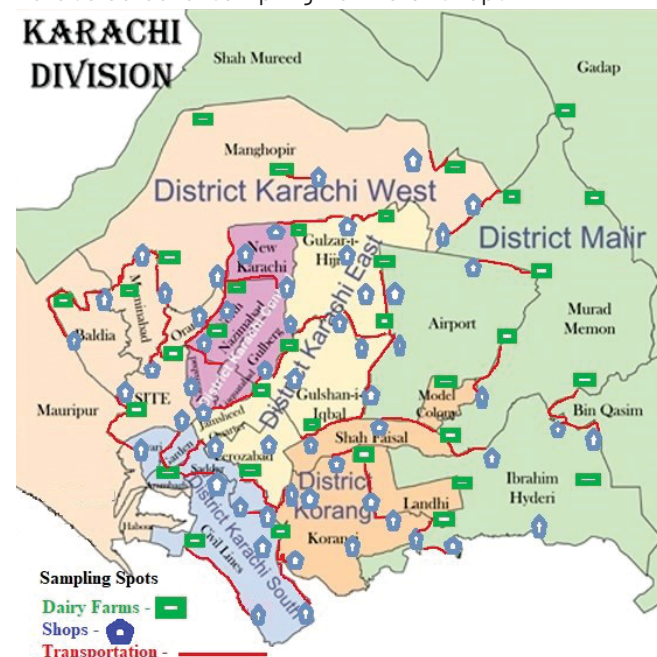


Figure 1: Source: map: dawn gis (November 29, 2015)

Three samples from each sampling point were collected. For sample collection, sterile, clean polythene plastic bags were used. The sample units were quickly and aseptically transferred to the lab in a clean, chilled container after being properly sealed. Most samples were examined for bacteriological examination as soon as they arrived or within 24 hours after being held at 0-4°C. APC, *Staph. aureus*, *Salmonella*, *Listeria*, *Coliforms* & *E. coli* isolates were analyzed in milk samples as per the US FDA (BAM - Ch#01, 12, 05, 10 and 04) protocol. APC, *E. coli* and *Staph. aureus* was enumerated by using (PCA, EMB and BPA) agar respectively by pour plate method while, MPN - Presumptive and confirmed tests were performed for *Coliforms*. However, *Salmonella* and *Listeria spp.* were analyzed by enrichment and culturing method.

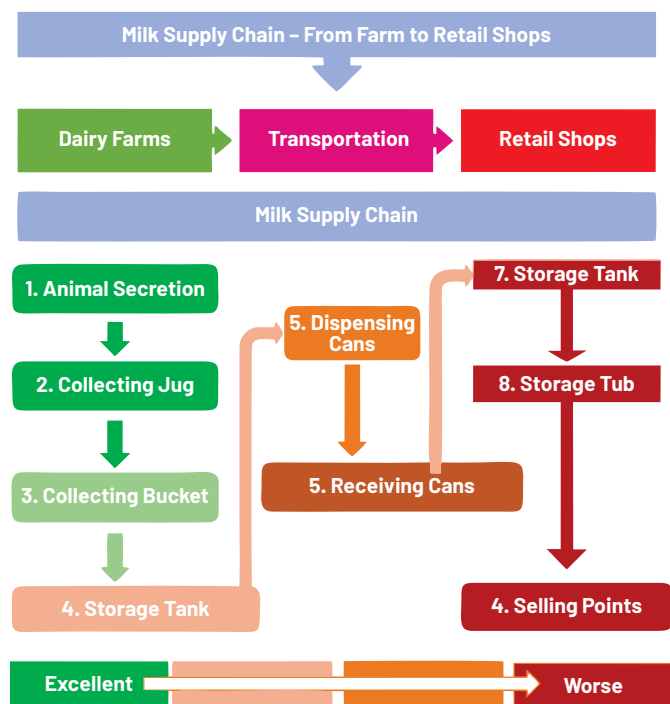


Figure 2: Flow chart of complete milk supply chain

For the detection of *Listeria monocytogenes*, 25 ml milk sample was transferred to 225 ml *Listeria* broth having 2.5 ml mixture of selective supplement and incubated it for 24 hours at 35°C. After the incubation 1 ml was transferred to BHI (brain heart infusion) agar plates and incubated for next 48 hours at 35°C. Similarly, for the *Salmonella* (isolation): 25 ml milk sample was mixed in 225 ml lactose broth and incubated for 24 hours at 35°C. 0.1 ml of the incubated sample was transferred to 10 ml RV (Rappaport Vassiliadis) medium and another 1 ml to 10 ml TT (Tetrathionate) broth. RV medium was incubated for 24 hours at 42°C and TT broth tubes for 24 hours at 43°C. A loopful (10 µl) of incubated TT broth was streaked on the BS (Bismuth Sulfite) agar, XLD (Xylose Lysine Deoxycholate) agar and HE (Hektoen enteric) agar plates and incubated for 24 hours at 35°C. Similar procedure was repeated for incubated RV medium. After incubation the plates were examined.

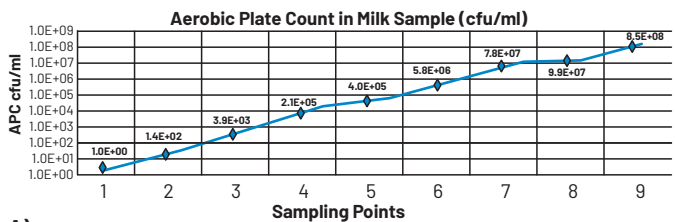
RESULTS

Raw milk directly obtained from the lactating animals showed good results when tested for total aerobic count, *Coliforms*, *E. coli*, and *Staph. aureus*. Average APC 135 CFU mL⁻¹ and average 2 CFU mL⁻¹ *Coliforms* were observed in samples taken from milk collection jugs at all dairy farms. It is illustrated that average 3.9x10³ CFU mL⁻¹ APC, 8 CFU mL⁻¹ *Coliform* and 5 CFU mL⁻¹ *Staph. aureus* count was observed in the collecting buckets samples. The total bacterial count was under the limitation however *Coliforms* and *Staph. aureus* counts slightly exceeded the limitation as shown in the table 3, on the bases the raw milk at this point was of

marginally acceptable quality. Milk samples from bulk storage tanks at dairy farms had a high microbiological count, with an average of 2.1x10⁵ CFU mL⁻¹ APC, 34 CFU mL⁻¹ *Coliforms*, 15 CFU mL⁻¹ *E. coli* and 12 CFU 25 mL⁻¹ *Staph. aureus* detected.

Organisms	Sampling Points								
	Dairy Farms			Supply Chain		Retail Shops			
	1	2	3	4	5	6	7	8	9
APC	2	135	3.9x10 ³	2.1x10 ⁵	4x10 ⁵	5.8x10 ⁶	7.8x10 ⁷	9.9x10 ⁷	8.5x10 ⁸
Coliforms	0	2	8	34	56	85	115	162	240
<i>E. coli</i>	0	0	0	15	20	25	49	70	90
<i>S. aureus</i>	0	0	5	12	15	19	30	37	77

Table 3: Microbiological assessment (cfu/ml) of raw milk from farm to shops. 1; Direct Animals 2; Milk Collecting Jug 3; Milk Collecting Bucket 4; Bulk Storage Tank, 5; Dispensing Cans, 6; Receiving Cans 7; Storage Tank 8; Milk storage tub 9; selling point. Since the count was beyond the defined microbiological parameters, the milk's quality was at this point was of poor quality. Further, boosted microbial count was recorded in milk samples collected during transportation the average APC counts 5.8x10⁶ CFU mL⁻¹ was noted in the milk supply cans, as average 85 CFU mL⁻¹ *Coliforms*, 25 CFU mL⁻¹ *E. coli* and 19 CFU mL⁻¹ *Staph. aureus* counts were shown in the shipment cans milk samples. The microbiological limits breached at these point and hence milk quality was account as of unacceptable quality. Average 7.8x10⁷ CFU mL⁻¹ APC count was noted in the retail shops storage tank milk samples while average 115 CFU mL⁻¹ and 49 CFU mL⁻¹ *Coliforms* and *E. coli* count was noted respectively while, 19 CFU mL⁻¹ *Staph. aureus* load was observed in the storage tank milk samples at the retail shops. These sites were judged to have poor milk quality because of the excessive bacterial growth in the samples. The retail shops selling point milk samples had the highest level of bacteria. As average 8.5x10⁸ CFU mL⁻¹ APC, 240 CFU mL⁻¹ *Coliforms*, 90 CFU mL⁻¹ *E. coli* and 77 CFU mL⁻¹ *Staph. aureus* count was recorded in the selling points milk samples. The count was beyond the acceptable bacterial limits, therefore, the quality of milk was declared unacceptable according to the microbiological criteria as shown in table 01. The average APC, *Coliforms*, *E. coli* and *Staph. aureus* count in milk samples from direct animals to selling points is illustrated in figure 3.



A)

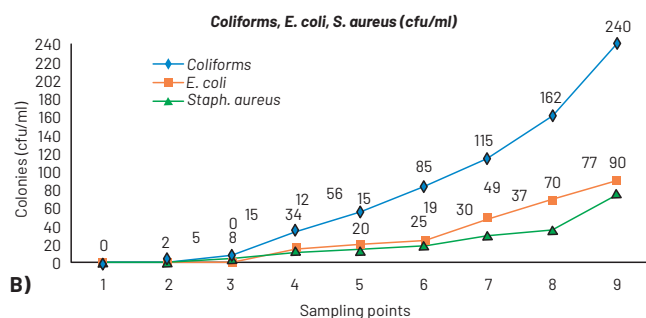


Figure 3: Average bacterial count at each sampling points
 A) Average APC count in milk samples collected from direct animals to the selling points at shops B) Average *Coliforms*, *E. coli* and *Staph. aureus* count in milk samples collected from direct animals to the selling points at shops.

DISCUSSION

Raw milk is a perfect medium to support the growth and proliferation of different bacteria [15]. Milk samples collected from direct animals were almost free of microorganism (average TAPC; 2 CFU/mL) this indicates that there is no inherent danger of bacterial contamination in dairy farm animals [16]. The majority of the dairy farms milk samples collected from storage tank showed exceeded microbiological limits (average 2.1×10^5 CFU/mL APC, 8 CFU/mL *Coliforms*, 5 CFU/mL *Staph. aureus*) due to lack of cooling systems, non-implementation of good management practices (GMP) and good hygiene practices [14, 17]. Likewise, the leading microbial flora associated with raw milk samples in and around were in the order of *aerobic count* > *Coliforms* > *Escherichia coli* > *Staphylococcus aureus* among the isolated pathogens. The existence of these bacteria in milk reflects the various sources of contamination such as animal, human, environment, utensils etc. [18]. The majority of the milk supplied to the city is transported mainly from Bhains Colony [15]. The delivery of milk from a great distance at ambient temperature encourages the growth and proliferation of bacteria. According to reports, lack of refrigeration and lengthy transportation lead to contamination in the milk supply chain [19]. The milk samples taken from bulk milk supply cans had a high bacterial counts (average APC; 5.8×10^6 CFU/mL, *Coliforms* 85 CFU/mL; *E. coli* 25 CFU/mL; *Staph. aureus* 19 CFU/mL). These high counts are related to improper milk handling practices, contamination from animal bedding, mixing of normal milk with milk taken from an animal with an infected udder, etc. *Total bacterial*, *Coliform*, *E. coli* and *Staph. aureus* count in milk samples collected from supply chain were beyond the acceptable limits [15]. The overall bacterial count in milk storage tanks without refrigeration and with refrigeration (-4°C) at uncontrolled temperature at various retail stores were high (average APC; 7.8×10^7

CFU/mL, *Coliforms* 115 CFU/mL; *E. coli* 49 CFU/mL, *Staph. aureus* 30 CFU/mL) highlights the same risks like lack of training for the staff, cleaning and disinfection plans, the absence of a waste management strategy and a failure to adhere to temperature limitations are all examples of noncompliance [20]. Milk samples collected from shop's selling points had high microbial count (average APC; 8.5×10^8 CFU/mL, *Coliforms* 240 CFU/mL; *E. coli* 90 CFU/mL, *Staph. aureus* 77 CFU/mL) its high number might be linked to the milk being collected in an unsanitary manner and being handled carelessly during transportation. Moreover, *coliforms* contaminated water in milk during transportation could be possible reason of its high count [15]. Although, the overall milk quality at the retail shops were unacceptable as it strongly exceeded the microbiological parameters of both national and international standards.

CONCLUSIONS

It could be concluded that the raw milk sold in Karachi at retail stores is of poor microbiological quality and is unsafe for human consumption because it contains pathogenic bacteria, which are responsible for a number of food-borne illnesses. A possible risk to consumer health is indicated by the high bacterial count and the presence of several pathogenic microorganisms. Overall, Karachi's raw milk is of poor quality for human consumption. It is therefore, significantly important to ensure high quality raw milk production under good hygienic conditions, beside this an effective sanitary and hygienic measures must be implement during milk handling, transportation and marketing to ensure its safety.

Conflicts of Interest

The authors declare no conflict of interest

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REFERENCES

- Verraes C, Vlaemyck G, Van Weyenberg S, De Zutter L, Daube G, Sindic M, et al. A review of the microbiological hazards of dairy products made from raw milk. *International Dairy Journal*. 2015 Nov; 50: 32-44. doi: 10.1016/j.idairyj.2015.05.011
- Haque ZF, Sabuj AA, Mahmud MM, Pondit A, Islam MA, Saha S. Characterization of *Staphylococcus aureus* from milk and dairy products sold in some local markets of Mymensingh district of Bangladesh. *J Nutr*. 2018; 8(6): 1000743. doi: 10.4172/2155-9600.1000743
- Dai J, Wu S, Huang J, Wu Q, Zhang F, Zhang J, et al. Prevalence and characterization of *Staphylococcus*

- aureus isolated from pasteurized milk in China. *Frontiers in microbiology*. 2019 Apr; 10: 641. doi: 10.3389/fmicb.2019.00641
- [4] Oliver SP, Jayarao BM, Almeida RA. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathogens & Disease*. 2005 Jun; 2(2): 115-29. doi: 10.1089/fpd.2005.2.115
- [5] Ekici K, Bozkurt H, Isleyici O. Isolation of some pathogens from raw milk of different milch animals. *Pakistan Journal of nutrition*. 2004; 3(3): 161-2.
- [6] Qamar A, Ismail T, Akhtar S. Prevalence and antibiotic resistance of *Salmonella* spp. in South Punjab-Pakistan. *Plos one*. 2020 Nov; 15(11): e0232382. doi: 10.1371/journal.pone.0232382
- [7] Wanjala WN, Nduko JM, Mwendu MC. Coliforms contamination and hygienic status of milk chain in emerging economies. *Journal of food quality and hazards control*. 2018 Mar 10; 5(1): 3-10. doi: 10.29252/jfqc.5.1.3
- [8] Meyer-Broseta S, Diot A, Bastian S, Rivière J, Cerf O. Estimation of low bacterial concentration: *Listeria monocytogenes* in raw milk. *International Journal of Food Microbiology*. 2003 Jan; 80(1): 1-5. doi: 10.1016/S0168-1605(02)00117-4
- [9] Ruegg PL. Practical food safety interventions for dairy production. *Journal of dairy science*. 2003 Jun; 86:E1-9. doi: 10.3168/jds.S0022-0302(03)74034-X
- [10] Jan T, Yadav KC, Borude S. Study of HACCP implementation in milk processing plant at Khyber Agro Pvt. Ltd in Jammu & Kashmir. *J Food Process Technol*. 2016; 7(610): 2. doi: 10.4172/2157-7110.1000610
- [11] Ishaq AR, Manzoor M, Hussain A, Altaf J, Javed Z, Afzal I, et al. Prospect of microbial food borne diseases in Pakistan: a review. *Brazilian Journal of Biology*. 2021 Jan; 81: 940-53. doi: 10.1590/1519-6984.232466
- [12] Akhtar S. Food safety challenges—a Pakistan's perspective. *Critical reviews in food science and nutrition*. 2015 Jan; 55(2): 219-26. doi: 10.1080/10408398.2011.650801
- [13] Azad T, Ahmed S. Common milk adulteration and their detection techniques. *International Journal of Food Contamination*. 2016 Dec; 3(1): 1-9. doi: 10.1186/s40550-016-0045-3
- [14] Quintana ÁR, Seseña S, Garzón A, Arias R. Factors affecting levels of airborne bacteria in dairy farms: A review. *Animals*. 2020 Mar; 10(3): 526. doi: 10.3390/ani10030526
- [15] Muhammad K, Altaf I, Hanif A, Anjum AA, Tipu MY. Monitoring of hygienic status of raw milk marketed in Lahore City, Pakistan. *The Journal of Animal & Plant Sciences*. 2009 Jan; 19(2): 74-7.
- [16] Angulo FJ, LeJeune JT, Rajala-Schultz PJ. Unpasteurized milk: a continued public health threat. *Clinical Infectious Diseases*. 2009 Jan; 48(1): 93-100. doi: 10.1086/595007
- [17] Shah T, Shah QA, Shah JM, Arain MA, Saeed M, Siyal FA, et al. Microbiological quality of raw milk and associated health risk in the Hyderabad region of Pakistan. *International Journal of Food and Nutrition. Saf*. 2016; 7(2): 61-77.
- [18] Mubarak HM, Doss A, Dhanabalan R, Balachander S. Microbial quality of raw milk samples collected from different villages of Coimbatore District, Tamilnadu, South India. *Indian Journal of Science and Technology*. 2010 Jan; 3(1): 61-3.
- [19] Lubote R, Shahada F, Matem A. Prevalence of *Salmonella* spp. and *Escherichia coli* in raw milk value chain in Arusha, Tanzania. *American Journal of Research Communication*. 2014; 2(9): 1-13.
- [20] Kamana O, Jacxsens L, Kimonyo A, Uyttendaele M. A survey on hygienic practices and their impact on the microbiological quality and safety in the Rwandan milk and dairy chain. *International Journal of Dairy Technology*. 2017 Feb; 70(1): 52-67. doi: 10.1111/1471-0307.12322