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

Sayed Zaheer Abbas¹, Muhammad Naseem Khan², Anjum Zehra Naqvi³, Nargis Tabassum⁴, Zulfiqar Ali Mirani⁵, Abdul Basit Khan¹ and Razim Ali³

¹Food and Marine Resources Research Centre, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Karachi, Pakistan
²Department of Microbiology, University of Karachi, Sindh, Pakistan
³Department of Pharmaceutical Chemistry, Federal Urdu University of Science and Technology, Karachi, Sindh, Pakistan
⁴Department of Biotechnology, University of Karachi, Sindh, Pakistan

A R T I C L E I N F O

Key Words:
Milk, Microbiological, Quality, Safety, Assessment

How to Cite:
Zaheer Abbas, S. ., Naseem Khan, M. ., Zehra Naqvi, A. ., Tabassum, N. ., Ali Mirani, Z. ., Basit Khan, A. . & Ali, R. . (2022). Microbiological assessment of raw milk available in the metropolitan city of Sindh, Karachi - Pakistan: Microbiological Assessment of Raw Milk . Pakistan Journal of Health Sciences, 3(06). https://doi.org/10.54393/pjhs.v3i06.288

*Corresponding Author:
Muhammad Naseem Khan
Food and Marine Resources Research Centre, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Karachi, Pakistan
micro.pcsir@yahoo.com

Received Date: 27th October, 2022
Acceptance Date: 16th November, 2022
Published Date: 30th November, 2022

I N T R O D U C T I O N

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.

M E T H O D S

Raw milk and their complete supply chain within the city were studied to assess 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      | $10^3$ CFU/mL        | $2.5\times10^4$ CFU/mL | $5\times10^5$ CFU/mL  |
| Coliforms     | -                        | $10^2$ CFU/mL        | $5\times10^3$ CFU/mL  |
| Escherichia coli | -                     | $3\times10^3$ CFU/mL | $2\times10^4$ CFU/mL  |
| Staph. aureus | -                        | $0$/CFU/mL           | $0$/CFU/mL            |
| Salmonella    | $0/25$ mL                | $0$/CFU/mL           | $0$/CFU/mL            |
| Listeria monocytogenes | $0/25$ mL      | $0/25$ mL           | $0/25$ mL            |

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 | Milk-Jug | Milk Collecting Bucket | Storage Tank |
| Milk Dispensing Cans | Milk Receiving Cans | Storage Tank | Temporary Storage Tub |
| Selling Point Tub | S |           |

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.

DOI: https://doi.org/10.54393/pjhs.v3i06.288
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 BHl (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 (Tetraphionate) 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 (Heftoent 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**

For the detection of Staph. aureus, samples taken from milk collection jugs at all dairy farms. It is estimated that average 3.9x10⁸ 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 Coliform 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 2.1x10⁸ CFU mL⁻¹ APC, 34 CFU mL⁻¹ Coliforms, 15 CFU mL⁻¹ E. coli and 12 CFU 25 mL⁻¹ Staph. aureus detected.

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 of poor quality. Further, boosted microbial count was recorded in milk samples collected during transportation the average APC counts 5.8x10⁸ 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 breeched at these point and hence milk quality was account as of unacceptable quality. Average 7.8x10⁹ 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.
DISCUSSION

Raw milk is a perfect medium to support the growth and proliferation of different bacteria [15]. Milk samples collected from direct animals to the selling points at shops 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 2.1x10^2 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 [18]. The milk samples taken from bulk milk supply cans had a high bacterial counts (average APC; 5.8x10^2 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; 7.8x10^1 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; 8.5x10^1 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

Source of Funding

The project was partially funded by a grant from the Dean, Faculty of Science, University of Karachi, Pakistan (AZN).

REFERENCES

[1] Verraes C, Vlaemynck G, Van Weyenbergh 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

[2] 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

[3] 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, Mwende 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: 10.29225/jfqhc.5.1.3

[8] Meyer-Broseta S, Diot A, Bastian S, Riviere 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; 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.