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Public Health Quality of Raw Milk sold in District Korangi, Karachi, Pakistan

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Abstract: This study was conducted to evaluate the public health quality of raw milk sold in district Korangi, Karachi. A total of 20 milk samples were collected randomly in a pre-sterilized glass bottles for the laboratory analysis comprises of methylene blue reduction test and isolation of bacterial and fungal species. The methylene blue test was done for raw milk samples indicated that out of 20 samples, six samples were poor, nine samples were fair, two samples were good and only three samples were excellent. Similarly, only five samples out of twenty were free from pathogens particularly from *E.coli*, *Salmonella* and *Lactobacillus species*. Fungal species were found in all the samples. *Aspergillus niger* are present in all the samples with abundance growth while 13 samples were contaminated with *Aspergillus flavus*. *Aspergillus terreus*, *Aspergillus candidus*, *Aspergillus fumigatus* were also observed in few samples. Similarly, *Rhizopus, Penicillium, Curvularia, Fusarium and Mucor* were also observed in few samples. Overall, the raw milk sold in district Korangi is unfit for human consumption.

Keywords: Raw milk, Methylene Blue Reductase Test, Public health, Korangi, Pathogens

INTRODUCTION

Humans consume milk as an important source of nutrients. It is considered as one of the primary and only available complete diets for the progeny of mammals [1]. It is mainly comprising of water (87%) while remaining part contains solid portion consisting of carbohydrates, fats, proteins and minerals that act as vital

elements for growth and development of the human and animal bodies[2]**.** Beside this, immuno-globulins present in milk provides protective shield to the infants against variety of diseases[1]**.** However, milk must be free from pathogens consumed by the human [3, 4]. Raw milk serves as a carrier for disease causing microorganisms, as it can be easily tainted during processing and transport[5].

Production of milk under poor sanitation and unhygienic conditions particularly in developing countries are of great concern to safeguard the dairy products from milk and food-borne microorganism[6,7]. Even though, milk and milk based products constitute negligible component in most of the diets but tainted milk are liable for almost 90% of milk borne infections in humans[8]. There are regulations available for maintaining the safety of public health against milk borne illness but these regulations hardly practice in developing countries. Such regulations are not given due importance and not been fully implemented, therefore a major concern for higher health risk in these countries [9]**.** In developing countries most of the marketed milk sold unpasteurized or raw through informal conduits [3, 10].

In general, pasteurization of milk is normally used to prevent bacterial contamination of milk to safeguard public health. High temperature and time regime for pasteurization is currently designed to kill *Coxiella burnetii*, responsible for causing Q- fever in humans. *Coxiella burnetii* is currently reported as one of the most heat tolerant pathogen causing infections in human through the consumption of milk [11]. In addition, milk must be obtained from healthy cows in the environment free from bacterial contamination. There are several sources of milk contamination that are classified as primary, secondary and tertiary. Primary source include contamination by sick or infected lactating animals. While the secondary sources of contamination may include poor sanitary conditions of utensils, equipment used for milking, personnel hygiene of milkman and contaminated area of milking used for storage and transportation. The tertiary sources are due to low or inappropriate handling and storage of milk during utilization [12].

Shirima *et al.* [13] reported that raw or unpasteurized milk are responsible for zoonotic diseases including brucellosis, tuberculosis and enterotoxaemia. There is a decline in the demand of raw and unpasteurized milk that discourages milk consumption due to the risk of milk-borne zoonotic infection [14]. Raw milk may contain a variety of pathogenic microorganism including *Escherichia coli, Proteus spp., Salmonella spp., Clostridium spp., Mycobacterium spp., Staphylococcus aureus, Pseudomonas aeruginosa, Listeria moncytogens, Campylobacter spp., Brucella abortus* and *Leptospira spp.,* [15]. Spoilage of milk is due to several reasons in which absence of refrigeration facilities at farm and family level is one of the major reasons of spoilage. Moreover, improper storage and transportation facilities with high ambient temperature could also be the other reasons for milk spoilage [16, 17]. Total microbial count in milk is a major feature to determine milk quality[18]. Presence and multiplication of microorganism in raw milk change the milk composition and influence the quality of the dairy product[17].

The outlets for the purchase of milk in developing countries are usually operated in deplorable conditions and are not often adequately monitored or regulated [19]. Even in many European countries where hygienic conditions are relatively better, several outbreaks of milk borne infection are reported due to the consumption of contaminated milk supplies [20].

Bacteria that are capable to produce lactic acid are responsible for most of the physiochemical and aromatic transformations in milk and dairy products [21]. These bacteria take milk sugars as a diet and convert them into lactic acid that is thus a major reason for high level of acidity, rendering milk proteins, especially casein, to denature and tangle into a solid mass or curd [22].

Landhi cattle colony is one of the world's largest cattle colony spread over an area of 6.5 km^2 located in the outskirts of district Korangi. The colony has about 1500 farms with more than 0.4 million animals in which 95% are buffaloes. The daily milking yield is 4.0 million liters while the daily cow dung production is about 7, 200 tons. The colony fulfills 70% of the milk demand for Karachi city. Only a small portion of cow dung (250 tons per day) is being picked up and used as an organic manure while liquid waste is drained into the main sewer thorough which it discharges into Port Qasim coastal area. The majority of the livestock are kept only for one lactation phase and thus nearly 10-12% of the inhabitants are interchanged each month. After the lactation period, most of the animals are vended to breeders or for slaughters and few are reserved by the dairy farmers for further breeding.

The aim of the study to evaluate the public health quality of raw milk available in the district Korangi, Karachi.

MATERIAL AND METHOD

Sampling: Twenty raw milk samples were collected in pre-sterilized glass containers randomly from different sites of district Korangi. They were transported to Institute of Environmental Studies, University of Karachi with ice packed insulated containers for analysis. The laboratory analysis comprises of the methylene blue reduction test and isolation of bacterial and fungal species.

Methylene Blue Reductase Test: Methylene Blue Reductase test was conducted to determine the hygienic quality of the raw marketed milk samples [23]. 1 ml of methylene blue solution (1:25000) was transferred to labeled and sterilized 20 ml screw caped test tube containing 10 ml of each of the samples. After complete mixing all the tubes were incubated at 37^0C and observed after every 30 minutes time interval up to 8 hours. The time taken by the methylene blue in milk to become colorless was recorded.

Isolation of Bacterial Species: The milk samples were inoculated on Nutrient Agar (Oxoid) and incubated aerobically for 24 hours at 37^0C. The plates were observed for the bacterial growth. The identification and characterization of the colonies were accomplished using morphological, cultural and biochemical tests up to the level of the genus [24].

Isolation of fungal species: Fungal species were isolated by transferring milk sample in a sterilized petri plate followed by the addition of 4% molten agar (Sabouraud Dextrose Agar, Merck) containing streptomycin (200 ppm). The petri plate was left for the solidification of agar. The plates were incubated at room temperature for 7 days. The fungal species were categorized by morphological and microscopic investigation by using the standard procedures as the fungal spores appears [25-28].

RESULTS AND DISCUSSION

Milk from healthy animals is most of the time free from bacteria however the environment in which procedure of lactating milk takes place comprise of so many unhygienic condition that make milk contaminated with pathogenic microorganisms [29]**. Table 1** describe the quality scale for methylene blue reductase test while **Table 2** showed the results on the basis of methylene blue reductase test on raw milk samples collected from district Korangi.

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Table 1: Milk samples grading on the basis of Methylene blue reduction test

S.No	Decolorization Time (Hours)	Quality
1	Less than 2 hours	Poor
2	Between 2-6 hours	Fair
3	Between 6-8 hours	Good
4	More than 8 hours	Excellent

Table 2. Quality of milk in district Korangi on the basis of Methylene blue reduction test

S.No	Samples Code	Area	Decolorization Time (Hours)	Quality
1	S-1	Cattle Colony, Landhi	1.5	Poor
2	S-2	Quaidabad	1.0	Poor
3	S-3	Korangi # 5	1.0	Poor
4	S-4	Korangi # 2.5	3	Fair
5	S-5	Bagh-e- Korangi	4.25	Fair
6	S-6	Korangi #1	5.5	Fair
7	S-7	Chakra Goth	1.5	Poor
8	S-8	Sharafi Goth	4.0	Fair
9	S-9	Korangi Nasir Jump	5.0	Fair
10	S-10	P and T colony	4.5	Fair
11	S-11	Korangi Crossing	3.0	Fair
12	S-12	Shah Faisal Colony #2 (Farm)	1.75	Poor
13	S-13	Shah Faisal Colony #3 (Farm)	2.0	Fair
14	S-14	Shama Centre, Shah Faisal colony # 2	2.5	Fair
15	S-15	Azeem Pura, Shah Faisal Colony	1.5	Poor
16	S-16	Shah Faisal Colony #1	11.5	Excellent
17	S-17	Shah Faisal Colony #5	10.25	Excellent
18	S-18	Alfalah Society, Shah Faisal Colony	9.0	Excellent
19	S-19	Landhi	7.25	Good
20	S-20	Landhi	6.25	Good

Samples from Cattle Colony, Quaidabad, Korangi #5, Chakra Goth, Shah Faisal colony # 2 and Azeem Pura contained poor quality milk shown by less time taken for decolorization of the methylene blue dye and this may be due to malpractices for milk handling in the conforming area [30]. However, the samples from Korangi

2.5, Bagh-e- Korangi, Korangi #1, Korangi Nasir Jump, P&T colony, Korangi Crossing, Shah Faisal Colony #3 (Farm) and Shama Centre, Shah Faisal colony # 2 contained fair quality milk that takes time more than 2 hours for color change. As per expectation, only 5 samples contained less number of bacteria or no microorganisms observed by decolorization time more than 6 hours. It has been reported that district Korangi occupied the second position in terms of epidemics and large numbers of patients[31].

Milk obtained from sick and unhealthy animals is grossly contaminated with pathogenic microorganisms like *Salmonella species*, *Streptococcus species*, *E. coli*, *micrococcus*, *lactobacillus* and *staphylococcus species*, etc. [32, 33]. The presence or absence of the pathogenic microorganisms in raw milk samples is given in **Table 3.** The majority of samples (approx. 75 %) contaminated with pathogenic bacteria that become the major reason for milk borne diseases especially in infants and people with low immune system[34]. The presence of these pathogens is associated with un-hygienic milk collection and distribution system, mixing of milk obtained from vigorous and sick ones and animal bedding. However, only five samples are free from most of the pathogens particularly from *E.coli, Salmonella* and *Lactobacillus species*

Table 3: Bacteriological Analysis of raw milk collected from district Korangi, Karachi

S.No	Samples code	Bacterial Isolate				
		Escherichia coli	*Salmonella sp.*	*Micrococcus sp.*	*Lactobacillus sp.*	*Staphylococcus aureus*
1	S-1	+	+	+	+	+
2	S-2	+	+	+	+	+
3	S-3	+	+	+	+	+
4	S-4	+	+	+	+	+
5	S-5	+	+	+	+	+
6	S-6	+	+	+	+	+
7	S-7	+	+	+	+	+
8	S-8	+	+	+	+	+
9	S-9	+	+	+	+	+
10	S-10	+	+	+	+	+
11	S-11	+	+	+	+	+
12	S-12	+	+	+	+	+
13	S-13	+	+	+	+	+
14	S-14	+	+	+	+	+
15	S-15	+	+	+	+	+
16	S-16	-	-	-	-	-
17	S-17	-	-	-	-	-
18	S-18	-	-	-	-	-
19	S-19	-	-	-	-	-
20	S-20	-	-	-	-	-

***Positive sign indicates the presence of particular microorganism**

***Negative sign indicates the absence of particular microorganism**

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Table 4: Result of the analysis of Fungal Species (CFU X 10^3 /100 ml) present in raw milk available in district Korangi

FUNGAL SPECIES	CFU X 10^3 /100 ml																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Aspergillus Niger	12.3	8.1	9.7	3.2	7.5	5.2	6.8	10.1	6.4	3.7	6.1	2.5	2.8	5.4	5.1	6.8	10.2	11.0	3.6	3.7
Aspergillusflaves	7.3	10.5	2.1	-	1.3	2.8	5.3	3.1	6.0	-	-	5.2	-	1.5	2.8	-	2.0	7.3	-	-
Aspergillusterreus	3.5	1.6	-	-	1.2	-	2.7	-	-	3.8	5.2	-	-	-	4.2	1.1	1.6	-	1.0	1.3
Aspergillus Candidus	-	4.2	-	3.6	-	-	-	-	4.2	-	-	-	-	2.1	-	-	1.3	-	-	-
Aspergillus Fumigatus	-	-	-	-	1.3	-	-	-	1.0	-	-	2.1	-	-	-	-	2.7	-	-	-
Rhizopus Stolonifera	2.5	-	-	1.2	1.6	1.0	-	-	-	2.6	-	1.4	-	-	-	-	1.6	2.4	2.0	-
Penicilliumsp	-	-	-	-	2.4	-	-	-	-	-	-	-	-	1.6	-	-	-	-	-	-
Curvulariasp	-	1.8	-	-	-	-	-	2.1	-	-	-	-	-	-	-	-	-	-	-	-
Fusariumsp	-	-	1.8	-	-	-	3.1	-	-	-	-	-	-	-	-	1.4	-	-	-	-
Mucormucedo	-	-	-	1.3	-	-	2.2	-	-	-	-	-	1.8	-	-	-	-	-	1.5	-

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Table 4 represents the results of different fungal species were found in all the samples. Generally, *Aspergillus* species were mostly found in milk samples and the genus is easily recognized by its typical conidiophore[35]. The most common isolated specie was *Aspergillus niger* that are present in all the samples with abundance growth. *Aspergillus niger* is one of the biologically active species that is used to produce several important compounds on industrial scale. Initially it was used for producing citric acid, gluconic and fumaric acid. However, since 1960, *Aspergillus niger* has become a source of a variety of enzymes that are used as an agents in baking, fruit processing, starch and for several other purposes in food industries[36]. On the other hand, *Aspergillus flavus* was also found in 13 of the milk samples. *Aspergillus flavus* produces frequent airborne conidia which also disperse through insects[37]. *Aspergillus flavus* grows well with water activity (aw) between 0.86 and 0.96 [38]. *Aspergillus terreus, Aspergillus candidus, Aspergillus fumigatus* were also isolated from the samples; however their growth was observed in few samples.

Fungal species other than *Aspergillus,* were *Rhizopus, Penicillium, Curvularia, Fusarium and Mucor* were also showed up the growth in very few numbers of the samples. These species are commonly found in milk and are capable to grow in it[39]. Raw milk provides all necessary nutrients and conditions for the growth of many fungal species, their occurrence is influenced by animal physiological state, weather and breeding conditions[40,41]. Alamgir *et al.*, (2015) [42]reported that the high fungal load was observed on street vended fresh fruit juices sold in Karachi.

CONCLUSION

The present study concluded at a point that raw milk that is supplied under various unsanitary and unhygienic practices comprises of pathogenic microorganisms as well as fungal species. Milk provides an enriched medium for the growth of these species. Methylene blue is the best and quick test for determining the milk quality and milk grading.

Overall, the raw milk sold in district Korangi is unfit for human consumption. Results showed that immediate and effective measures must be taken by the local authority as well as by the public for improving the quality of raw milk, which can only be done by proper sanitary practices and no compromise should be made on hygienic conditions regarding milk handling, transportation and preservation.

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