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ASSESSMENT OF MICROBIOLOGICAL QUALITY AND ASSOCIATED HEALTH RISKS OF RAW MILK SOLD IN AND AROUND HYDERABAD CITY

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ABSTRACT

The present study was done to assess the microbiological quality and related health risks of raw milk sold in and around Hyderabad city. A total of 60 milk samples were collected from milk sellers on bicycle (15) and two wheelers (15), local dairy farms (10), small hotels (10) and tea stalls (10) in and around Hyderabad city from January to March, 2012. Quality and milk borne hazards were assessed with combination of tests to quantify the total bacteria, the pathogenic microbes such as *Escherichia coli, Salmonella* and *Staphylococcus* and the occurrence of brucellosis (Abortus bang ring test). Plate count agar for total bacterial count and specific media for the pathogenic microbes were used in the study. The average total bacterial count in all the samples was recorded as 162 colonies at 10^6 dilutions. 27, 21 and 33 samples gave positive results out of 60 samples for *Escherichia coli, Salmonella* and *Staphylococcus* respectively with mean count ranged from 10^3 to 10^4 CFU/ml. Out of 60 samples 15 samples (25%) were positive for brucellosis.

KEY WORDS: Microbiological quality-raw milk-public health significance-Hyderabad city.

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INTRODUCTION

At present, public health concern associated with microbial food safety has arisen. Numerous epidemiological reports have implicated non-heat treated milk and raw-milk products are the major factors responsible for illnesses caused by food-borne pathogens[1, 2]. Cross-contamination with pathogenic microorganisms can gain access to milk either by faecal contamination or by direct excretion from the udder into milk.

Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections/intoxications in consumers[3]. Microbes may gain entry into raw milk directly from dairy cows which have sub clinical or clinical mastitis, from the farm environment particularly the water sources and utensils used for the storage of milk on farm or during transportation. Generally, bacteria in the milk can occur through colonization of the teat canal or an infected udder gets contaminated at various stages whether it is from the animal, milker (manual as well as automated), extraneous dirt or unclean process water. When milk is produced under poor hygienic conditions and is not cooled enough, the main contaminants, usually lactic acid producers cause rapid souring. Lactic acid has an inhibitory effect on pathogenic bacteria but this cannot be depended upon to provide a safe milk product.

The presence of food-borne pathogens in unpasteurized raw milk either directly or indirectly, increases the risk of ingestion and transmission of food-borne pathogens and ingestion of potentially harmful toxins. The diseases transmissible to humans through the consumption of milk are brucellosis, tuberculosis, salmonellosis, listeriosis, *E.coli* infections[4]. Pathogenic organisms in milk can derive from the cow itself, from the human handlers and from the environment. Unfortunately, due to unorganized and non-regulated marketing system, the quality of milk is hardly maintained at consumer level. Addition of water and ice is common which affects the physical, chemical as well as microbiological quality of milk by altering the proportion of milk constituents.

Supply of milk to the people in Hyderabad city through different marketing channels such as directly from dairy farms, milk sellers, milk collection centers and milk venders of the surrounding areas and is based on unorganized marketing system. Hence, the present study was undertaken to evaluate the quality of raw milk and its associated health risks of public living in and around Hyderabad city.

MATERIALS AND METHODS

Study area

The samples were collected from in and around Hyderabad city, the capital city of Andhra Pradesh in India. The study area is located between 17^0 12′ N, 78^0 18′ E and characterized by combination of a tropical wet and dry climate that borders on a hot semi-arid climate. The summer months of April and May are hot and the city frequently records temperatures exceeding 40 °C

Collection of samples

A total of 60 milk samples of each 50ml were collected aseptically from milk sellers on bicycle (15) and two wheelers (15), local dairy farms (10), small hotels (10) and tea stalls (10) in sterilized bottles in and around Hyderabad city from January to March, 2012. All the samples collected were immediately transferred on to ice and later stored in refrigerator at 4^oC for analysis. The samples were analyzed in the department of Veterinary Public Health, College of Veterinary Science, Rajendranagar, Hyderabad.

Analysis of Samples

a. Isolation and identification of pathogenic microbes in raw milk samples

Before starting analysis, the samples were brought to room temperature and mixed properly. Serial tenfold dilutions of each sample from 10^{-3} to 10^{-6} were made in phosphate buffer saline (PBS) using sterile

pipettes. The wide range in dilutions was selected due to the expected wide variation in bacterial counts. From each dilution, 1 ml was placed on a sterile Petri dish followed by the addition of 15-20 ml autoclaved eosin methylene blue agar (EMB agar) and then cooled to 45^{o}C onto the dish for the count of *Escherichia coli*. Likewise 1ml of diluted sample was placed on sterile Petri dishes followed by the addition of Plate count agar (PCA), Xylose lysine deoxycholate agar *(*XLD agar) and Mannitol salt agar (MSA) for the count of total bacteria *Salmonella* and *Staphylococcus* respectively. The sample and agar were then mixed and left to solidify after which the plates were incubated in inverted positions at 37^{o}C for 24-48 hrs. EMB Plates showing green colonies with metallic sheen, XLD plates showing red colonies with or without black center, MSA plates showing yellow colonies and colonies on plate count agar in the countable range of 30-300 colony forming unit per plate (C.F.U/plate) were chosen and counted. Selected suspected colonies were confirmed by colony morphology, Gram's staining and biochemical tests.

b. Abortus bang ring test (ABRT) for brucellosis

The ABRT was performed by adding 30µl of stained *Brucella abortus* antigen (IVRI, Izatnagar) both to a volume of 1 ml of whole milk that has been stored at 4^{o}C for at least 24 hrs. The tubes were thereafter incubated at 37^{o}C for 1 hr. The test is read using a uniform light source. If the blue colour in the cream layer at the top of the fluid column is deeper than the remaining milk column (i.e. presence of a blue coloured ring) the test is considered positive. If the intensity of colour in the cream layer is equal to or less than that of in the milk portion, then the test is considered negative.

RESULTS AND DISCUSSION

The means of total bacterial count and counts of *E. coli, Salmonella, and Staphylococcus* were given in Table 1. The total bacterial count ranges from 1.24x10^{6} to 8.58x10^{6} and these high bacterial loads are may be due to poor cleaning of milking system. These results are lower when compared to the results like 1.75x10^{6} to 1.22x10^{8}cfu/ml in raw milk[5]. The higher total bacterial count in the present study is may be due to dirty udders maintaining an unclean milking and housing environment and failing to cool milk rapidly to less than 40^{o}F. The mean of total bacterial count (TBC) of raw milk were collected from dairy farms, milk sellers on bicycle and two wheelers, tea stalls and small hotels is 3.71x10^{6}, 4.52x10^{6}, 8.13x10^{6}, 2.34x10^{6} and 2.57x10^{6} cfu/ml respectively. The means of TBC were increased till the vendors who sold milk on bicycles and two wheeler later decreased due to maintenance of cooling system and frequent boiling in tea stalls and small hotels. Similar results were given[6] where the mean of TBC of raw milk collected from collection centers and restaurants was lower than that of raw milk collected from milk sellers on bicycle. The highest total counts were obtained in raw milk handled by milk sellers on bicycle and two wheelers are usually take more time to distribute to consumers after milking. It is also due to improper cleaning of milk cans by the traders, the conditions of roads and the distance travelled by the traders which creates good environment for multiplications of bacteria in milk.

Table 1
Microbiological quality of raw milk collected from different sources

Sample collection points	Sample size	Total bacterial count	*E. Coli* count		*Salmonella* count		*Staphylococcus* count	
		Mean	n	Mean	n	Mean	n	Mean
Dairy farms	10	$3.71x10^6$	6	$1.06x10^5$	5	$2.17x10^5$	6	$1.27x10^5$
Bicycle sellers	15	$4.52x10^6$	8	$1.39x10^5$	6	$2.45x10^5$	11	$1.83x10^5$
Two wheelers	15	$8.13x10^6$	10	$2.47x10^5$	7	$3.79x10^5$	12	$3.07x10^5$
Tea stalls	10	$2.34x10^6$	1	$0.89x10^5$	2	$1.71x10^5$	2	$0.92x10^5$
Small hotels	10	$2.57x10^6$	2	$0.56x10^5$	1	$1.63x10^5$	2	$0.85x10^5$
Total	60		27		21		33	

The mean counts of E.Coli, Salmonella and Staphylococcus are higher in the raw milk collected from milk sellers on bicycle and two wheelers than from dairy farms, tea stalls and small hotels. The mean E.Coli count of raw milk samples ranges from $0.56x10^5$-$2.47x10^5$ cfu/ml and these mean counts are lower than the findings[5,7 and 8] in which, $4.5x\ 10^3$-$2.03x10^6$, <1000 cfu/ml and <30-$2.08x10^7$ cfu /ml respectively. The mean Salmonella count of raw milk ranges from $1.63x10^5$ - $3.79x10^5$ which were lower[5] reports and were ranges from $1.4x10^5$- $5.9x10^5$ cfu/ml. They[9] didn't obtain any Salmonella counts from raw milk samples.

Among the three pathogenic organisms studied, the mean count of Salmonella is higher whereas[5] found that the mean count of Salmonella is lower than the mean Staphylococcal count. Mean Staphylococcal count in the raw milk collected from different sources ranges from $3.07x10^5$- $0.85x10^5$ cfu/ml and these counts are less than the findings of[9] where the mean staphylococcal counts were 4.4x106 cfu/ml. The mean Staphylococcal count range of raw milk in the present study is higher than the findings which range from $5.7x10^4$-$1.48x10^6$ cfu/ml and [8]found mean ranges from 30-10820 cfu/ml.

Among the isolated pathogens E.coli, Salmonella and S.aureus contamination possibly due to water used in unclean farm conditions, unhygienic practices followed in the farm during milking and storage environment in the farm. *Staphylococcus aureus* has been linked to gastroenteritis by producing enterotoxins, boils, skin infections, pneumonia, deep abscesses and meningitis in debilitated persons[10]. High microbial count and contamination with pathogens will affect the keeping quality and safety of raw milk as well as products derived from such milk samples. The hygienic management in dairy farm directly influences the production oriented economic results and health safety perspectives in human beings.

The results of milk samples tested for bovine brucellosis by milk ring test (MRT) were given in Table 2. The samples showing positive and negative results for brucella milk ring test were depicted in Fig 1. The proportion of Brucellosis positive samples from dairy farms was lower than from bicycle boys and two wheelers. This could be due to the fact that bicycle boys and two wheelers were collecting raw milk from a larger number of animals and herds as compared to a single dairy farm. The detection of Brucella antibodies in the milk samples might be due to excretion of the antibodies by infected carrier or vaccinated cows. This result differs from the findings of[6.]

Figure 1
Milk ring test showing Positive (left) and negative (right) results for Brucellosis

Table 2
Results of MRT for Brucellosis

Sample collection points	Sample size	Number of samples positive for Brucellosis
Dairy farms	10	3
Bicycle sellers	15	4
Two wheelers	15	6
Tea stalls	10	1
Small hotels	10	1
Total	60	15

Pathogenic bacteria like *Brucella* in milk have been a major factor for public health concern since from the early days of the dairy industry. Many diseases are transmissible via milk products. Traditionally raw or unpasteurized milk has been a major vehicle for transmission of pathogens. The health of dairy herd and milking conditions basically determine the milk quality. Another source of contamination by microorganisms is unclean teats. The use of unclean milking and transport equipments also contributed to the poor hygienic quality[11] .

CONCLUSION

The present study indicated that the dominant micro flora in the raw milk samples in and around Hyderabad city are as follows Salmonella>Staphylococcus aureus>E.coli among the isolated species. The presence of those pathogens in milk indicated that contamination from various sources such as animal, human, environment, utensils and others (Murphy and Boor, 2000). The high numbers of the isolated microorganisms not only contaminate the milk but also multiply and grow in milk. The present study insists that the production of high quality raw milk from healthy animals under good hygienic conditions protects human health. Therefore, it is recommended that training and guidance should be given to farm owners, workers, and small milk traders involved in milking. Regular screening of animals for Brucellosis is recommended.

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REFERENCES

1. De Buyser M.L., Dufour B., Maire M and Lafarge V. Implication of milk and milk products in food-borne disease in France and different industrialized countries. Int.J. Food Microbiol. 67: 1-17, (2001).
2. Harrington P., Archer J., Davis J.P., Croft D.R. and Varma, J.K. EIC officers: Outbreak of Campylobacter jejuni infections associated with drinking unpasteurized milk through a cow-leasing program-Wisconsin, 2001. MMWR 51: 548-549, (2002).
3. Oliver S.P., Jayarao B.M. and Almadiea R.A. Food borne pathogen in milk and the dairy farm environment. Food safety and public health implications. Food borne pathog, Dis, 2:115-129, (2005).
4. Murphy S. C and Boor K. J. Trouble shooting sources and causes of high bacteria counts in raw milk. Dairy Food Environ Sanitat. 20: 606-611, (2000).
5. Hossain T.J., Khorahed Alam and Dwipayan Sikdar. Chemical and microbiological quality assessment of raw and processed liquid market milk of Bangladesh, Res. Jrnal. of dairy sci 4: 28-34, (2010).
6. Swai E.S and Schoonman. Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania, Asian Pacific Journal of Tropical Biomedicine 217-222, (2011).
7. Saitanu I.A., Chuanchuen K.R, Nuanuarsuwan C., Koowatananukul and Rugkhaw V. Microbiological quality of raw cow milk. Thai J.Vet. Med., 26:193-214, (1996).
8. Srairi M.T., Moudnib J. Rahho L. and Hamama A. How do milking conditions affect the hygienic quality of raw milk? Case study from Moroccan dairy farms. Livest. Res.Rural Dev., 18, (2006).
9. Ghose A.K. and Maharanjan K.L. Milk marketing channels in Bangladesh: A case study of three villages from 3 districts. J. Int. Dev. Cooperat., 8:87-101, (2002).
10. Okpalugo J., Ibrahim K., Izebe K.S. and Inyang, U.S. Aspects of microbial quality of some milk products in Abuja, Nigeria. Trop. J. Pharma. Res.7:1169-1177, (2008).
11. Parekh T.S and Subhash R. Molecular and bacteriological examination of milk from different milch animals with special references to coliforms. Curr. Res. Bacteriol. 1(2), 56-63, (2008).