ISSN:2319-2607 IISUniv.J.Sc.Tech. Vol.6(1), 41-44 (2017)
RESEARCH ARTICLE

Incidence of *E. coli, Salmonella* and *Staphylococcus* in Camel Milk Collected from Bikaner District, India

Mahima Verma[1], Sant Prakash[2] , Alka Prakash[1]*
[1]Cell Biotechnology Lab, Dept. of Zoology, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra, India
[2]Molecular Genetics Lab, Dept. of Zoology, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra, India

Abstract

Unprocessed camel milk is consumed in some parts of our country. It is known to have therapeutic properties. In the present study incidence of three major food borne pathogens, *Escherichia coli, Salmonella* and *Staphylococcus* in raw camel milk samples collected from Bikaner district, India was studied. A seasonal variation with respect to their presence was observed though *E. coli* was found to predominate in all the seasons. Antibiograms determined for the isolates exhibited different antibiotic susceptibility patterns. Resistant coagulase positive *Staphylococcus* isolates were obtained whereas; *E. coli* and *Salmonella* isolates were susceptible to Co-Trimoxazole, Ofloxacin, Tetracycline and Gentamicin. *Lactobacillus fermentum* proved to be an effective alternative probiotic control against all the pathogenic bacteria.

Keywords: Camel milk, Food-borne pathogens, Antibiotics, Probiotics

Introduction

Milk, a rich nutritional source has always been an important part of human diet. Apart from nutritional values, therapeutic properties associated with camel milk are thrusting it over the more popular cow and buffalo milk. Contributing to only 0.4% of total milk produced around the world camel milk is known to have unique medicinal properties. As the animal is confined to arid regions, its milk mainly supports the tribes raising these animals.

Camel milk contains three times more vitamin C and ten times iron content than cow's milk (Farah *et al.,* 1992) and is lower in lactose (Sestuzheva, 1958). It helps to increase the haemoglobin level, enhances blood circulation and oxygenation of the body's organ systems and extremities. However, levels of potassium, magnesium, iron, copper, manganese, sodium and zinc are higher than in cow's milk contributing to its nutritional value (Safari, 2012). Camel milk contains some proteins having potential antimicrobial and protective activities (Hailu *et al.,* 2016, Kumar *et al.,* 2016); Camel milk has more fat and protein than cow milk and contains lower cholesterol than cow or goat milk (Medhammar *et al.,* 2011). It is used for treating dropsy, jaundice, spleen ailments, tuberculosis, asthma, anemia and piles (Rao *et al.,* 1970); multiple sclerosis, lupus, psoriasis, allergies-asthma (Wernery, 2006); for treating crohn's disease (Shabo *et al.,* 2008), Tuberculosis and Hepatitis B and C (Korish *et al.,* 2013, El-Fakharany *et al.,* 2013). It has also been shown to help protect against aluminum toxicity in rats (Al-Hashem, 2009) heal stomach ulcers (Korashy *et al.,* 2012) and helps insulin dependent type 1 diabetes patients in maintaining a natural glycemic control (Agrawal *et al.,* 2009; Agrawal *et al.,* 2011; Malik *et al.,* 2012). Furthermore, it has been reported to have anti-carcinogenic properties (Magjeed, 2005) reversing effects in leukemia, and is also used to cure other types of cancer infecting the lung, liver and breast (Korashy *et al.,* 2012).

The properties of fresh and raw camel milk are being reviewed as an emerging natural alternative in future medicine science in countries like Saudi Arabia, Kazakhstan, UAE and India. In many countries where camel dairies exist, camel milk and milk products are being marketed under standard safe measures. On the other hand, in India this falls under the unorganized dairy sector where collection and marketing of this milk is at random. This further impresses upon an urgent need to the study of the indigenous microbial flora of raw milk.

Epidemiological reports say untreated raw milk is responsible for many outbreaks (Buyser *et al.,* 2001; Harrington *et al.,* 2002). Food borne illnesses through consumption of contaminated milk and milk products are due to microbes like *Escherichia coli, Listeria monocytogenes, Enterobacter sakazakii, Salmonella spp., Staphylococcus aureus* and *Camphylobacter jejuni* have been grouped as major food borne pathogens. Ziney and Turki (2007) reported the same in camel milk in Saudi Arabia. *E. coli*, *Salmonella* and *Staphylococcus* are the major food-borne bacteria associated with milk. They frequently cause

*Corresponding Author : Email : prakashdr.dei@gmail.com

food illnesses in children, immune compromised, pregnant and aged persons.

Quality of milk is assessed not only by its protein fraction, fatty acid composition, and biomolecules but more importantly by its microbial flora. As the environmental conditions affect the biochemistry of milk, the microbial modules of raw milk may be indigenous owing to the specific environmental conditions or may be subjective to the health of the animal. Therefore, different seasons are conducive for different microbes thus, varying the composition of micro flora seasonally.

The incidence of these food borne pathogens in milk and milk products being sold under market conditions have been reported earlier (Verma and Prakash, 2016; Singh and Prakash, 2008). The present study has been extended to the analysis of untreated camel milk, collected directly from the udder of the animal in different seasons, from the unorganized sectors (local people raising camels) in Bikaner district, India.

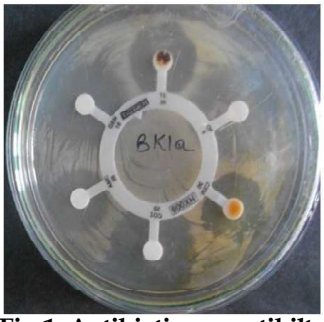
Materials and Methods

Bikaner district, situated in the northwest of the Rajasthan state of India, 242 m above sea level encounters a characteristic hot desert climate with very little rainfall and extreme temperatures. For the present study camel milk samples were collected by convenience sampling method, from local people (unorganized sector) of Bikaner. These animals were raised by the local people for their domestic use.

A total of 62 camel milk samples were procured from Bikaner region during all the three seasons' viz., summer, monsoon and winter from four different dromedary breeds, Bikaneri, Marwari, Jaisalmeri and Kachchi. This many samples were collected as per the availability of animals of different breeds in different seasons in the sampling area over two consecutive years. Milk was collected aseptically, directly from the udder of animal under field conditions in sterilized collection tubes. It was transferred under cold conditions to the lab and analyzed within 24 hours.

Milk samples (1 ml) were enriched in buffered peptone saline (9 ml, 0.5% w/v; peptone; 0.85% w/v; NaCl), and incubated for 48 hours in a microbiological incubator. Dilution plating on selective media was applied for isolation (Singh and Prakash, 2008; Singh *et al.*, 2009; Ziney and Turki, 2007). The distinct colonies were selected, purified and confirmed through various biochemical and sugar tests (Agarwal, 2003). *Staphylococcus* isolates were subjected to coagulase test.

Incidence of these pathogenic bacteria was statistically analyzed using GraphPad Prism 7.02. Antibiotic susceptibility by disc diffusion method (Bauer –Kirby, 1966) using separate antibiotics for gram positive (*Staphylococcus*) and gram negative (*Escherichia coli* and *Salmonella*) bacteria was studied. Probiotic control using agar well diffusion method (Toba *et al.*, 1991) was also determined (Fig. 1).



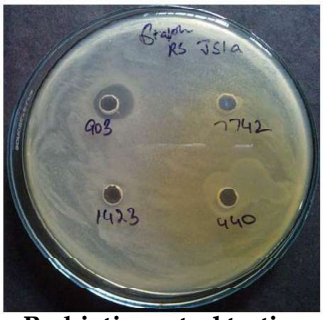


Fig.1. Antibiotic susceptibilty testing by disc diffusion methods showing zones of inhibition

Probiotic control testing by agar well diffusion methods showing zones of inhibition

Results and Discussion

Of the total samples 14.5% samples show the presence of *E. coli*, 12.9% show *Salmonella*, 8% show coagulase positive *Staphylococcus* and 2% show coagulase negative *Staphylococcus*. 85.5% of the total milk samples were free from these food borne bacteria (Fig. 2). Contamination was observed to be higher in the samples collected in monsoon and summer seasons than those collected in the winter season (Fig. 3).

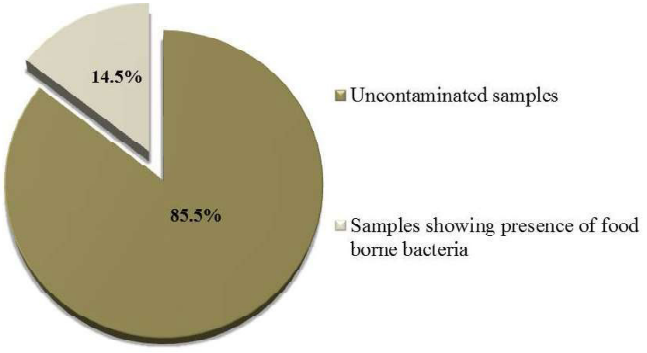


Fig.2. Percentage occrence of food borne pathogenic bacteria in camel milk

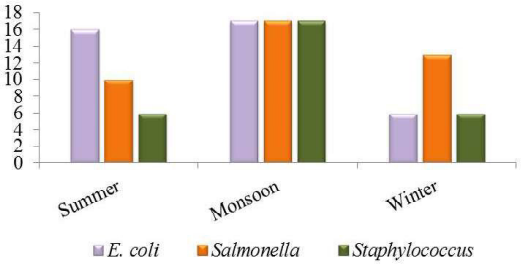


Fig. 3. Percentage occurrence of *E. Coli*, *Salmonella* and *Staphylococcus* as recorded in camel milk collected in different seasons.

Statistical analysis indicates that the samples collected in summers show a significant difference ($p < 0.05$) from those collected in monsoon as well as winters. This relates to the fact that the environmental conditions have an impact on the biochemical composition of milk, which directly affects the composition of indigenous micro-flora. No significant difference was observed in samples collected from different breeds or in occurrence of different microbes in milk samples collected in different seasons or from different breeds.

Different antibiotic susceptibility patterns were observed for *E. coli*, *Salmonella* and *Staphylococcus* by the disc diffusion method as per CLSI standards. It was found that all the *E. coli* and *Salmonella* isolates were susceptible to Co-Trimoxazole 25mcg, Ofloxacin 05mcg, Tetracycline 30mcg and Gentamicin 30mcg. Isolates from samples obtained during the monsoon season exhibited resistance to Cefuroxime 30mcg and Amoxycillin 30mcg. The *Staphylococcus* isolates from samples collected in winter and summer season were resistant to Methicillin 05mcg, Penicillin-G 10mcg, Vancomycin 30mcg and Ampicillin 10mcg and show intermediate susceptibility to Oxacillin 01mcg and Erythromycin 15mcg. All the isolates were inhibited by *Lactobacillus fermentum* (MTCC 903) showing a zone of inhibition of more than 8mm (Table 1).

The occurrence of the pathogens in raw camel milk presses for an urgent need towards healthy and hygienic milking practices. Raw camel milk is being used in the treatment and control of many diseases and disorders. As reflected in the present findings, its consumption by immune compromised people should be avoided in its unpasteurized state. Presence of the methicillin resistant coagulase positive *S. aureus* and other bacterial pathogenic strains can prove to be major threat to individuals depending on one's immunity.

Due to the extensive use of antibiotics, multidrug resistance is occurring globally. In order to overcome, this menace probiotics are emerging as a natural control with relatively no side effects on body metabolism. As observed in the present study *Lb. fermentum* promises to provide an alternative to control the pathogenic bacteria.

References

Agarwal, R.K., Bhilegaonkar, K.N., Singh, D.K., Kumar, A., Rathore, R.S. (2003) Laboratory Manual for the isolation and identification of food borne pathogens. *Jai Ambey, Bareilly*. ISBN 81-7525-409-2.

Table 1. Antibiotic and probiotic susceptibility patterns of food borne pathogenic bacteria

Season of sample collection	Food borne pathogenic bacteria	No of isolates	Antibiotics						Probiotics		
			COT 25	CXM 30	OF 05	TE 30	GEN 10	AMC 30	*Lb. fermentum* (MTCC 903)	*Lc. Lactis* (MTCC 1423)	*P. acidilactici* (MTCC 7442)
Winter	*Escherichia coli*	1	S	S	S	S	S	S	S	-	+
	Salmonella	2	S	S	S	S	S	S	S	-	±
Summer	*Escherichia coli*	5	S	S	S	S	S	S	S	-	±
	Salmonella	3	S	S	S	S	S	S	S	-	+
Monsoon	*Escherichia coli*	3	S	S	S	S	S	I	S	-	-
	Salmonella	2	S	R	S	S	S	I	S	-	-
		1	S	R	S	S	S	I	S	-	-
			Antibiotics								
			MET 05	P 10	VA 30	AMP 10	OX 01	E 15			
Winter	*Staphylococcus*	1	R	R	R	R	I	I	S	-	-
Summer	*Staphylococcus*	2	R	R	R	R	I	I	S	-	-
Monsoon	*Staphylococcus*	2	S	R		R	S	I	S	-	-
		1	S	R	S	R	S	I	S	-	-

S susceptible, I intermediate, R resistant (as per CLSI standards)
COT 25 - Cotrimoxazole 25 mcg, CXM 30 - Cefuroxime 30mcg, OF 05-Ofloxacin 5 mcg, TE 30 - Tetracycline 30 mcg, GEN 10-Gentamicin 10 mcg, AMC 30 - Amoxicillin-Clavulanate (2.1) 30 mcg, MET 5-Methicillin 5 mcg, P10-Penicillin –G 10 mcg, VA30-Vancomycin 30 mcg, AMP 10-Ampicillin 10 mcg, OX 1-Oxacillin 1 mcg, E 15 - Erythromycin 15 mcg.

Agarwal, R.P., Jain, S., Shah, S., Chopra, A., Agarwal, V. (2011) Effect of camel milk on glycemic control and insulin requirement in patients with type-1 diabetes: 2-years randomized controlled trial. *Eur J Clin Nutr* **65**(9):1048-52.

Agrawal, R.P., Dogra, R., Mohta, N., Tiwari, R., Singhal, S., Sultania, S. (2009) Beneficial effect of camel milk in diabetic nephropathy. *Acta Biomed* **80**(2): 131-134.

Al-Hashem, F. (2009) Camel's milk protects against aluminum chloride-induced toxicity in the liver and kidney of white albino rats. ***Am J Biochem Biotechnol*** **5**(3):98-108.

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**(4):493–496.

De Buyser, M L., Dufour, B., Maire, M., Lafarge, V. (2001). Implication of milk and milk products in food-borne disease in France and different industrialized countries. – *Int. J. Food Microbiol* **67**(1-2): 1-17.

El-Fakharany, E.M., Sanchez, L., Al-Mehdar, H.A., Redwan, E.M. (2013) Effectiveness of human, camel, bovine and sheep lactoferrin on the hepatitis C virus cellular infectivity: comparison study. *Virol J* **10**:199.

El-Ziney, M. G., Al-Turki, A. I. (2007) Microbiological quality and safety assessment of camel milk (*Camelus dromedaries*) in Saudi Arabia (Qassim Region). *Appl Ecol Env Res* **5**(2): 115-122.

Farah, Z., Rettenmaier, R., Atkins, D. (1992) Vitamin Content of Camel Milk. *Int J Vit Nutr Res* **62**(1):30-33.

Hailu, Y., Hansen, E.B., Seifu, E., Eshetu, M., Ipsen, R., Kappeler, S. (2016) Functional and technological properties of camel milk proteins: a review. *J Dairy Res* **83**(4):422–429.

Harrington, P., Archer, J., Davis, J.P., Croft, D.R., Varma, J.K., EIC officers. (2002) Outbreak of *Campylobacter jejuni* infections associated with drinking unpasteurized milk through a cow-leasing program-Wisconsin, 2001. *MMWR Weekly* **51**(25): 548-549. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5125a2.htm Accessed 26 Apr 2017

Korashy, H. M., Maayah, Z. H., Abd-Allah, A. R., El-Kadi, A. O. S., & Alhaider, A. A. (2012) Camel Milk Triggers Apoptotic Signaling Pathways in Human Hepatoma HepG2 and Breast Cancer MCF7 Cell Lines through Transcriptional Mechanism. *J Biomed Biotechnol* **2012**:593195. doi:10.1155/2012/593195

Korashy, H.M., El Gendy, M.A., Alhaider, A.A., El-Kadi, A.O. (2012) Camel milk modulates the expression of aryl hydrocarbon receptor-regulated genes, Cyp1a1, Nqo1, and Gsta1, in murine hepatoma Hepa 1c1c7 cells. *J Biomed Biotechnol* **2012**:782642. doi: 10.1155/2012/782642.

Korish, A.A., Arafah, M.M. (2013) Camel milk ameliorates steatohepatitis, insulin resistance and lipid peroxidation in experimental non-alcoholic fatty liver disease. *BMC Complement Altern Med* **13**:264.

Kumar, D., Chatli, M.K., Singh, R., Mehta, N., Kumar, P. (2016) Enzymatic hydrolysis of camel milk casein and its antioxidant properties. *Dairy Sci & Technol* **96**(3):391-404.

Magjeed, N.A. (2005) Corrective effect of milk camel on some cancer biomarkers in blood of rats intoxicated with aflatoxin B1. *J Saudi Chem Soc* **9**(2):253-263.

Malik, A., Al-Senaidy, A., Jankun, E.S., Jankun, J. (2012) A study of the anti-diabetic agents of camel milk. *Int J Mol Med* **30**(3):585-592.

Medhammar, E., Wijesinha-Bettoni, R., Stadlmayr, B., Nilsson, E., Charrondiere, U.R., Burlingame, B. (2011) Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective. *J Sci Food Agric* **92**(3):445-474.

Rao, M.B., Gupta, R.C., Dastur, N.N. (1970) Camel's milk and milk products. *Indian J Dairy Sci* **23**:71-78.

Safari, C. (2012) The amazing characteristics of the camels. *Int J Antimicrob Agents* **22**:360-366.

Sestuzheva, S. (1958) Effect of stage of lactation on camels' milk. *Molecular Prom* **19**:33–39.

Shabo, Y., Barzel, R., Yagil, R. (2008) Etiology of crohn's disease and camel milk treatment. *J Camel Prac Res* **15**(1):55-59.

Singh, P., Manjeera, G., and Prakash. A. (2009) In vitro antimicrobial control measures for *Staphylococcus aureus* under the influence of specific physical, chemical and biological factors. *DEI Journal for Science and Engineering* **15** (1&2):53-57.

Singh, P., Prakash, A. (2008) Isolation of *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* from milk products sold under market conditions at Agra region. *Acta agriculturae Slovenica* **92** (1): 83–88.

Toba, T., Samant, S.K., Itoh, T. (1991) Assay system for detecting bacteriocin in microdilution wells. *Lett Appl Microbiol* **13**: 102–104. doi:10.1111/j.1472-765X.1991.tb00581.x.

Verma, M. and Prakash, A. (2016) Incidence of Coagulase positive *Staphylococcus* in raw camel milk from different regions of India: A possible threat to Diabetic Consumers. *Adv Appl Sci Res* **7**(4):121-126.

Wernery, U. (2006) Camel milk, the white gold of the desert. *J Camel Practice & Res* **13**(1):15-26.