Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Pelagia Research Library

Advances in Applied Science Research, 2016, 7(4):121-126

Pelagia Research Library

ISSN: 0976-8610
CODEN (USA): AASRFC

Incidence of Coagulase positive *Staphylococcus* in raw camel milk from different regions of India: A possible threat to Diabetic Consumers

Mahima Verma and Alka Prakash

Cell Biotechnology Lab, Dept. of Zoology, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra, 282010, India

---

ABSTRACT

Camel milk forms a significant part of tribal diet in arid and semi-arid regions. With the ever-increasing knowledge of its therapeutic value in diabetes and other health concerns, study of indigenous micro flora becomes very important. Staphis reported to be a threat to diabetic patients and immune-compromised people. In the present study 62 raw milk samples were analyzed for incidence of Staphylococcus. Results revealed a high rate of incidence in samples i.e., in 12.9% samples. 62.5% of isolates were coagulase positive whereas, coagulase negative isolates were methicillin resistant. Ciprofloxacin (MIC 1-2 µg/ml) and Imipenem emerged as effective antibiotic drugs for control and Lactobacillus fermentum(MTCC 903) was spotted as an operative probiotic control against these isolates. Statistical analysis of antibiograms showed significant differences in coagulase positive and coagulase negative isolates. Probiotics are emerging as a rapid alternative to antibiotics.

Keywords: Dromedary, *Staphylococcus*, Public health, Probiotics, Antibiotics.

INTRODUCTION

Camel milk has been an important food for nomads and its use was restricted to them, till interesting discoveries of its unusually useful properties triggered its demand. Absence of β-lacto-globulin and low content of α-casein in camel milk, grades it fit for consumption by individuals allergic to protein fraction of cow, buffalo, goat or ewe's milk [13]. Improvement in conditions of MDR tuberculosis patients [12], cancer cases [1] autism patients [2]along with antiviral, anti-bacterial properties [15] have been found to be associated with camel milk. Camel's milk is reported to have a stronger inhibitory system than that of cow's milk [15]. Consumption of camel milk reduces insulin dosage in Type 1 Diabetes [8], and reduced number of diabetic patients has been observed in tribes consuming camel milk as staple diet [9].

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

Food borne illnesses through consumption of contaminated milk and milk products are due to microbes like *Escherichia coli, Listeria monocytogenes, Enterobacter sakazakii, Salmonella spp.* and *Staphylococcus aureus*[3]. *Staph isubiquitous* and is the cause of many infections in humans and other animals. Many global outbreaks have

beendue to *Staph* contamination in food; these infections are fatal and contagious until the infection has been taken care of. These bacteria produce heat stable enterotoxins[10] that are not inactivated during pasteurization or during preparation of milk products and can incite food intoxication (vomiting and diarrhea)[18]. Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent nosocomial pathogen. In 1990 to 1995, the National Nosocomial Infection Surveillance (NNIS) program informed that coagulase-negative *Staphylococci* are the causative agent in 11% of all nosocomial infections. CDC is presently running two big programs to get a full picture of invasive MRSA infections. Pathogenicity of *Staph* is even coupled with coagulase production[17]Diabetic people have been known to be more prone to *Staph* infections as they are immune compromised [14]. Even the insulin injections provide a gateway for penetration of such pathogens. Thus it can be said that the benefits of the use of raw camel milk for diabetes control may contrarily develop the undesirable infections if *Staph* contamination occurs in milk.

Antibiotics are being extensively used to resolve bacterial infections. Penicillins, carbapenebs, aminoglycosides, vancomycin, macrolides and quinolones are diverse groups of antibiotics with varying working mechanisms used against *Staph* infections. Due to development of antibiotic resistance[11]Probiotics are also emerging as an operational control against food borne pathogens bacteria [5]. Lactic Acid Bacteria are well known probiotic bacteria widely in use to check the growth of food borne pathogenic bacteria. This study aimed to detect the presence of *Staphylococcus aureu*s and MRSA in raw milk of camels' population in use for commercial milking in India. The unorganized sectors in two regions of India were taken under this study. Control for indigenous strains through antibiotics and probiotics was also studied.

MATERIALS AND METHODS:

The selected area of study was the villages on the outskirts of Bikaner city in Rajasthan, India where camels are brought up for personal purpose and villages on the outskirts of Agra city in Uttar Pradesh, India where the locals rear camels for commercial milking. Bikaner is situated in the center of the Thar Desert whereas, Agra city lies at its border.

Sampling: Total of 62 Camel milk samples were procured in summer, winter and monsoon seasons. Milk was collected directly from the udder in sterilized autoclaved sample collection tubes. The tubes were handled carefully and aseptically to prevent any contamination from surroundings. The samples were transferred immediately to the lab in insulated, ice containers at 4^0C and were further analyzed.

Isolation and Identification: Isolation was carried out according to[7] [6]. Milk samples were diluted in 1:9 ratios with peptone water (v/v), mixed properly and incubated at 37^0C for 48-50 hours. Sterilized plates of Baird Parker Agar complemented with 5% egg yolk emulsion and 0.35% potassium tellurite were used for surface plating of serial dilutions of samples (Fig 1).The plates were incubated at 37^0C for 24 hrs. Shiny black colonies with a halo were picked and cultured in Brain Heart Infusion Broth. *Staphylococcus aureus* MTCC 3381 was taken as a positive control.

(a) (b)

Fig. 1: Characteristic Growth of *Staphylococcus* on (a) Baird Parker Agar (b) TSYA

Gram staining and various physiochemical tests including Catalase, Oxidase, Methyl Red, Voges Proskauer, Indole test and Nitrate Reduction test were performed according to [4]. Sugar fermentation patterns were also observed. These tests were performed in triplicates.

Coagulase test: The confirmed isolates were subjected to the coagulase test by inoculating 100µl24 hours culture in 0.5 ml of 1:10 diluted rabbit blood plasma and analyzed after incubating for 4 hrs.at 37^0C (Fig. 2). The isolates

showing negative results were left for 24 hours at room temperature and results were noted [16]. Coagulase test is associated with pathogenicity of microbe.

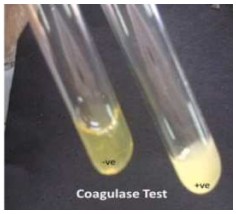


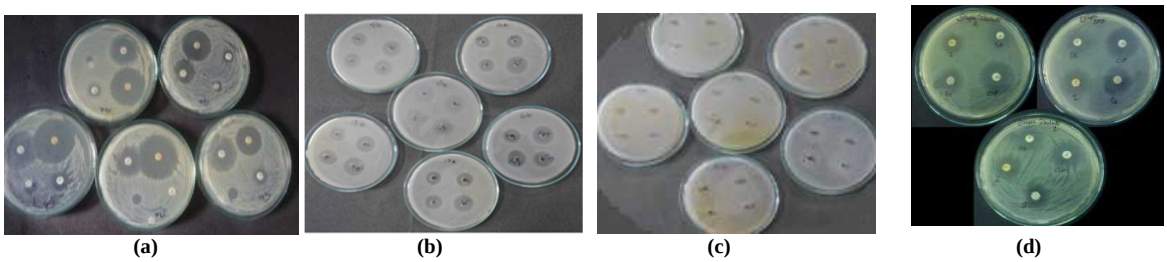
Fig 2: Coagulase test

Antibiograms: The sensitivity of isolates against Ciprofloxacin, Imipenem, Gentamycin, Spectinomycin, Methicillin, Penicillin–G, Vancomycin, Ampicillin, Oxacillin and Erythromycin were determined using disc diffusion method. The zones of inhibitions were noted after 24 hours incubation and interpreted according to CLSI (formerly NCCLS) standards (Fig. 3 a, b).

Analysis of Variance was performed.
Minimum Inhibitory Concentration: MIC of the drug ciprofloxacin was determined by well diffusion assay using different concentrations of Ciprofloxacin viz., 0.1µg/ml, 1µg/ml, 2µg/ml, 3µg/ml, 5µg/ml, 10µg/ml, 15µg/ml, 17µg/ml, 20µg/ml and 25µg/ml (Fig. 3 c).

Antimicrobial susceptibility test: Lactic acid bacteria were used to control these isolates, the strains used were MTCC 903 (*Lactobacillus fermentum*), MTCC 7742 *(Pediococcus acidilactici*), MTCC 1423 (*Lactococcus casei*) and MTCC 440 (*Lactococcus lactissubsplactis*). 150 µl of 24 hours cultures of isolates were seeded in 15ml Muller Hinton Agar media in separate plates. Wells were bored using 6mm borer and 40 µl of cell free supernatant of each lactic acid bacteria was inoculated in wells and plates incubated for 24 hours before measuring the zones of inhibition (Fig. 3 d).

Fig 3: Antibiograms of isolates (a,b) Antibiotic Sensitivity testing, (c) Determining Minimum Inhibitory concentration of Ciprofloxacin by well diffusion method (d) Probiotic control



(a) **(b)** **(c)** **(d)**

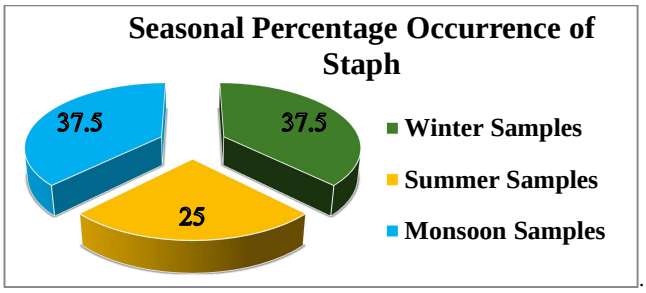
RESULTS AND DISCUSSION

The study showed the presence of *Staphylococcus* in 8 out of 62raw camel milk samples(Fig 4). The isolates were confirmed to be *Staphylococcus* biochemically. Out of the confirmed isolates 3 isolates from the winter samples were coagulase negative whereas, the 2 from summer samples 3 from monsoon samples were confirmed to be coagulase positive(Table 1).

Table 1: Seasonal Occurrence of Staph isolates

Season	No of tested Samples	Positive Samples	As percent of total occurrence	Coagulase
Winter (Nov to Mar)	16	3	37.5%	Negative
Summer (Apr to June)	30	2	25%	Positive
Monsoon (July to Oct)	16	3	37.5%	Positive

Fig.4. Seasonal Occurrence of *Staph* isolates



In this study the two populations of camels are from two different regions of India. The population from villages of Bikaner is raised for personal use of tribal families (presence of coagulase negative isolates) and the ones from villages near Agra are being raised for their commercial use mainly to yield milk (presence of coagulase positive isolates). Coagulase positive *Staphylococcus aureus* has been considered pathogenic by many researchers actively working in this field as it causes coagulation of blood. Results show the presence of Coagulase positive staph in milk samples collected in the summer and monsoon season from Agra region. Probable reason for this may be the infected micro-environment in which these animals are housed or the vegetation on which they are fed differs from the feed of camel population in desert area.

Various antibiotic resistant patterns were shown by the isolates (Table 2, Fig. 5). Coagulase negative isolates were methicillin, penicillin, vancomycin and ampicillin resistant whereas coagulase positive were all gentamycin resistant (Table 4).Methicillin resistant strains are a matter of concern in researchers. Ciprofloxacin and Imipenem were a control for all the isolates, methicillin resistant as well non-resistant.

Table 2: Antibiograms of *Staph* isolates. [ ] colour denotes resistant, [ ] colour denotes intermediate susceptible and [ ] denotes susceptible isolates according to NCCLS standards. CIP 5: Ciprofloxacin 5 mcg; IMP 10: Imipenem 10 mcg; GEN 10: Gentamycin 10 mcg; SPT 100: Spectinomycin 100 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.

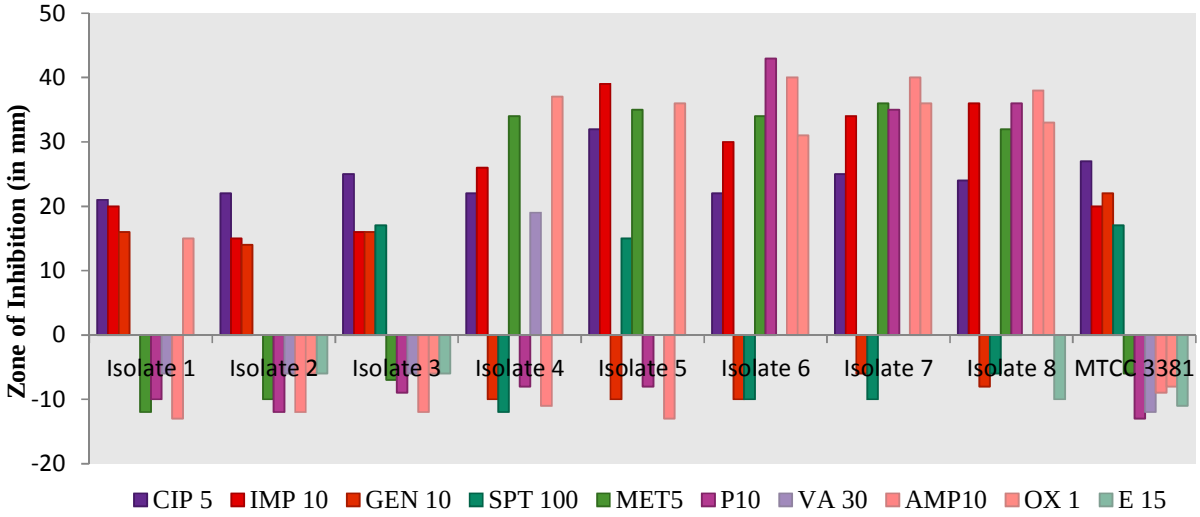
Isolates name	Zones of Inhibition for antibiotics (in mm)									
	CIP 5	IMP 10	GEN 10	SPT 100	MET5	P10	VA 30	AMP10	OX 1	E 15
Isolate 1	21	20	16	15	12	10	0	13	15	19
Isolate 2	22	15	14	13	10	12	0	12	0	0
Isolate 3	25	16	16	17	7	9	0	12	0	0
Isolate 4	22	26	10	12	34	8	19	11	37	22
Isolate 5	32	39	10	15	35	8	17	13	36	21
Isolate 6	22	30	10	10	34	43	16	40	31	22
Isolate 7	25	34	0	10	36	35	16	40	36	23
Isolate 8	24	36	8	0	32	36	16	38	33	10
MTCC 3381	27	20	22	17	0	13	12	9	8	11

The pattern clearly shows the variance in isolates from two areas. Also the methicillin resistant isolates were found sensitive to only three antibiotics showing the isolates to be more resistant and difficult to be controlled.

ANOVA ($p<0.01$) shows that there exist a significant difference between the coagulase positive and negative isolates with respect to their susceptibility to the various antibiotics (Table 2). There is a significant difference in the incidence of coagulase positive and coagulase negative isolates obtained in different seasons.

MICof ciprofloxacin for the isolates was<2 µg/ml (Fig.3(c)) by well diffusion method. Ciprofloxacin can be implemented to control Staph infections

Fig. 5.Antibiograms in form of bar graph showing susceptible zones on positive axis and resistant zones on negative axis

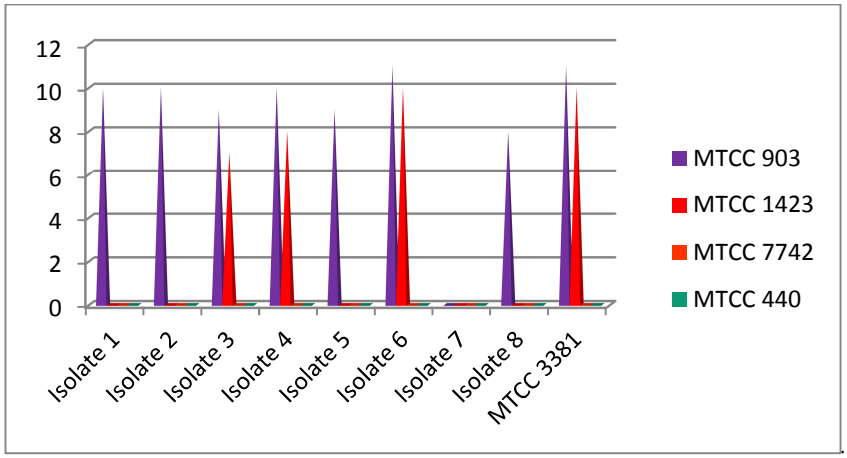


Lactobacillus fermentum(MTCC 903) emerged as an effective antimicrobial control against all the isolates (Table 3, Fig 6). Probiotics are emerging as an effective natural control to many diseases.

Table 3: Probiotic control of isolates

Isolates name	Zones of Inhibition for Lactic Acid Bacteria (in mm)			
	Lactobacillus fermentum MTCC 903	*Lactobacillus casei* MTCC 1423	*Pediococcus acidilactici* MTCC 7742	*Lactococcuslactis subsp. Lactis* MTCC 440
Isolate 1	10	-	-	-
Isolate 2	10	-	-	-
Isolate 3	09	07	-	-
Isolate 4	10	08	-	-
Isolate 5	09	-	-	-
Isolate 6	11	10	-	-
Isolate 7	-	-	-	-
Isolate 8	08	-	-	-
MTCC 3381	11	10	-	-

Fig.6. Graph showing zones of inhibition of probiotics



CONCLUSION

The high incidence rate of Staph in raw camel milk is a matter of serious concern specifically because of its consumption by diabetic patients who are already immune compromised. The animals may not be properly tended and the collection of milk may not be done hygienically therefore the microbe persists in the sample making it unfit for consumption by diabetic and immune compromised individuals. In India there is an urgent need to spread the awareness regarding the hygienic practices and possible infections to avoid any outbreaks. Still the question of consumption of such contaminated milk by diabetic patients remains in place. It may be suggested that the milk be inoculated artificially with probiotics before being marketed for its antimicrobial properties to eliminate the chances of probable infection.

Table 4: Probiotic control of antibiotic resistant indigenous Staphylococci

Isolate numbers	Season	Coagulase test	Resistance to antibiotics	Probiotic control
Isolate 1, Isolate 2, Isolate 3	Winter	Negative	Methicillin, penicillin-G, Vancomycin, Ampicillin	*Lactobacillus fermentum*
Isolate 4, Isolate 5	Summer	Positive	Gentamycin, Penicillin_G, Ampicillin	*Lactobacillus fermentum*
Isolate 6, Isolate 7, Isolate 8	Monsoon	Positive	Gentamycin, Spectinomycin	*Lactobacillus fermentum*

Acknowledgment
We would like to thank the Almighty and our department for successful completion of this work.

REFERENCES

[1] HabibHM, IbrahimWH, Schneider-StockR, HassanHM,*Food Chemistry*, **2013**, 141:1, 148.
[2] Laila Y,Ayadhi Al, Elamin NE,*AlternatMedv*, **2013**,8, Article ID: 602834.
[3] Abeer, AlAll A, Gouda, Assa SA, Dardir HA, Ibrahim AK,*Global Veterinaria*, **2012**,8:1, 76.
[4] Singh P, Prakash A,*ActaargiculturaeSlovenica*,20**1**0,96:1, 37.
[5] SuskovicJ, Kos B, Beganovic J,Pavunc AL,Habjanic K,MatosicS, *Food Technol. Biotechnol*,**2010**,48:3, 296.
[6] Singh P, Prakash A,*ActaAgriculturaeSlovenica*,**2008**,92:1, 83.
[7] ZineyEl,TurkiAl,*Applied Ecology and Environmental Research*,**2007**,5:2, 115.
[8] Agrawal RP, Beniwal R, Sharma S, Kochar DK, Tuteja FC, Ghorui SK, Sahani MS,*Journal of Camel Practice and Research*, **2005**,12:1, 27.
[9] Agrawal RP, SwaniSC, Beniwall R, Kochar DK, Sahani MS, Tuteja FC, Ghorui SK,*India Journal of Animal Sciences*, **2003**, 73:10, 1105.
[10] Presscott LM, Harley JP, Klein DA,*Text book of microbiology*, Brown Publishers, **2002**, pp441–42.
[11] LevySB, *J. Antimicrob. Chemother*,**2002**,49:1, 25.
[12] Mal G,Suchitra-Sena D, Jain VK, Sahani MS, *Israel Journal of Veterinary Medicine*,**2000**, 61:3-4, 88.
[13] Restani P, GaiaschiA, PlebaniA, BerettaB, CavagniG, FiocchiA, PoiesiC, VelonaT, Ugazio AG,GalliCL,*Clin. Exp. Allergy*,**1999**,29, 997.
[14] Geerlings SE,Hoepelman AI,*FEMS Immunol Med Microbiol*, **1999**, 26:3-4, 259.
[15] elAgamy EI, Ruppanner R, Ismail A, Champagne CP, Assaf R,*J Dairy Res*,**1992**, 59:2, 169.
[16] Hemker HC, Bas BM, Muller AD,*Biochim. Biophys. Acta*, **1975**, 379, 180.
[17] Kloos WE,Musselwhite MS,*Microbiol*,**1975**,30, 381.
[18] Payne DN, Wood JM,*J Applied Bacteriol*,**1974**,37:3,319.