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Prevalence and Antibiogram of *Staphylococcus aureus*, a Camel Mastitogen from Pakistan

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ABSTRACT

Camels from Cholistan desert of Pakistan were studied for *Staphylococcus aureus* mastitis infection, associated risk factors and subsequently their antimicrobial susceptibility. The milk samples were screened with surf field mastitis test and furthered to biochemical analysis. Pearson' Chi-square test at nighty five percent confidence interval was used to analyze the collected data. Antibiotic sensitivity was checked with twenty four antibiotics by disc diffusion method. Over all mastitis was found 52.5% (63/120) with leading subclinical (41.67%, 50/120) form of mastitis on overall milk collected data. Pathogen type was crowned by *Staph. aureus* with 74.5% (47/63) prevalence, following which were *Streptococcus* species (17.45%, 11/63), *E. coli* (3.17%, 2/63), and *Bacillus cereus* (4.76%, 3/63). Coagulase positive *Staph. aureus* (41 out of 47), and hemolysin producing *Staph. aureus* (39 out of 47) primed among *Staph. aureus* isolates indicating very pathogenic nature of infection in the area. Risk factors determinants were found significantly (P<0.05) associated with mastitis occurrence except frequency of milking per day. Antibiogram of *Staph. aureus* indicated very strong resistance to oxacillin, ticarcillin, ampicilline, amoxicillin, azlocine, chloramphenicol, mupirocin, vancomycin, cefixinme, cefuroxime, and cefotaxime. In contrast to this sulphaphenazole, gentamicin, amikacin, and ciprofloxacin were highly sensitive. Piperacillin, Tazabactam and cinxacin were moderately effective against *Staph. aureus*. The concluded remarks of research staged *Staph. aureus* to the most obvious pathogen and widely resistant to antimicrobials camel mastitogen. The risk factors were found soul determinants of pathogen spread among mammary glands of camels.

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AIA, AAA and MI conceived and designed the project, conducted research work, analyzed the data and wrote the manuscript. SH and SS contributed in experimental work. AZD, RH and SSA helped in data analysis and writing of manuscript.

Key words
Camel (she-camel) mastitis, *Staphylococcus aureus*, Risk factors, Antibiotics.

INTRODUCTION

Camel dwells in arid and semi-arid areas of Africa (15 million) and Asia (4 million) accounting 19 million numbers (FAO, 2001). Camel population is growing faster compared to cattle and sheep in the world (Faye and Bonnet, 2012). Pakistan holds 1 million numbers of camels which makes 1% of total animal in Pakistan but emerges as 3rd highest milk producer after buffalo and cattle (Anonymous, 2015). Camel occupies individuality for its characteristic milk, which is consumed raw, and in pasteurized form alongwith its products that have access to markets of Gulf countries (El-Agamy and Khatab, 1992). Low level of lactose, fewer short-chain fatty acids, high voltaic linoleic and polyunsaturated acids, 3 times greater vitamin C, 10 times higher iron, antibodies compatible to that of human's with short chain in specificity (Walstra *et al*., 2006), natured with lysozyme and lactoferrin, equipped with immunoglobulin that fight against anti-DNA agents, lacking β−lactoglobulin and a "new" β-casein (Beg *et al*., 1986) allergens, and featured with 150 U/ml insulin (Zagorski *et al*., 1998) make it solution to hungry and diseased community. The milk is being sold in the name of Shubat, sour milk in Kazakhstan; Kefir, the Caucasian fermented milk; Lehban, fermented products in Syria and Egypt (Rihab *et al*., 2008). Non-cattle milk production has been estimated to be 16.5% of the total milk yield at world level by 2009, camel milk making a big share (Nagy *et al*., 2013). The consumption of camel milk is ten times more than that of its meat. The milk production is expected to double in the near future.

Mastitis in clinical subclinical form (Ahmad *et al*., 2012) is prevalent in dairy animal worldwide reducing milk quality and quantity, lowering shelf life

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and processing for further products. Maximum daily milk production may fall under 35-40 liter yielding total of 1000-12,000 liter in lactation length of 8-18 month (Faye, 2005). Camel mastitis, especially subclinical, has been neglected and infact irrational use of antibiotics has resulted in development of resistant bacteria, which has rendered antibiotic therapy inefficient (Yousaf, 2009). Among various bacteriological etiology of mastitis, *Staph. aureus* is the major pathogen that induces intramammary infection. Mere this pathogen accounts for 20.35% mean prevalence in the world in 52.3% in Pakistan (Ahmad *et al*., 2012; Sarwar, 2013).

Intramammary infections caused by *Staph. aureus* impair alveolar physiology, decrease milk production, and impart harmful effects on milk composition (Leitner *et al*., 2000; Dego and Tareke, 2003). *Staph. aureus* executed intramammary infection used to be long term and chronic because of ability of bacteria to hide itself in mammary epithelial cells (Yousaf, 2009). Use of antibiotics against this bacterium has been global focus among various species. Keeping in view the very nature of pathogen, present study was undertaken to determine the prevalence of mastitis, risk factors, and response towards various antibiotics.

MATERIALS AND METHODS

Animal screening for mastitis

120 Camels (n=95 lactating; n= 25 dry) from Cholistan desert of Pakistan (28°15'O north latitudes and 70°45'O east longitudes) residing in temperature range of 6-50°C and annual average rain fall of 100 millimeter were screened for mastitis during year 2015 in localities of district Rahim Yar Khan of Cholistan desert. The clinical mastitis was screened as per description of Radostits *et al*. (2007), while subclinical screening of mastitis was done using Surf Field Mastitis Test (SFMT), proposed by Muhammad *et al*. (1995). Precautions and regulations were strictly observed while collecting and storing of milk samples for laboratory examination (NMC, 1987).

Risk factors assessment

For risk factor assessment additional information like age, parity, udder ticks, teat dipping, milking frequency, stage of lactation, feeding pattern, and animal health status was gathered through a questionnaire. The collected information was refined by formal and informal testing according to Thrusfield (2007).

Biochemical confirmation of bacteria

A 0.5 ml of milk sample was spread out primarily on blood agar and incubated for 24 and 48 h at 37°C (Lafi and Hailat, 1998). The bacterial isolates were purified by multiple streaking and characterized according to Holt *et al*. (1994).

Antibiotic sensitivity test against Staph. aureus

An antibiogram consisting of all classes of antibiotics was developed against biochemically confirmed *Staph. aureus* isolates following standard procedures stated by Bauer *et al*. (1966). To carry out sensitivity test revived broth growth of *Staph. aureus* was adjusted by spectrophotometric method at 0.5 McFarland units, equals to 10^8 CFU, to be swabbed subsequently on Muller Hinton agar. Antibiotics discs used in the experiment included Ticarcillin (70µg), Cefixime (5µg), Cloxacillin (1µg), Sulphaphenazol (200µg), Gentamicine (10µg), Amoxicillin (30µg),Vancomycin (30µg), Ceftaxime (30µg), Ceftazidine (30µg), Amoxicillin (25µg), Cefuroxime (30µg), Ciprofloxacillin (5µg), Ampicillin (10µg), Oxacillin (1µg), Amicacin (30µg), Azlocillin (75µg), Piperacillin Tazabactam (100:10 µg), Ticarcillin Calvanic acid (75:10µg), Triple Sulphas (300µg), Cinoxacin (100µg), Mupiracin (5µg), Spectinomycin (100µg), Chloramphenicol (25µg), Trimethoprim (30µg). Antibiotic discs were placed under sterilized conditioned and later incubated at 37˚C for 24 h (Baur *et al*., 1996). Zones around antibiotic discs measured in millimeter were compared with standard zone of inhibition (CLSI, 2015) to declare *Staph. aureus* eventually sensitive, intermediate or resistant to the drugs.

Statistical analysis

Prevalence of mastitis and bacterial isolates was calculated by formula described by Thrusfield (2007). Pearson's chi square test with 95% confidence interval ($P<0.05$) was used to identify the significant relation using SPSS version 22 (IBM Corp., 2013).

RESULTS

Prevalence of mastitis and mastitogens

Out of 120 animals screened by SFMT and confirmed with biochemical tests 52.5% (63/120) camels were found to be suffering from mastitis (Table I). Subclinical mastitis (41.67%) was four times more than the clinical (10.83%) form of mastitis. Mastitis positive samples, however, constituted 79.4% (50/63) subclinical and 20.6% (13/63) clinical mastitis. Isolates identified from collected milk samples showed *Staph. aureus* to be prevalent bacterium, 74.6% (47/63), causing intramammary infection. The other major isolates found were *Streptococcus agalactiae, Strep. dysgalactiae, E. coli* and *Bacillus cereus* which were 85, 91, 95, and 93% fewer than *Staph. aureus,* respectively, in mastitis milk samples.

Table I.- Prevalence of mastitis in camels with *Staphylococcus aureus* and other major isolates.

		Tested samples	Positive samples	% mastitis	Clinical	Sub-clinical
Overall mastitis/isolate based mastitis		120	63	52.5	13 (10.83%)	50 (41.67%)
Staphylococcus aureus		63	47	74.6	7 (11.11%)	40 (63.49%)
Coagulase positive		47	41	87.23	5 (10.64%)	36 (76.60%)
Hemolytic		47	39	82.98	4 (8.51%)	35(74.47%)
Types of hemolysin produced	Alpha	47	10	21.28	2 (4.26%)	8 (17.02%)
	beta	47	5	10.64	1 (2.13%)	4 (8.51%)
	Alpha-beta	47	24	51.06	3(6.38%)	21 (44.68%)
Streptococcus agalactiae		63	7	11.1	2 (3.17%)	5 (7.94%)
Streptococcus dysgalactiae		63	4	6.35	1 (1.59%)	3 (4.76%)
E. coli		63	2	3.17	2 (3.17%)	0
Bacillus cereus		63	3	4.76	1 (1.58%)	2 (3.17%)

Table II.- Percentage of risk factors along with their univariate occurrence as mastitis determinants.

Risk factors	Mastitis % (no. of cases positive/ no. of cases observed)	P value
Udder hygiene		
Satisfactory	20% (6/30)	0.00
Unsatisfactory	63.3% (57/90)	
Udder ticks		
Present	71.4% (55/77)	0.00
Absent	18 % (8/43)	
Teat dips		
Yes	22.2% (4/18)	0.005
No	57.8% (59/102)	
Body condition		
Normal	27.3% (15/55)	0.00
Thin	73.8% (48/65)	
Milking frequency		
Once a day	63.6% (14/22)	0.247
More than once	50% (49/98)	
Milking status		
Lactating	42% (40/95)	0.008
Dry	72% (18/25)	
Milk yield		
<5 liter or less	31% (18/58)	0.014
> 7 liter	53% (33/62)	
Feeding status		
Well fed	21.7% (10/46)	0.00
Under fed	66.2% (53/80)	
Age		
2-5 year	26.8% (11/41)	0.00
6-9 year	69.6% (32/46)	
10-13 year	60.6% (20/33)	
Parity		
1-2	34.4% (11/32)	0.00
3-4	77.8% (35/45)	
>5	39.5% (17/43)	

P<0.05 indicates significance difference.

The percentile of all isolates was higher in subclinical cases except for *E. coli*. The coagulase and hemolytic activity of *Staph. aureus* is connected to its pathogenicity. The study observed substantial pathogenic form in camel population. The coagulase positive *Staph. aureus* were 87.23% (41/47) whereas hemolytic *Staph. aureus* constituted 82.98% (39/47) of all *Staph. aureus* isolates. On isolate basis, calculating from mastitis milk samples, coagulase positive *Staph. aureus* showed 65% (41/63) while among overall camel population it was 34.1% (41/120). Similar pattern was observed for hemolytic pathogenic *Staph. aureus* in overall camel constituted 32.5%, and from mastitis milk samples reference it was 61.9% (39/63). Among hemolytic *Staph. aureus,* higher percentage noted was for alpha-beta type of hemolysis followed by alpha and beta hemolytics. It was noticed that complete and partial hemolysine producing *Staph. aureus* isolates were 58.3 and 79% greater, respectively compared to alpha hemolytic and beta hemolytic *Staph. aureus*.

Risk factors

The analysis of determinants of mastitis (Table II) showed overall significant (P<0.05) association with mastitis in this study except frequency of milking which was noted non-significant (P>0.05) for causing mastitis. Poor udder hygiene (63.3%) was noted significantly (P<0.05) associating with mastitis occurrence. The study found 57.8% of camel suffering mastitis where no teat dipping was applied, but 22.2% of camel had mastitis though teat dipping was practiced. Other risk factors for mastitis included thin body condition, under feeding, high milk yield, and dry period. Older age (>5 year) and 3rd to 4th parity number presented two times higher mastitis compared to other age and parity numbers in their respective categories.

Table III.- Antimicrobial sensitivity against *Staphylococcus aureus.*

Class of antibiotic	Name of antibiotic	Individual drug (%)		
		R	I	S
Penicillin				
Ticarcillin		100	-	-
Cloxacillin		100	-	-
Amoxicillin Calvalanic acid		70	-	30
Ampicillin		100	-	-
Amoxicillin		100	-	-
Oxacillin		100	-	-
Piperacillin Tazabactam		46	-	54
Ticarcillin Calvanic acid		100	-	-
Azlocillin		100	-	-
	Penicillin (overall)	90.67 (816/ 900*100)	-	9.33 (84/ 900*100)
Cephalosporin				
Cefixime		100	-	-
cefotaxime		16	-	84
Ceftazidine		93	-	7
Cefuroxime		100	-	-
	Cephalosporin (overall)	77.25 (309/ 400*100)	-	22.75 (91/ 400*100)
Sulphonamide				
Sulphaphenazol		7	-	93
Triple Sulphas		15	-	85
Trimethoprim		-	-	100
	Sulphonamide (overall)	7.33 (22/ 300*100)	-	92.67(278/ 300*100)
Aminoglycoside				
Gentamicine		7	-	93
Amikacin		-	23	77
Spectinomycin		85	-	15
	Aminoglycoside (overall)	30.66 (92/ 300*100)	7.67 (23/ 300*100)	61.67 (185/ 300*100)
Quinolones				
Ciprofloxacillin		-	-	100
Cinoxacin		45	-	55
	Quinolones (overall)	22.5 (45/ 200*100)	-	77.5 (155/ 200*100)
Miscellaneous				
Vancomycin	100	-	-	-
Mupirocin	100	-	-	-
Chloramphenicol	100	-	-	-
	Miscellaneous (overall)	100 (300/ 300*100)	-	-
Over all response (%age)				
		54.7	1.3	44

R, resistant; I, intermediate; S, sensitive.

Antibiotic sensitivity

Antibiotic resistance measured by disc diffusion method revealed higher range of *Staph. aureus* resistance towards various classes of antibiotics (Table III). The overall average 54.7% of *Staph. aureus* isolates were resistant to antibiotics collectively on class-wise (sum of susceptibility percentages of each class/sum of total expected percentages in each). Among different classes of antibiotics tested in this experiment, *Staph. aureus* show greater resistance (90%) to penicillin group (beta lactam) followed by Cephalosporin (77.25%). In contrast to these, Sulphonamides were 92.7% effective whereas aminoglycosides and quinolones were found 61.7% and 77.5%, respectively, efficacious against *Staph. aureus* isolates. On individual antibiotic basis 100% resistance was observed for Oxacilline, Ticarcillin, Cloxacillin,

Ampicillin, Ticarcillin calvanic acid, Azlocillin, Cefixime, Cefurosime, vancomycin, Mupirocin, and Chloramphenicol. Contrary to this, *Staph. aureus* were found 100% sensitive to Trimethoprim and Ciprofloxacin in this experiment. The sensitivity shown by *Staph. aureus* to other antibiotics included Sulphaphenazol (93%), Gentamicin (93%), Cefotaxime (84%), Triple sulpha, Amikacin (77%), Cinoxacin (55%), and Piperacillin Tazabactam combination (54%).

DISCUSSION

Prevalence of mastitis and mastitogens

Overall mastitis in the present study is in line with the finding of Sarwar (2013) reporting nearly 50% mastitis in camels from Cholistan area of Pakistan. The study finds close resemblance with findings of Ahmad *et al*. (2012) from Pakistan, Abdulkadhim (2012) from Alqadsia province of Kuwait, and Wanjohi *et al*. (2013) from northern east province of Kenya who reported 46, 43, and 60% overall mastitis in camel community. In contrast, Abera *et al*. (2010), Abdurahman (2006), and Bekele and Molla (2001) reported lower mastitis prevalence than the present study. This discrepancy might be because of different geographical area, climate, breed, and system of rearing. The higher prevalence of *Staph. aureus* among other bacterial isolates in studies conducted by Al-Dughaym and Fadlelmul (2015), Wanjohi *et al*. (2013), Hawari and Hassawai (2008), Abdurrehman (2006) and Woubit *et al*. (2001) were similar the results of current research. Ahmad *et al*. (2012) reported 42.19% *Staph. aureus* which was higher among other isolates but lower compared with that of present study. The strains variability exists that may confuse conventional techniques of identification (Arslan and Mutlu, 2016).

Risk factors

The findings of risk factors association with mastitis occurrence in camel of present study are in line with those of Husein *et al*. (2013), Ahmad *et al*. (2012), Abdurrehman (2006), Teketelwa and Bayleyegen (2001), Woubit *et al*. (2001), Sena *et al*. (2000) and Mulei (1999) except divergent pattern in age and parity. In the current study middle age (6-8 year) and middle parity (3-4th) were most vulnerable to mastitis than to early years (before 5years). This contrast might be because of higher milk production in current study during middle age and middle parity number which is predisposing factor for mastitis (Radostitis *et al*., 2007). The tick infestation was found strongly associated with mastitis. Ticks facilitate entrance of bacteria to udder, which is predilection site for mastitogens (Megersa, 2010). Under feeding, poor udder hygiene, and thin body condition allows bacteria find opportunity to play trouble, which renders animal suffering diseases. Scratches on skin are usual for camels grazing on thorny plants which favor lesion development adding to this used to be ticks involvement. In addition to this the habits of locals to tie teat by fiber with intention to avoid calf suckling promotes skin lesions (Woubit *et al*., 2001). Higher prevalence of dry animal mastitis might be because of delayed keratin plug formation at teat, dilution of lactoferrin, inhibited leukocyte, immune suppression, and no flushing action (Smith *et al*., 1985).

Antibiotic sensitivity

The higher sensitivity of aminoglycosides in the current study agreed with findings of Abdulkadhim (2012), Hawari and Hassawi (2008), Najeeb *et al*. (2013) and Sarwar (2013). The susceptibility of *Staph. aureus* against sulphonamides (sulphaphenazole) is supported by Fazlani *et al*. (2011); Rind and Sheik (2001), Methews *et al*. (1992) and Ayhan and Aydin (1991) declaring antibiotic showing high sensitivity. However, in contrast to the findings of aforementioned researchers the organisms were highly resistant to penicillin. This could be the reason of higher use of this group as it is common practice by locals to use this group in almost all general ailments. The resistant *Staph. aureus* is a common problem in cattle of developing countries which can be source of spread of resistance in other milk producing species of animals. There is contradiction with results of cefixime, cefotaxine with some studies but it is generally stated that *Staph. aureus* resistant to cefexitin are resistant to other drugs of this group with few exceptions (EUCAST, 2015). The discrimination in results with chloramphenicol and other drugs can be correlated with geopraphical area, climate, breed, system of rearing and exposure to various infections.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Abdurahman, O.A.S.H., 2006. Udder health and milk quality among camels in the Errer Valley of Eastern Ethiopia. *Livest. Res. Rural Develop*., **18**: 1-9.

Abera, M., Demie, B., Aragaw, K., Regassa, F. and Regassa, A., 2010. Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama Town, Ethiopia. *J. Vet. Med. Anim. Hlth.*, **2**: 29-34.

Abdulkadhim, M.H., 2012. Prevalence of methicillin resistance staphylococcus aureus in cattle and she-camels milk at Al-Qadisyia Province. *Al-Anbar J.*

Vet. Sci., **5**: 63-67.

Ahmad, S., Yaqoob, M., Bilal, M.Q., Muhammad, G., Yang, L.G., Khan, M.K. and Tariq, M., 2012. Risk factors associated with prevalence and major bacterial causes of mastitis in dromedary camels (*Camelus dromedarius*) under different production systems. *Trop. Anim. Hlth. Prod*., **44**: 107-112. https://doi.org/10.1007/s11250-011-9895-0

Arslan, E. and Mutlu, E.G., 2016. Genotyping of *Staphylococcus aureus* strains isolated from bovine mastitis in turkey by using ERIC-PCR method. *Pakistan J. Zool*., **48**: 1747-1752.

Ali, M., Chaudhry, M.S. and Farooq, U., 2009. Camel rearing in Cholistan desert of Pakistan. *Pakistan Vet. J*., **29**: 85-92.

Al-Dughaym, A.M. and Fadlelmula, A., 2015. Prevalence, etiology and its seasonal prevalence of clinical and subclinical camel mastitis in Saudi Arabia. *Br. J. appl. Sci. Techn*., **9**: 441-449.

Anonymous, 2015. *Economic survey of Pakistan*. Finance Division, Government of Pakistan, Economic Advisors Wing. Islamabad, Pakistan.

Ayhan, H. and Aydin, N., 1991. Adherence, bacteriocin activity and in-vitro bacterial interference of staphylococci isolated from fowl. *Doga. Turk. Vet. Hay. Derg*., **5**: 129-139.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. clin. Pathol*., **45**: 493-496.

Bekele, T. and Molla, B., 2001. Mastitis in lactating camels (*Camelus dromedarius*) in Afar Region, north-eastern Ethiopia. *Berl. Munch. Tierarztl. Wochenschr*., **114**: 169–172

Beg, O.U., Von-Bahr-Lindststrom H, Zaidi, Z.H. and Jornvall, H., 1986. Characterisation of camel milk protein rich proline identifies new beta casein fragment. *Regul. Pept.,* **15**: 55-62. https://doi.org/10.1016/0167-0115(86)90075-3

CLSI, 2015. *Performance standards for antimicrobial susceptibility testing;* Twenty-Fifth informational supplement. CLSI document M100-25. Clinical and Laboratory Standards Institute, Wayne, PA.

Dego, O. and Tareke, F., 2003. Bovine mastitis in selected areas of Southern Ethiopia. *Trop. Anim. Hlth. Prod*., **3**: 197-205. https://doi.org/10.1023/A:1023352811751

El-Agamy, E.I. and Khatab, A.A., 1992. Physicochemical and microbiological characteristics of Egyptian human milk. *Alexandria J. agric. Res*., **37**: 115-126.

Fazlani, S.A., Khan, S.A., Faraz, S. and Awan, M.S., 2011. Antimicrobial susceptibility of bacterial species identified from mastitic milk samples of camel. *Afr. J. Biotech*., **10**: 2959-2964. https://doi.org/10.5897/AJB10.716

IBM CORP, 2013. *IBM SPSS Statistics for Windows*, Version 22.0. IBM Corp., Armonk, NY.

FAO, 2001. *Production year book.* Food and agriculture Organization, Rome, Italy.

Faye, B., Bonnet P., 2012. Camel sciences and economy in the world: current situation and perspectives. Proc. 3rd ISOCARD conference. Keynote presentations. 29th January -1st February, 2012, Mascate (Sultanate of Oman), 2-15.

Faye, B., 2005. *Productivity potential of camels.* Proc. of Int. Workshop. Desertification Combat and Food Safety – The added value of camel producers. Ashkabad (Turkmenistan), 19-22 April 2004. NATO Science Series, In: Life and Behavioral Sciences, **362**: 127-134.

Hawari, A.D. and Hassawi, D.S., 2008. Mastitis in one-humped shecamels (*Camelus dromedarius*) in Jordan. *J. biol. Sci*., **8**: 958-961. https://doi.org/10.3923/jbs.2008.958.961

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T., 1994. *Bergey's Manual of determinative pacteriology*, 9th ed. Williams & Wilkins, Baltimore.

Husein, A., Haftu, B., Hunde, A. and Tesfaye, A., 2013. Prevalence of camel (*Camelus dromedaries*) mastitis in Jijiga Town, Ethiopia. *Afric. J. agric. Res*., **8**: 3113-3120.

Lafi, S.Q. and Hailat, N.Q., 1998. Bovine and ovine mastitis in Dhuleil valley of Jordan. *Vet. Arch.,* **68**: 51-57.

Leitner, G., Yadlin, B., Glickman, A., Chaffer, M., Saran, A., 2000. Systemic and local immune response of cows to intramammary infection with *Staphylococcus aureus*. *Res. Vet. Sci.*, **69**: 181-184. https://doi.org/10.1053/rvsc.2000.0409

Megersa, B., 2010. An epidemiological study of major camel diseases in the Borana Lowland, Southern Ethiopia. *Dry Lands Coordi. Group Rep.,* **58**: 32-33.

Methews, K.R., Oliver, S.P. and Jayarao, B.M., 1992. Susceptibility of *Staphylococci* and *Streptococci* isolated from bovine milk to antibiotics. *Agric. Pract*., **13**: 18-24.

Muhammad, G., Akhtar, M., Shakoor, A., Khan, M.Z., Rehman, F. and Tauseef, M., 1995. Surf field mastitis test: an inexpensive new tool for evaluation of wholesomeness of fresh milk, Pakistan. *J. Fd. Sci*., **5**: 91-93.

Mulei, M., 1999. Teat lesions their relationship to intra-

mammary infections on small scale dairy farms in Kiambu district in Kenya. *J. S. Afri. Vet. Assoc*., **70**: 156-157. https://doi.org/10.4102/jsava.v70i4.786

Najeeb, M.F., Anjum, A.A., Ahmad, M.U.D., Khan, H.M., Ali, M.A. and Sattar, M.M.K., 2013. Bacterial etiology of subclinical mastitis in dairy goats and multiple drug resistance of the isolates. *J. Anim. Pl. Sci.*, **23**: 1541-1544.

Nagy, P., Faye, B., Marko, O., Thomas, S., Wernery, U. and Juhasz. 2013. Microbiological quality and somatic cell count in bulk milk of dromedary camels (*Camelus dromedarius*): descriptive statistics, correlations and factors of variation. *J. Dairy Sci*., **96**: 5625-5640. https://doi.org/10.3168/jds.2013-6990

NMC, 1987. *National Mastitis Council. Laboratory and field handbook on bovine mastitis*. National Mastitis Council (NMC) Inc., Madison.

Radostits, O.M., Blood, D.C., Gay, C.C., and Hinchcliff, K.W., 2007. Veterinary medicine: a text book of the disease of cattle, sheep, pigs, goats, and horses. 9th eds., Saunders, London, pp. 603-700.

Rihab, A.H., Ibtisam, E.M. Zubeir, E.L. and Babiker, S.A., 2008. Chemical and microbial measurements of fermented camel milk ''Gariss'' from transhumance and nomadic herds in Sudan. *Austr. J. Basic appl. Sci*., **2**: 800-804.

Rind, R. and Shaikh, S.N., 2001. *In vitro* antibiotics susceptibility of bacterial species, identified from uteri of slaughter goats. *Pak. J. biol. Sci*., **4**: 861-865. https://doi.org/10.3923/pjbs.2001.861.865

Sena, D.S., Mal, G., Kumar, R. and Sahani, M.S., 2001. A preliminary study of prevalence of mastitis in camel. *J. appl. Anim. Res*., **20**: 27-31. https://doi.org/10.1080/09712119.2001.9706733

Scales, F.M., 1922. A new method for differential staining of bacteria. *J. Inf. Dis*., **31**: 494-498. https://doi.org/10.1093/infdis/31.5.494

Sarwar, N.U., 2013. *Clinic-bacteriological characterization of mastitis in Cholistan camel breeds in Rahim-Yar Khan, Pakistan*. M.Phil. thesis, Faculty Veterinary Sciences University. Veterinary Animal Sciences, Lahore, Pakistan.

Smith, K.L., Todhunter, D.A. and Schoenberger, P.S., 1985. Environmental mastitis: Cause, prevalence, prevention. *J. Dairy Sci*., **68**: 1531-1553. https://doi.org/10.3168/jds.S0022-0302(85)80993-0

Teketelew, B. and Bayeleyeg, M., 2001. Mastitis in lactating camels (*Camelus dromedaries*) in Afar Region, North Eastern Ethiopia. *Berlmunch. Tierztlwochenschr*., **1145**: 169-72.

Thrusfield, M., 2007. *Veterinary epidemiology.* Blackwell Science, USA, pp. 180-181.

UCAST, 2015. *European committee on antimicrobial susceptibility testing MIC distributions,* EUCAST, Basel. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf

Walstra, P., Wouters, J.T.M. and Geurts, T.J., 2006. *Dairy science and technology*. 2nd ed. CRC Press Taylor & Francis Group, Boca Raton, Fla., pp. 783.

Wanjohi, M., Gitao, C.G. and Bebora, L., 2013. Subclinical mastitis affecting hygienic quality of marketed camel milk from North-Eastern Province, Kenya. *Micr. Res. Int*., **1**: 6-15.

Woubit, S., Bayleyegn, M., Bonnet, P. and Jean-Baptiste, S., 2001. Camel (*Camelus dromedarius*) mastitis in Borena lowland pastoral area, southwestern Ethiopia. *Rev. Elev. Med. Vet. Pays Trop.*, **54**: 207-212.

Yousaf, M., 2009. *Evaluation of some non-antibiotic antibacterials in the treatment of bubaline mastitis*. PhD thesis, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.

Zagorski, O., Maman, A., Yaffe, A., Meisles, A., Creveld, V.C. and Yagil, R., 1998. Insulin in milk – a comparative study. *Int. J. Anim. Sci*., **13**: 241-244.