The Prevalence of Common Milk Borne Pathogens of Camelus Mastitis Origin and their Antibiotic Resistance in North Eastern Province, Kenya

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ABSTRACT

In most pastoral communities, camel milk is consumed raw without any heat treatment and this can pose a health hazard to consumers. Camels are milked in poor sanitary conditions, with all the predisposing factors to mastitis that include dust, flies and scarce water resources among others. Whenever mastitis is encountered in the field, antibiotic treatment is the basis of most treatment regimes. In most of the cases the treatment is given without the benefit of bacteriological examination as laboratories are non-existent in many such areas. Use of inadequate or inappropriate dosage of antibiotics may lead to generation of bacteria resistant to the commonly used antibiotics. It is therefore important to determine prevalence of the most common milk pathogens in camel milk such as *Staphylococcus* and *Streptococcus spp,* from North Eastern province marketed in urban areas, as well as the extent of their antibiotic resistance. Isolation and identification of the bacteria was done with a total of 207 milk samples that were collected in Garissa and Wajir.. The prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae* in the whole region were found to be 22.76% and 22.74%, respectively. However, the prevalence in the two districts differed with Garissa having higher percentage prevalence both for *Staphylococcus aureus* (34.95%) and *Streptococcus agalactiae* (37.79%) compared to Wajir (10.58% and 7.69%, respectively).

The antibiotic sensitivity showed that S. *aureus and S. agalactiae* were resistant to sulphamethoxazole (0.13), Co-Trimoxazole (0.25) and Ampicillin (0.30) and sensitive to Gentamicin(1.89) and Tetracycline(1.58).

Some of the recommendations for improved milk quality include provision of more veterinary personnel to arid areas to provide basic camel health services; provision of simple testing kits before pooling of camel milk; provision of cooling points and aluminum cans for transport of camel milk. It would be advisable to investigate the impact of high level of milk contamination by surveying clinical and sub-clinical infection, if any, in humans consuming camel milk and also the antibiotic resistance of the prevalent bacteria.

Key words: Camel milk, Garissa, Wajir, *Streptococcus*, *Staphylococcus*

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INTRODUCTION

Camel meat and milk are the key foods in arid and semi arid areas (ASALS) of Africa and Asian countries. The Food and Agriculture Organization (FAO) has reported that 18 million camels around the world support the survival of millions of people in ASALS (FAO, 2003). Camel milk not only contains higher amount of nutrients compared to cow milk but it has also medicinal properties (Barbour *et al.*, 1985; Elagamy *et al.*, 1992; FAO, 2003). In pastoral condition the camel milk is

mostly consumed raw without any heat treatment or varying degrees of sourness and this can pose a health hazard to consumers. Milk and milk products derived from any animal can harbor a variety of microorganisms and can be important sources of food borne pathogens. The bacterial content of freshly drawn milk is significantly increased by infectious mastitis.

Mastitis is a multifactorial disease caused under specific circumstances and the most common and significant causal pathogens responsible for economic losses are *Staphylococcus aureus, Streptococcus agalactiae* and *Escherichia coli,* which are also pathogenic to humans (Brakstad *et al*., 1992 Brooks *et al.*, 2001 and Gillespie *et al.*, 2000).

Mastitis eradication programs have been successful in dairy cattle herds and are justified economically (Radostits *et al.*, 1997; Edmondson, 1989*). S. agalactiae* and *S. aureus* have been isolated from intramammary infections (IMIs) in different camel populations (Karamy, 1990; Abdurahman *et al*., 1995; Obied and Bagadi, 1996). According to Anderson (1989), *Staphylococcus spp.* and *Streptococcus spp.* cause more than 80% of clinical mastitis. The antibiotics treatment selected based on the severity of the mastitis, history of the farm (including previous milk culture results and responses to treatment), the field experience of the farmer and the prescribing veterinarian. This may lead to development of bacteria resistant to antibiotics. Azmi *et al*., (2008) in their study in Jordanian camels found mastitis organisms including *Staphylococcus spp*.. and *Streptococcus spp.* greatly resistant to Penicillin and Streptomycin which were the common drugs for domestic animals. There is limited information on the rate that bacteria are developing resistance to antibiotics commonly used to treat mastitis in camel population. Determination of prevalence of *S.. agalactiae* and *S. aureus* in camel milk in Kenya and their antibiotic resistance will help to advise the pastoralists on management of mastitis in order to curb the incidences of the disease.

MATERIALS AND METHODOLOGY

Sampling

Milk samples were drawn from individual producers in five litre containers ready for sale in markets. Milk sampling was done in the morning. Samples of 20 – 50 ml of milk were drawn from the container using a clean cup, into a sterile tube for bacteriological analysis. The milk samples were transported in ice-box to the laboratory and stored at 4 º C for analysis. One calibrated loop of milk was streaked on nutrient media slants for transportation to Nairobi.

Sampling frame

The study was a cross-sectional survey of potential public health hazards and associated health risks with an aim of establishing the prevalence of selected hazards in camel milk. The sampling frame was constructed with the assistance of key stakeholders. The primary marketing agents in Wajir were from Griftu, Khorl-Haral, Tarbaj and Wajir-Bor. In Garissa district Karokora, Kulan and Damajale were the sampling site. 104 camel milk samples were collected from Garissa and 103 from Wajir to make a total of 207.

Bacteriological analysis

The bacteria grown on nutrient media slants were streaked out on Blood Agar (BA; Oxoid No. CM 271) containing 5% defibrinated sheep blood and on Mannitol Salt Agar (MSA; Oxoid No. CM 85). The BA and MSA cultures were examined after overnight incubation at 37 °C and re-incubated for another 24 hours if there was no growth. Type of haemolysis colony morphology, Gram stain (3.7.1) and catalase reaction (3.7.2) were recorded. Catalase-positive, Gram-positive cocci were tested for coagulase reaction (Oxoid Staphylase Test No. DR 596, DR 597, DR 500) (3.7.3) Catalase negative

Gram- positive cocci were subjected to CAMP TEST (Christie, Atkins, Munch-Petersen, 1944) (3.7.4).

All isolates referred to as *S. agalactiae* (Lancefield group B) produced a characteristically shaped (arrow head) clear zone of haemolysis in the cloudy zone of haemolysis due to *Staphylococcus* beta toxin on BA. All isolates referred to as *S. aureus* reacted positively in the Oxoid Staphylase test and negative with the negative control and showed beta – haemolysis on BA. The Gram stain, CAMP test and catalase test were performed according to Forbes et al (2002).

Antibiotic-Sensitivity Test

The confirmed *S.. agalactiae* and *S. aureus* isolates were subjected to agar diffusion sensitivity test using Nutrient Agar (Oxoid No. CM 3) with 5% defribrinate sheep blood for *S. agalactiae.* The antibiotic test discs used were: Ampicillin 25µg, Tetracycline 100µg, Nitrofurantoin 200µg, Nalidixic acid 30µg., Streptomycin 25µg, Sulphamethoxazole 200µg, Co-Trimoxazole 25µg and Gentamicin 10 µg.

RESULTS

Prevalence of *S. aureus* and *S. agalactiae*

Out of 207 milk samples examined, 49 (24%) were contaminated with *S. aureus* while 48 (23%) with *S. agalactiae.* Table 3 shows the prevalence of the two organisms in Garissa and Wajir districts. The incidence of *S. aureus* in Garissa was almost three and half times more (34.95%) than in Wajir (10.58%). *S. agalactiae* prevalence in Garissa was even higher with 37.79% which is about five times that of Wajir (7.59%). On average the prevalence of *S. aureus* and *S. agalactiae* is 22.77% and 22.74% in camel milk in the two districts, respectively. Only 23 (22.33%) of camel milk samples were

contaminated by the two organisms in Garissa district. It can be assumed from the result that the origin of the two organisms is both endogenous and exogenous.

Table 1: Prevalence of S. aureus and St. agalactiae in Percentage

District	Mastitis organisms status				Prevalence of both the two organisms in same sample	
	Staph. aureus		*Strept. agalactiae*		*Staph. aureus* and *Strept. agalactiae*	
	Absent (%)	Present (%)	Absent (%)	Present (%)	Absent (%)	Present (%)
Garissa	65.05	34.95	62.13	37.79	77.67	22.33
Wajir	89.42	10.58	92.31	7.69	0.00	0.00
Mean	77.24	22.77	77.22	22.74	38.83	11.17

Antibiotic Resistance of *S. aureus* and *S. agalactiae*

The susceptibility or resistance of each organism was determined by measuring the diameter of the zone of inhibition. The highest diameter was 2.4 cm. From Table 4 it is indicated that *S. aureus* is resistant to Ampicillin (0.53 cm) and Co-Trimoxazole (0.40 cm), Nalidixic acid (0.10 cm), Sulphamethoxazole (0.22 cm).The organism is susceptible to Gentamicin (1.90 cm) and Tetracycline (1.70 cm). Nitrofurantoin (1.05 cm) and Streptomycin (1.18 cm) are not very effective. *S. agalactiae* is more resistant to Ampicillin (0.06 cm), Co-Trimazole (0.09 cm), Nitrofurantoin (0.58 cm), Streptomycin (0.56 cm) and Sulphamethoxazole (0.04 cm). The organism is inhibited by Gentamicin (1.87 cm) and Tetracycline (1.45 cm)..

Table 2: Mean Zone of Inhibition in Centimetres for Each Antibiotic on Each Organism

Micro-organisms	Antibiotic								Micro-organism Mean
	Ampicilin	Co-Trimoxazole	Gentamicin	Nalidixic acid	Nitrofurantoin	Streptomycin	Sulphamethoxazole	Tetracycline	
S. aureus	0.53	0.40	1.90	0.10	1.05	1.18	0.22	1.70	0.88
S.agalactiae	0.06	0.09	1.87	0.92	0.58	0.56	0.04	1.45	0.70
Antibiotic Mean	0.30	0.25	1.89	0.50	0.82	0.88	0.13	1.58	0.79

Table 3: Analysis of Variance Tables

Source of variation	Df	Ss	Ms	Vr	Fpr
Microorganisms	1	0.6937	0.6937	0.92	0.343
Residual	54	40.8835	0.7571	1.99	
Microorganisms	1	5.6257	5.6257	14.76	<0.001
Antibiotics	7	224. 5852	32.0836	84.20	<0.001
Microorganism. Antibiotics	7	27.1534	3.8791	10.18	<0.001
Residual	553	210.7100	0.3810		
Total	**623**	**509.6515**			

Where: Df = degree of freedom, Ss = sum of squares, Ms = mean square, Vr = variance ratio and Fpr = level of significant

Table 4: Least Significant Differences of Means

	Microorganisms	Antibiotic	Microorganism antibiotic
			0.2913 min. rep
Lsd	0.1293	0.1942	0.2876 max-min
			0.2839 max.rep

The ANOVA table 4 indicates that the way in which antibiotic is clearing the organism is different among the microbes. There is a high significant difference between the different antibiotics to each organism. By comparing the difference between the means with the Lsd value, five had values greater than the Lsd (0.1293) as in Table 4 meaning they had a significant effect on the organisms. Co-Trimoxazole (0.12) and Ampicillin (0.17) have values less than the lsd hence had no significant effect.

DISCUSION

Prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae* in Camel Milk

Garissa

In this study *S. aureus* and *S. agalactiae* prevalence in Garissa had values of 34.95% and 37.79% respectively, Table 1. A study by Abdurahman (2006) in the Errer Valley of Eastern Ethiopia indicated a prevalence of 12.7% for *S. aureus* while 8.8% for *S. agalactiae*. IMIs udder – prevalence were 12% for *S. agalactiae* and 11% for *S. aureus* in six Kenyan camel herds (Younan *et al.*, 2001). In two other studies on Sudanese camels, *S. agalactiae* were isolated from 26.7% of composite

udder-milk samples (Obied and Bagadi, 1996) and from 17.6% of quarter-milk samples (Abdurahman *et al.,* 1995) while *S. aureus* were isolated from 17% (udder milk sample) and 5.4% (quarter milk samples). The results of composite udder-milk samples are close to the findings in Garissa. The results are also in agreement with those of Younan (2001) which showed a higher percentage of multiple quarter infections for *S. agalactiae* infected udders (44%) compared to udders infected with *S. aureus* (23%). The incidence of the two organisms existing together in the same sample was found from this study to be 22.33%, Table 1. There is currently no data to compare this value with.

Muli *et al.,* (2008), from their survey, found that small scale camel milk producers who do not wash the camel udders before milking usually contaminated the milk with dirt, the camel's urine and other debris. In this study all camel milkers stated that they do not use water for milking. In many cases, use of little scarce water or dirty water may be a source of spread of microorganism.

Garissa being more active in business compared to Wajir, pooling and exchange of the camel milk in many hands could be a contributing factor to the high figures. Pooling of different camel milk batches along the collection and marketing chain can result in increased prevalence of *S. agalactiae.* Abduraham and Younan, (2004) found *S. agalactiae* in 50% of milk transport containers coming from producing herds, 62% of milk containers sampled at primary collection sites, 70% of milk containers sampled from an urban market of the same region and 89% of raw milk baths received at a dairy processing plant. These findings indicate a very widespread occurrence of the pathogens in milk producing camel herds and in the milk collection and distribution system. From the results, Garissa district showed higher values especially for *S. agalactiae* indicating there is actually pooling of milk. The same study showed that, farmers with camel herds of up to 500 will have only up to 35% lactating camels and with the general low yield per camel, even the largest farmers will have milk production of not more than 500 litres per day at the best of times. The majority of farmers, with an average of 20 lactating camels are able to produce only up to 10 litres of milk per day. None

of these levels of production are large enough for farmers to operate on their own. It is therefore essential that milk from different farmers is assembled together for economies of scale in transportation and market access.

According to a study by Dargent *et al.*, (1988), high *S. aureus* prevalence generally increased with increasing herd-size among herds infected with *S. agalactiae*. There was no consistent pattern within market or district category, but a high prevalence of *S. aureus* and *S. agalactiae* is more likely in a wider market. The current study cannot explain the variations in results, however poor management and unhygienic milking practices prevalent in the traditional husbandry systems including tying the teats with soft tree barks to prevent the calf from suckling, tick infestations and cauterization of the udder skin (Abdurrahman *et al.*, 1995, Obeid *et al.*, 1996 and Woubit *et al.*, 2001) seem to be common in Garissa. Although there is no data, from the researcher's observation, pastoralists in this region have poor hygienic practices.

The Garissa market serves a wide market, including Nairobi, from where some milk is forwarded to other traders, particularly Kakuma refugee camp, Kisumu and Kampala. A number of wholesale traders also have occasional buyers who take up to 10 litres every time they are traveling abroad (especially Turkey). The high levels of *S. aureus* and *S. agalactiae* in the "highly esteemed" camel milk, potentially reach and expose a wide and diverse market beyond the Garissa market to pathogens. From this study 28% (92 383) of Garissa population takes raw camel milk contaminated, with *Staph. aureus* (34.95%) and *S. agalactiae* (37.79%), Table1. The milk is consumed daily (75%) and by all members of the family (75%). These values are worrying to any one who understands the harm the pathogens can cause to the people in the district thus serious measures should be taken.

Wajir

Wajir had lower prevalence for both *Staph. aureus* (10.58%) and *Strept. agalactiae* (7.69%), Table 1. These values look impressively small in the eyes of camel milk handlers from this district; however, Kenya Bureau of Standards indicates that camel milk for human consumption should have "Nil

value" for the two organisms. *S. aureus* has been ranked as the most frequent (Karamy, 1990; Al-Ani and Al-shareeti; 1994) or second most-frequent (Barbour *et al*., 1985; Obied and Bagadi, 1996) microorganism involved in intramammary infection in camels. This was reflected in the results from Wajir, (Table 1). Other studies (Benkerroum *et al.*, 2003; Semereab and Molla, 2001; Buyser, 2001 and El-ziney and Al-Turki, 2007) have indicated *S. aureus* as the most frequent pathogen associated with many disease outbreaks.

S. aureus and *S. agalactiae* are common in the environment, their presence in the bulk milk is often attributed to poor milking time, and unhygienic practices in milking. Generally it was not determined if the pathogens originated from the camel or the environment. This would have been possible by comparing the strains found in the milk. The variation in results from Garissa and Wajir is a clear indication that environmental contamination may play a great role. The mean prevalence in both districts for *S. aureus* was 22.77% while for *S. agalactiae* was 22.74%, Table 1. These are almost twice the finding of Younan *et al.*, 2001 in Kenya. According to their study, intramammary infection udder prevalence was 12% for *S. agalactiae* and 11% for *S. aureus*. Since their research was based on milk from camel herds, it is expected that the percent prevalence be lower than what is expected from bulk or pooled milk.

Camels affected by mastitis are not treated in traditionally managed camels and will often take a natural course to chronicity resulting in a permanent loss of milk production (Obied *et al.*, 1996). The potential danger of this is that before the loss, the pathogenic mastitis organism will have caused much damage to the humans, including hospitalization and even death of many individuals. Neonatal mortality is primarily due to diarrhea following failure of passive transfer and exposure to *Escherichia coli*, rotavirus, Corona virus, Coccidia and Salmonella. Now that this milk is becoming popular, the problem of mastitis in camels should not be looked at single handedly as affecting not only pastoralists but the whole public.

Antibiotic Sensitivity

The antibiotic sensitivity screening showed that *S. aureus* and *St. agalactiae* were resistant to Sulphamethoxazole (0.13), Co-Trimoxazole (0.25) and Ampicillin (0.30) and sensitive to Gentamicin (1.89) and Tetracycline (1.58), Table 2. The latter two antibiotics had a wider zone of clearance on the organisms growing on the blood agar. A study by Mody *et al*., (1998) showed that all mastitis bacterial isolates were resistant to Nitrofurantoin, Furazolidone and Penicillin. Antimicrobial drugs against which the bacterial isolates showed good sensitivity were Gentamicin and Chlorophenicol. Cotrimoxazole, Sulphamethoxazole and Streptomycin were also found to be highly effective against some bacterial isolates. Antimicrobial drugs against which bacterial isolates showed moderate susceptibility were Oxytetracycline, Co-trimoxazole and Chlorophenicol. In yet another study by Azmi *et al*., (2008), Gentamicin, Ampicillin and Tetracycline were the most effective drugs against mastitis bacterial isolates. The bacteria flora showed greatest resistance to Penicillin and Streptomycin. These two drugs were said to be the most commonly used for domestic animals in Jordan.

According to the study done in Kenya, by Younan *et al*., (2000), 56% of the tested *S. agalactiae* and 50% of the tested *S. aureus* isolates were fully sensitive to Tetracycline. Nitrofurantoin and Streptomycin affected the two organisms differently. *S. aureus* is sensitive to the latter named antibiotics with a mean zone clear of 1.05 cm and 1.18 cm respectively while *S. agalactiae* is less sensitive.

There is limited information on the rate at which bacteria are developing resistance to antibiotics commonly used to treat infections in camels. The likelihood of antibiotics resistance developing broadly depends on the: prevalence of resistant bacteria in the animal population, frequency of antibiotic use and type of exposure to the antibiotic e.g. short treatment courses of high doses of antibiotic confer less selective pressure than long term exposure to low doses of antibiotic (Guterbock *et al* .1993 and Hallberg *et a*l., 1994). Myllys *et al.*, (1998) reported an increase of 27%

in the proportion of *S. aureus* strains resistant to at least one antibiotic (mostly due to strains capable of producing beta lactamase). There is currently no substantial data set in Kenya that enables comparison of these findings with what is happening in Garissa and Wajir camel population.

The population of mastitis bacteria changes over time. This is true for the occurrence of bacteria species and for the occurrence of antimicrobial resistance within bacterial species. According to a study done in 1985, and 2000 in New York Dairy Herds by Linda *et al.*, 2004, there was a significant decrease in the susceptibility of *Streptococcus Spp* to Ampicillin, Cloxacilin, Penicillin, Erythromycin, Pirlimycin and Tetracycline. The trend for *S. aureus* was very different than the trend for *Strepococcus. Spp.* Where *Streptococcus Spp* showed a decrease in susceptibility, *S. aureus* showed significant increase in susceptibility to both Ampicillin and Penicillin. Susceptibility to Amoxicillin (94%) and Cephalothin (98%) remained stable. Susceptibility to Erythromycin, Eirlimycin and Tetracycline did not change significantly over time either. In 1975, Davidson performed a similar analysis that covered 10 years, on antimicrobial resistance (Davidson, 1980). The contrast between the 1979 and the 1999 data, a 20 year span, were striking. Davidson found that 95% of *Streptococcus Spp* tests were susceptible to Ampicillin while Linda *et al.*, data indicated as little as 26% susceptibility of *Streptococcus Spp* in 1999. By contrast only 49% of *Staphylococcus aureus* isolates tested in 1975 were susceptible to Ampicillin, in 1999, the overall susceptibility to Ampicillin was 79%. These changes in susceptibility reflected changes in mastitis treatment used over the years. The data suggests that long term trends in antimicrobial resistance may be different from trends measured over a limited number of years.

CONCLUSION

The prevalence for *S. aureus* was found to be 22.76% and 22.74% for *S agalactiae.* The two districts however differed with Garissa having high percent incidence both for *S. aureus* (34.95%) and *S.*

agalactiae (37.79%). Wajir, on the other hand, had low prevalence of 10.58% and 7.69% respectively. It was only Garissa district which had samples (22.33%) contaminated by the two pathogenic organisms.

S. aureus was resistant to Ampicillin (0.53 cm), Co-Trimoxazole (0.40 cm) Sulphamethoxazole (0.22 cm) and Nalidixic acid (0.10 cm). *St. agalactiae* was even more resistant to the above antibiotics except Nalidixic acid, with zones of inhibition being, 0.05 cm, 0.09 cm, 0.04 cm and 0.92 cm respectively.

From this study, the prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae* differed in Garissa and Wajir, there is need to find out the causes of those variations. It would be very useful to investigate the impact of these levels of milk contamination on human health by surveying clinical and sub clinical infection in humans consuming camel milk Health intervention strategies need to be put in place in Garissa market since it serves a wide and diverse market including Nairobi, Kakuma refugee camp, Kisumu, Kampala and other countries.The trend in *Staphylococcus aureus* and *Streptococcus agalactiae* susceptibility to antibiotics in camels over time needs to be studied. This is necessary in order to come up with an effective program for fighting mastitis in camels. The Government should set up a research organization for camels; this will take care of many problems in the camel milk industry. There is need to harmonize camel milk prices. Camels are animals which can survive in very harsh areas and about two thirds of Kenyan land is either semi-arid or arid. This can be utilized in rearing camels that can go for even a month without water, for abundant milk production.

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