

High Prevalence of Subclinical Mastitis and Multidrug Resistant *Staphylococcus aureus* Are a Threat to Dairy Cattle Production in Kiboga District (Uganda)

Keneth Iceland Kasozi^{1,2}, John Bosco Tingiira², Patrick Vudriko^{1*}

¹Central Diagnostic Laboratory, Department of Pharmacy, Clinics and Comparative Medicine, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda

²Kiboga District Regional Veterinary Laboratory, Department of Production and Marketing, Kiboga District Local Government, Kiboga, Uganda

Email: *vpato@vetmed.mak.ac.ug, vpato2009@gmail.com

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Abstract

Subclinical mastitis (SCM) is one of the major factors affecting the productivity of dairy cattle all over the world. This study established the burden of SCM and determined the potent antibacterial formulation for control of *Staphylococcus aureus* (SA) related SCM in selected dairy cattle farms in Kiboga district. A total of 124 dairy cattle from 12 farms were screened for SCM using California Mastitis Test (CMT) from Kiboga Town-Council, Kapeke and Lwamata sub-counties. The offending bacteria were cultured and the antibiogram of SA was carried out using antibacterial susceptibility by the modified Kirby-Bauer disc diffusion method. Additional qualitative data on the factors that predispose cows to SCM was obtained through questionnaires and observation of milking practice. The prevalence of SCM in the three sub-counties was 87.9%. Over 70% of the dairy cattle screened for SCM had more than 2 udder quarters affected. The majority (90%) of SCM was caused by mixed bacterial infections: Coagulase negative staphylococci (64.4%) and SA (16.6%) being the most prevalent. All the farmers (100%) lacked knowledge on SCM, udder towels, teat dipping and drug cow therapy. Overall, 71.4% of SA isolated was multi-drug resistant. There was a high level of resistance against penicillin (100%), neomycin (85.7%) and tetracycline (71.4%). In contrast, all the above isolates were susceptible to Trimethoprim-Sulphamethazole. In conclusion, the high burden of SCM and emergence of multidrug resistant SA are one of the constraints to dairy production in Kiboga district. Therefore, sensitization of dairy farmers in Kiboga district on proper hygienic, appropriate milking techniques and dry cow therapy using potentiated sulfonamide in-

*Corresponding author.

tra-mammary preparations are highly recommended in SA associated SCM.

Keywords

Antibacterial Susceptibility, Dairy Cattle, Subclinical Mastitis, *Staphylococcus aureus*

1. Introduction

Subclinical mastitis (SCM) continues to be a silent nightmare to the economic propensity of the dairy sector in Uganda. In fact, 75% of economic losses of mastitis is caused by SCM [1] [2], thus contributing to the poor performance associated with the dairy sector in Uganda. As the disease takes toll on the dairy industry in Uganda, early detection and management remains elusive due to lack of diagnostic services at local government levels. This has been accentuated by the weak extension services and poor animal health seeking behavior of farmers. Moreover, recent studies [1] [3] have revealed that dairy farmers lacked knowledge on the ideal milking techniques, milking hygiene and SCM. The major contagious mastitis causative pathogens are *Staphylococcus aureus* (SA) and *Streptococcus agalactiae* (SAG), while the minor contagious pathogen is *Corynebacterium bovis* (CB) [1]. The major environmental mastitis causative pathogens are other Streptococci (OS) and Coliforms (CO), while Coagulase negative Staphylococci (CNS) are minor environmental pathogens [1] [4] [5].

SA is the fourth most common subclinical mastitis pathogen in Uganda [6]. SA is considered one of the most problematic mastitis pathogens due to its strong virulence factors [4]. Its α -haemolysis virulence factor was reported [2] to cause fatal gangrenous mastitis. The infection due to SA is easily spread within a herd, and cows get infected primarily during the milking process. The most commonly isolated CNS species in SCM are *S. epidermidis*, *S. simulans*, *S. chomogenes*, *S. xylosus* and *S. haemolyticus*; they are generally considered less virulent than SA thus causing milder forms of SCM [4]. Therefore, udder tissue damage due to CNS is limited, hence the prognosis is fairly good. Most CNS mastitis can be prevented through good milking procedures, accurate management measures and overall hygiene [1]. SCM caused by CNS can be locally treated with antibiotics during the period. However, of concern is the increasing prevalence of β -lactamase-producing CNS [2] which are resistant to penicillins. Although SA is often sensitive to penicillin, such treatment may be unsuccessful due to the bacteria's ability to hide deep in the udder tissue, thus leading to chronic SCM [1] [4].

Dairy cattle with chronic infection or with penicillin resistant bacteria should be eliminated to avoid new infections. Various antibiotics-based intra-mammary infusions for both dry-cow therapy and treatment of clinical mastitis are available on the Ugandan market. However, the increasing emergence of antibiotic resistant pathogens in the country [2] [7] [8] is further suspected to complicate the effectiveness of mastitis treatment. The treatment of clinical mastitis by farmers using antibacterial drugs without seeking prescription was reported [2] [7] as predisposing factor to the emergence of drug resistance and treatment failures. Therefore, this study established the prevalence of SCM and determined the potent antibacterial formulation for possible control of SA related SCM in selected dairy cattle farms in Kiboga district.

2. Materials and Method

2.1. Study Design

This was a basic cross-sectional study that was carried out between August and November 2012 to determine the prevalence, causes and choice of antibacterial drug for effective control of SA related SCM in Kiboga district. The study area included three randomly selected sub-counties of Kapeke, Lwamata, and Kiboga Town-Council.

2.2. Sample Size Determination

The sample size for this study was determined using the formula by Martinez *et al.*, [9] at a previous prevalence of sub-clinical mastitis of 86.2% at dairy cattle level [5]. Furthermore, 5% Error at 95% confidence was considered for the calculation. The list of dairy farms in each sub-county was obtained from the District Veterinary Of-

fice and used as a sampling frame for random selection of farms for sampling. A total of 12 farms were chosen with 2 from Kapeke, 7 from the Town Council, and 3 from Lwamata. The selection criteria for these farms were based on herd structure, district veterinary records and accessibility as advised by the District Veterinary Office. All lactating dairy cattle in the selected herd that had no history of clinical mastitis were eligible for selection. However, lactating cattle that were on antibiotic therapy in the last one month prior to the study were excluded.

2.3. Selection of Cows and SCM Screening Using CMT

In each herd, the number of lactating dairy cattle that were sampled was determined by the herd size. A preliminary visit was made to the farms within the sub-counties of Kiboga district to ascertain the average herd size for small, medium and large herd categories in the area. Individual herds of dairy cattle were selected randomly and the entire herd was selected if it met the inclusion criteria. The cattle were restrained in a crush to avoid injury whenever found available on the farms. Each teat and its orifice were thoroughly cleaned with cotton wool soaked in 70% ethanol starting with teats that were furthest. Using a gloved hand, approximately 2 ml of milk from each quarter (starting with the furthest teats) were squirted in corresponding quadrants of the CMT paddle. An equivalent volume (2 ml) of CMT reagent was then added to each quadrant and mixed gently. The results were then interpreted qualitatively depending on the thickness of the gel formed [10].

2.4. Collection of Milk Samples for Microbial Analysis

All dairy cattle that were lactating were included in the study after analyzing their individual clinical history against the inclusion criteria. Milk samples were collected from all the quarters for bacterial culture and isolation. The teats and the orifices of the quarters were cleaned again using cotton wool soaked in 70% ethanol as described above. Approximately 2 ml of milk was stripped using clean gloved hands into sterile sample vial from the affected quarter(s) to make a composite sample. Each sample was labeled and the information regarding the cow was entered in the data sheet. The sample was immediately transferred into a cool box maintained at 5°C with ice packs and then taken to Kiboga District Veterinary Laboratory for bacterial analysis.

2.5. Bacterial Analysis

2.5.1. Microbial Culture of Milk Samples

Bacterial culture and isolation was done according to methods by Mornica [11] with minor modifications [1]. Briefly, 50 µl of composite milk was inoculated on 5% sheep blood agar medium and incubated for 18 - 20 hrs at 37°C. Identification of the bacteria was carried out basing on colony characteristics such as size, shape, color and hemolysis patterns [1].

2.5.2. Antimicrobial Susceptibility Test for SA

This was done using Mueller-Hinton agar (MHA) according to Kirby-Bauer disc diffusion method [12]. The SA inoculum for each dairy farm was prepared as follows; one SA colony was mixed with 100 µl of distilled sterile water in sterile tubes. The mixture (inoculant) was applied on MHA plates and surface spread using a sterile spreader. Antibiotic discs were then applied using sterile forceps. The zones of inhibition were measured using a ruler and Oxford mathematical set divider, and the resultant diameters compared to the European Committee on Antimicrobial Susceptibility Testing [13] reference values for the different antimicrobial agents.

2.6. Questionnaire and Observations of Farm Practice

An open ended questionnaire was used to determine the hygiene status, occurrence of clinical mastitis as well as antibiotic use practices on the farm. The key lead questions were on milking practices, udder hygiene, personnel hygiene, knowledge on mastitis prevention and therapy. Observation of hygiene and milking practices were made for purposes of validation of the farmers' responses.

2.7. Data Analysis and Presentation

The data was analyzed descriptively using SPSS Version 16 software and presented as frequency tables.

3. Results

3.1. Demographics

A total of 124 dairy cattle were screened for SCM from three sub-counties in Kiboga district. The majority (40%) of the dairy cattle sampled were from Lwamata while the rest were from Kapeke (30%) and Town Council (30%). Majority (70%) of the farms had over 20 heads of dairy cattle in both Lwamata and Kapeke. However, the Town Council was dominated by only small-holder dairy farmers who owned less than 10 heads of cattle. The overall proportion of lactating dairy cattle in the herd for the three sub-counties was 40%.

3.2. Prevalence of SCM

The prevalence of SCM for the three sub-counties ranged from 80% to 94.59%. Kapeke sub-county had the highest burden of SCM at a prevalence of 94.59% followed by Town Council with 91.89%; Lwamata recorded a prevalence of 80%. The district prevalence was found to be at 87.9% (Table 1).

3.3. Number of Udder Quarters Affected by SCM

Over 70% of the dairy cattle screened for SCM had more than 2 quarters affected. Majority (54.8%) of them had all the 4 quarters diagnosed with SCM while 21.0% of the cows had 3 quarters affected. However, 6.5% and 13.7% of the dairy cattle tested had SCM in 1 and 2 quarters of the udder respectively. Only 4% of all the dairy cattle screened tested negative for SCM (Table 2).

Table 1. Distribution of dairy cattle sampled for SCM across the study area.

Sub-county	No. of dairy cattle screened	No. of dairy cattle with SCM	% of dairy cattle SCM
Kapeke	37	35	94.59
Town council	37	34	91.89
Lwamata	50	40	80
Total	124	109	87.9% ^a

Key:^a = Overall prevalence for the district; No. = Number.

Table 2. Number of udder quarters affected by SCM in the different dairy farms.

Location	Farm	1 Qtr	2 Qtrs	3 Qtrs	4 Qtrs	C.Qtrs	N
Kapeke	F1	0	1	3	13	0	17
	F2	0	3	8	9	0	20
	F3	0	0	0	3	0	3
	F4	0	0	1	4	0	5
	F5	1	4	1	2	0	8
Town Council	F6	0	2	1	1	0	4
	F7	0	0	1	3	0	4
	F8	0	0	1	3	0	4
	F12	1	1	2	4	1	9
Lwamata	F9	4	1	7	16	2	30
	F10	0	1	1	5	1	8
	F11	2	4	0	5	1	12
Total		8	17	26	68	5	124
Percentage		6.5	13.7	21.0	54.8	4.0	100

Key: F₁-F₁₂ = farms; 1 Qtr-Dairy cattle in which one quarter of the udder had SCM, 2 Qtrs-Dairy cattle in which two quarters of the udder had SCM, 3 Qtrs-Dairy cattle in which three quarters of the udder had SCM, 4 Qtrs-Dairy cattle in which all the Four quarters had SCM and C. Qtrs-Dairy cattle in which all the 4 quarters had no SCM, N is the sample size from individual farms sampled.

3.4. Degree of Severity of SCM Based on CMT Scores

Over 60% of the cows tested had CMT scores ranging from 1 to 2 in the milk. However, 16.13% of the dairy cattle had more severe SCM as evidenced by scores of 3 in the milk as shown below (**Table 3**).

3.5. Bacterial Pathogens Isolated from Milk in the Different Dairy Farms Visited

The most prevalent bacterial pathogens associated with SCM were Staphylococci such as Coagulase negative Staphylococci (64.42%) and SA (16.56%). Streptococci species were below 10%; SAG at 3.68% and 5.52% for the other Streptococci. However, other bacterial pathogens isolated included CB (6.13%), and CO (3.68%) as shown in the **Table 4**.

3.6. Susceptibility of SA against Common Antibacterial Drugs Used for Treatment of Mastitis

Majority (71.4%) of SA isolated were resistant to at least four antibacterial drugs. The prevalence of drug resistance was over 70% in the different farms in the Town Council compared to those in Kapeke and Lwamata in which resistance ranged from 42.6% to 57.1%. The SA isolated from all the 7 farms were highly resistant to Penicillin (100%) followed by Neomycin (85.7%) and tetracycline (71.4%). Resistance to Streptomycin and Gentamicin were 42.6% and 28.6% respectively. Trimethoprim-Sulphamethoxazole (Sulfamethoxazole), was potent and no resistance was recorded in SA isolated from the 7 farms in Kiboga district (**Table 5**).

3.7. Hygiene Status, Milking Practices and Management of Mastitis on the Dairy Farms

The factors that could have contributed to high prevalence of SCM included; lack of knowledge on SCM (100%), cleaning towels (100%), teat dipping (100%), dry cow therapy (100%) and teat pulling (100%) during milking as well as no usage of disinfectant (100%). Over 50% of the farmers had poor farm hygiene and visible dirt on the udder of lactating cows. Antimastitic formulations containing penicillins (60%), tetracycline (30%) sulfonamides (5%) and aminoglycoside (5%) were used for treatment of clinical mastitis. Unfortunately, 68% of the treatment was done by the farmers and herdsman while only 32% was done by veterinarians (**Table 6**).

Table 3. Severity of subclinical mastitis in the various farms based on CMT scores.

Location	Farm	CMT score				N
		0	1	2	3	
Kapeke	F ₁	7	26	12	23	68
	F ₂	16	27	22	15	80
	F ₃	0	4	4	4	12
	F ₄	1	8	6	5	20
	F ₅	13	11	3	5	32
Town council	F ₆	5	9	2	0	16
	F ₇	1	8	6	1	16
	F ₈	1	8	3	4	16
	F ₁₂	10	11	9	6	36
Lwamata	F ₉	29	46	35	10	120
	F ₁₀	7	17	5	3	32
	F ₁₁	17	17	10	4	48
Total		107	192	117	80	496
Percentage		21.57	38.71	23.59	16.13	100

Key: F₁-F₁₂-farms, 0-negative, no thickening, 1-distinct thickening, no gel formation; 2-distinct thickening, slight gel formation; 3-gel is formed; N is the sample size.

Table 4. Prevalence of bacteria isolated from milk in the different dairy farms.

Farm (F)	SA	CNS	SAG	OS	CB	CO
F ₁	5	12	2	3	0	3
F ₂	11	18	3	2	0	1
F ₃	0	3	0	0	0	1
F ₄	2	5	0	1	0	0
F ₅	1	8	0	0	0	0
F ₆	0	4	0	0	0	0
F ₇	1	4	0	0	0	0
F ₈	0	4	0	0	0	0
F ₉	6	20	0	2	10	1
F ₁₀	1	8	1	1	0	0
F ₁₁	0	12	0	0	0	0
F ₁₂	0	7	0	0	0	0
Total:	27	105	6	9	10	6
Prev.	16.56	64.42	3.68	5.52	6.13	3.68

Key: F₁-F₁₂-farms; SA = *Staphylococcus aureus*, CNS = Coagulase negative staphylococcus; SAG = *Streptococcus agalactiae*, OS = Other Streptococcus, CB = *Corynebacterium bovis*; CO = Coliforms; Prev. = prevalence; total number of bacterial isolates = 163.

Table 5. Susceptibility of *Staphylococcus aureus* against common antibacterial drugs used for treatment of mastitis in Uganda.

Location	Farm	PG10C	T30C	G10C	CX5C	SXT25	N10C	S10C
Kapeke	F ₁	R	R	S	R	S	S	S
	F ₂	R	R	S	R	S	R	S
Town Council	F ₄	R	R	R	R	S	R	R
	F ₅	R	R	S	R	S	R	R
	F ₇	R	R	R	R	S	R	S
Lwamata	F ₉	R	S	S	R	S	R	S
	F ₁₀	R	S	S	R	S	R	R
	%RD	100	71.4	28.6	100	0	85.7	42.6

Key: F₁-F₁₀ = farm; R = resistant, S = susceptible; PG10C = penicillin G; T30C = tetracycline; G10C = gentamicin; CX5C = cloxacillin; SXT25 = trimethoprim-sulphamethazole; N10C = neomycin; S10C = streptomycin; % RD = Resistance per drug basis.

4. Discussion

The current prevalence of SCM in Kiboga district (87.9%) was higher than what has been previously reported. Byarugaba *et al.* [6] found a prevalence of 61.3% in Jinja district. In a related study, Abrahmsén [5] reported prevalence of SCM of 86.2% among dairy cattle reared in urban and peri-urban areas of Kampala. Byarugaba *et al.* [6], carried out the study on mastitis on dairy farms that were predominantly managed under zero grazing system unlike the current study where cattle were kept under extensive system. Within Kiboga dis-

Table 6. Hygiene status, milking practices and management of mastitis on the farms.

Variable	Frequency of response by dairy farmers				
	Scale/action	Kapeke	Town Council	Rwamatta	
Hygiene status	Cleanliness of resting area	Good	0	2	1
		Fair	0	2	0
		Poor	2	3	2
	Personal hygiene	Good	1	4	1
		Fair	1	0	0
		Poor	0	3	2
Udder hygiene	Dirty	1	4	2	
	Relatively clean	1	3	1	
Milking practice	Use of cleaning towel		0	0	0
		Fore-stripping	2	2	2
		Teat pulling	2	7	3
		Calf stimulation	2	7	3
		Hand milking	0	0	0
		Disinfection of milking equipment	0	0	0
Mastitis prevention	Teat dipping	0	0	0	
	Dry cow therapy	0	0	0	
Drug types	Multiject®	2	7	3	
	Duofast®	1	0	0	
	Gentamast®	0	0	1	
	Tetracycline	2	2	2	
Who treats mastitic cows	Herdsman	2	4	0	
	Area veterinarian	2	4	0	
	Farmer	0	4	3	
	Home use	2	4	0	
What happens to the milk during drug withdrawal period	Feed to the dogs	0	1	3	
	Sold	0	2	0	
	Poured away	0	1	1	

Key: Multiject® (neomycin, streptomycin and penicillin); Duofast® (trimethoprim and sulfamethazole); Gentamast® (gentamicin); n = 12 farms.

trict, variations in prevalence of SCM among different sub-counties could be due to the extent of accessibility to extension services and clean water. Highly contaminated water carries bacteria that can gain entry into the udder

to cause SCM when used for cleaning the teats especially with the absence of post teat dipping.

The study also revealed that 70% of the dairy cattle tested had SCM in more than 2 quarters of the udder and 54.8% having all the 4 quarters affected. This has a serious implication on economics of milk production in the affected dairy farms. It is recognized that SCM can cause reduction in milk production from the affected quarter by up to 60% [1]. Moreover, at least 60% of the dairy cattle tested had CMT scores ranging from 1 to 2. This indicates that majority of the dairy cattle that tested positive had severe SCM. Similarly, Hand *et al.* [14] reported significant milk production losses associated with increase in SCC due to SCM. Other economic losses attributed to SCM include increase in treatment cost and culling [15].

In this study, the major constraints to mastitis control were primarily farmers' poor hygienic practices, largely attributed to lack of knowledge on the disease. No wonder, all the farmers assessed had no prior knowledge about SCM although they were aware of clinical mastitis. Poor environment and udder hygiene characterized by extensive soiling of the udder and the teats with manure were amongst factors predisposing cattle to SCM. This was further exacerbated by poor personnel hygiene; contaminated water coupled with lack of cleaning towels thus compounding the spread of contagious and environmental pathogens that cause SCM. Moreover, the few dairy farms that used milking salve had high level of bacterial load during preparation due to poor hygiene. Unfortunately, such poor hygiene also leads to possible contamination of milk thus arousing genuine milk safety concern.

While Uganda Dairy Development Authority (DDA), urges all dairy producers to use metallic buckets, a majority (10/12) of the farms assessed use plastic milking jars which are difficult to effectively clean thus encouraging bacterial multiplication. Bacterial culture results indicate that the most prevalent causative pathogens associated with SCM were Staphylococci (over 80%). Anri [1] also reported that Staphylococci are the dominant causes of SCM and are associated with low CMT scores. The contagious pathogens isolated were SA (16.56%), SAG (3.68%), CB (6.13%) which accounts for the high prevalence of SCM in the dairy cattle and being propagated by the poor hygienic practices at the dairy farms visited. However, environmental pathogens isolated included: CNS (64.42%), OS (5.52%), and CO (3.68%). Since CO are usually associated with clinical mastitis, this proportion was probably due to the contamination of the milk samples during sample collection [1].

Antibacterial sensitivity of SA isolated from the 7 dairy farms revealed 100% resistance against penicillin followed by neomycin (85.7%) and tetracycline (71.4%). At least 71.4% of SA isolated from the above farms were resistant to at least four antibacterial drugs. This multi-drug resistance is a real threat to effective control of the disease since the options are limited. Such high prevalence of resistance by udder pathogens was previously reported [6]. The highest level of resistance was registered from samples obtained from the Town Council (over 50%), suggestive of high drug pressure and irrational use of antibiotics. Indeed, over 80% of the farmers used Multiject[®] (penicillin, neomycin and streptomycin) and tetracycline for treatment of mammary and systemic bacterial diseases. The sale of antibiotics over-the-counter without prescription [16] has offered unprecedented access to the drugs by farmers. No wonder, only 30% of the farmers relied on veterinarians for treatment but the majority treated their animals with the aid of herdsmen. Such treatments were carried without strict adherence to dosage requirements and treatment period due to lack of technical knowledge among farmers and herdsmen considering their low level of education. Such abuse of drugs is responsible for the emergence of multi-drug resistant SA [17] which is a threat to both animal and public health. However, Trimethoprim-sulphamethazole was 100% effective against penicillin, tetracycline and aminoglycoside resistant SA.

5. Conclusion

The high burden of SCM and emergence of multidrug resistant SA are one of the constraints to dairy production in Kiboga district. Therefore, sensitization of dairy farmers in Kiboga district on proper hygiene, appropriate milking techniques and dry cow therapy based on the results of antibiotic sensitivity tests are highly recommended in SA associated SCM. Further research is also needed to identify the most appropriate extension materials on mastitis that can have a significant impact on effective control of the disease.

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