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Antimicrobial Resistance Patterns and Bovine Sub-Clinical Mastitis Burden in Low and High Tick Acaricide Resistance Regions of Uganda

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Abstract

Mastitis, antimicrobial resistance and acaricide resistance pose significant threats to the development of the dairy industry in sub-Saharan Africa. This study aimed to determine the prevalence of antimicrobial resistance in bacteria isolated from CMT positive cows on farms located in high (HARA) and low (LARA) acaricide resistance challenge regions of Uganda. Among selected herds in both regions, subclinical mastitis (SCM) screening was performed using CMT. CMT positive samples were collected, cultured, bacteria isolated and antibiotic sensitivity tests conducted. Overall, the prevalence of SCM in cows was 71.5% and 27.7% for HARA and LARA respectively. A SCM herd prevalence of 66.3% and 28.2% was recorded for HARA and LARA respectively. Furthermore, 67.5% and 20.8% of the cows in the HARA and LARA groups, respectively, had three out of four udder quarters infected with SCM. *Staphylococcus aureus* (44.2%) and coagulase-negative *Staphylococcus* (CNS) (47.6%) were the most prevalent causative agents of SCM isolated from cows from HARA and LARA, respectively. Most isolates from both regions were highly resistant to penicillin (HARA, 84.3%; LARA, 95.6%) and colistin (HARA, 100%; LARA, 97.8%). Tetracycline (77.1%) and oxacillin (76.1%) resistance was high in isolates from HARA and LARA, respectively. Intermediate responses (neither susceptible nor resistant) to antibiotics were

more common in isolates from HARA than in those from LARA. With this level of antibiotic resistance, there is a potential risk of failure to control mastitis in dairy cattle using antibiotics, especially in the HARA region, which may possibly jeopardize the growth of the dairy industry in Uganda.

Keywords

Antimicrobial Resistance, Sub Clinical Mastitis, Tick Resistance

1. Introduction

Inflammation of mammary tissue, referred to as mastitis, is a common disease in dairy cattle. It is very expensive to manage mastitis in the dairy industry [1] because it is an infectious bacterial disease capable of significantly reducing the productivity of a dairy herd [2] [3]. Mastitis is categorized as either clinical (CM) or subclinical (SCM). Clinical mastitis manifests as an abnormal appearance of milk with visible signs of inflammation (swelling and reddening) in the udder tissues. In contrast, SCM does not present with any clear and visible clinical signs, but its effects on the udder and milk can only be confirmed by examining milk samples for somatic cell counts (SCC) using the California mastitis test (CMT) or automated methods [4]. Clinical and subclinical mastitis is often caused by *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Streptococcus dysgalactiae*, and *Streptococcus agalactiae* [5] [6] [7] [8] [9]. Coliform bacteria such as *Escherichia coli* and *Klebsiella* spp. It causes mainly CM and rarely gives rise to SCM cases. *S. aureus* and *S. agalactiae* which easily spread from cow to cow, especially through unhygienic milking processes cause SCM [1]. Antibiotics such as tetracyclines, aminoglycosides, penicillin, sulfonamides, macrolides, and quinolones continue to be important in the treatment of CM and SCM in cattle [10]. However, the misuse of antibiotics, especially in dairy cattle in Uganda, poses a great risk in the development of antibiotic resistance [8] [11] [12]. Several studies have been conducted in Uganda and East Africa region at large to assess the prevalence of both CM and SCM in dairy cattle and the antibiotic susceptibility of mastitis-causing pathogens [1] [5] [8] [9] [11] [12] [13] [14].

On the other hand, several scholars have reported the challenge of tick acaricide resistance which has adversely affected the dairy sector in Uganda [15] [16] [17]. The dairy cattle sector is dominated by exotic cattle breeds and crosses between exotic and indigenous cattle breeds. These breeds tend to be more susceptible to ticks and tick-borne diseases (TBDs) than indigenous cattle breeds. The challenge of tick resistance has been linked to a surge in tick-borne diseases (TBDs), especially among the high milk-producing exotic breeds of cattle and their crosses [18]. We recently reported evidence of widespread antimicrobial use for controlling TBDs in HARA [2]. Similarly, in some pastoralist communities in northeastern Uganda, antibacterial drugs such as tetracyclines and penicil-

lin-streptomycin formulations have been used to treat TBDs [19]. Since cases of TBDs have been reported to be more common on dairy farms than on farms that have beef cattle or keep indigenous cattle, frequent use of antibiotics to treat TBDs may affect the way mastitis-causing bacteria respond to the same antibiotics. This is due to the frequent unprecedented exposure of mastitis-causing bacteria to antibiotics that are sometimes irrationally used to treat TBDs. Both mastitis and tick resistance [17] appear to be adversely affecting the dairy cattle industry in Uganda [1] [2] [9] [14]. We evaluated and compared the SCM burden and antibiotic susceptibility patterns for mastitis-causing pathogens in the high acaricide resistance areas (HARA) and low acaricide resistance areas (LARA) of Uganda.

2. Materials and Methods

2.1. Study Area

The study was conducted in two districts of Kiruhura and Adjumani, representing a high acaricide resistance area (HARA) and a low acaricide resistance area (LARA) in southwestern and northern Uganda [20], respectively (**Figure 1**). Kiruhura district was selected because it is documented to be one of the districts hard-hit by acaricide resistance, where multi-acaricide resistance to all three classes of conventional acaricides on the market was detected, namely, organophosphates, amides, and synthetic pyrethroids. In contrast, the Adjumani district was reported to be relatively free from acaricide resistance [17] [20]. The cattle population in Adjumani district is estimated to be 220,000, while Kiruhura district has approximately 340,000 cattle [21]. The majority of the cattle kept in Kiruhura District are cross breeds of Ankole cattle and other exotic breeds, the most common being the Holstein Friesian breed [18]. On the other hand, small East African Zebu, Ankole cattle, Boran, and their crosses are the dominant breeds of cattle maintained by farmers in the Adjumani district. Communal grazing is common in Adjumani (LARA), whereas paddock systems are common in Kiruhura (HARA).

2.2. Study Design and Sample Size

This cross-sectional study was conducted in August 2017 in selected districts of the HARA and LARA regions in Uganda. This study involved screening all milking herds at a randomly selected farm for SCM using CMT. Ten farms were selected in each region (HARA and LARA), making a total of 20 farms in the two regions. All the milking cows from each farm were screened for SCM. A total of 187 (748 teats) and 101 (404 teats) cows were tested at HARA and LARA, respectively. Individual teat/quarter milk samples were collected from all CMT-positive teats/quarters for bacterial culture, isolation, and antibiotic sensitivity tests (AST). CMT positivity was scored from 1 to 3 according to previously reported methods [1].

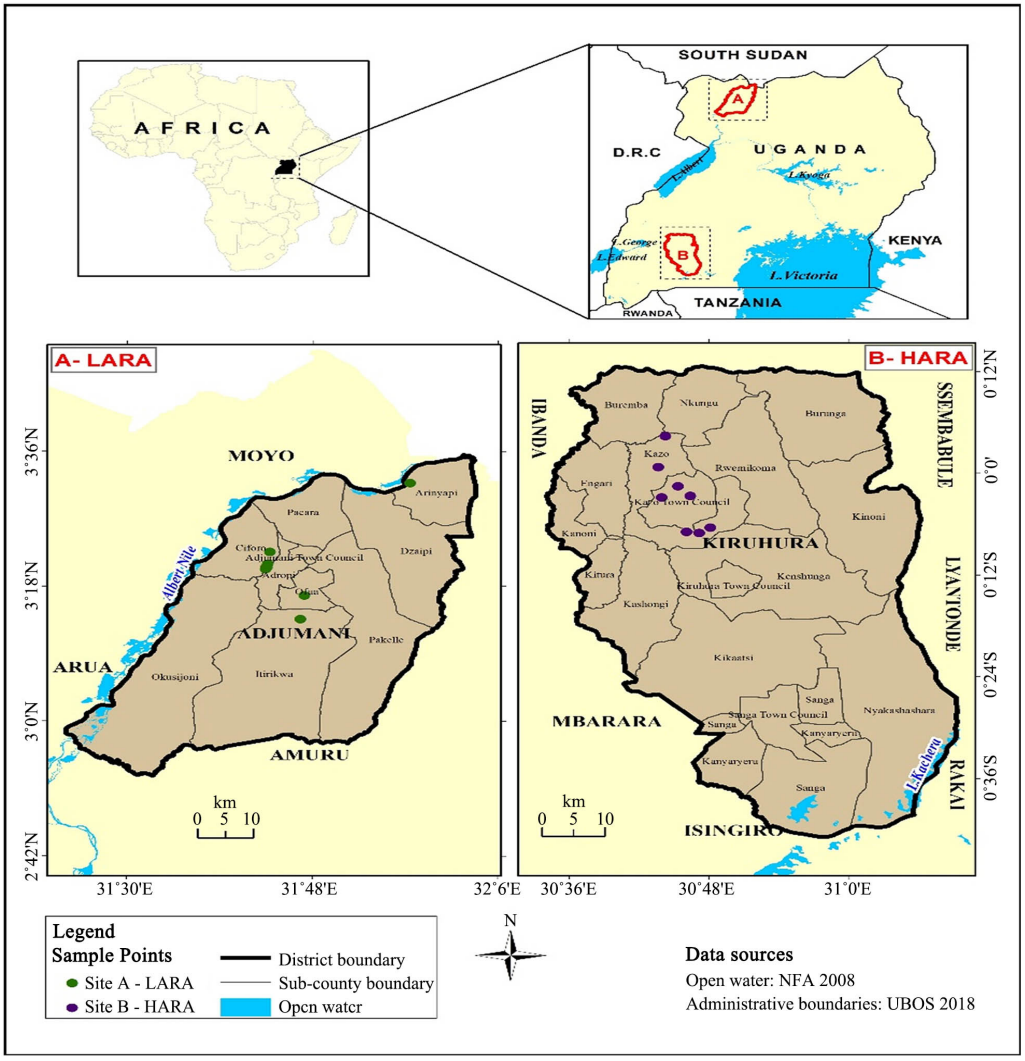

Figure 1. Map showing the study sites (LARA and HARA). Some of the sampling points are not visible due to overlapping. Source: Byaruhanga *et al.*, 2020.

2.3. Milk Sample Collection

Individual teat CMT-positive milk samples were collected from each CMT-positive cow. Before collecting the milk sample, the teat was cleaned for 20 seconds with cotton wool soaked in 70% ethanol and wiped dry with a sterile paper towel. Hand milking method was then used to squeeze and release the milk into a sterile 15 ml falcon tube labeled with the sample code, which included the codes for cow identification, teat, farm, and date of collection. The sample tubes were then transferred to a holding wrack inside a cool box containing ice packs for storage and transportation.

2.4. Sample Culture and Isolation

Individual milk samples were cultured on 5% sheep blood agar for 24 - 48 h in an aerobic incubator kept at 37˚C and 80% relative humidity. Ten microliters of each milk sample were streaked on blood agar plates using chromium wire

loops. Plates with uncertain growth were allowed to remain in the incubator for >24 h before the final examination. To confirm positive bacterial growth in any given sample, at least one colony-forming unit (CFU) was required for *Staphylococcus aureus* and *Streptococcus agalactiae* and at least three CFUs for the other bacterial genera. Bacterial culture and isolation were performed following methods described by several researchers [13] [22]. Bacterial identification was based on colony characteristics such as size, shape, color, and hemolysis patterns, as well as biochemical tests, following the procedure described by Sears [22].

2.5. Antibiotic Sensitivity Tests (AST)

Randomly selected bacterial isolates of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, coagulase-negative *staphylococcus* (CNS), and other *Streptococcus* spp. were subjected to antibiotic sensitivity tests using the disc method [23] [24]. Colonies of individual bacterial isolates were mixed in 50 µL of double distilled sterile water in a sterile 1.5 ml Eppendorf tube to form a homogenate. The homogenate (30 µL) was inoculated on Mueller Hinton agar using the spreading method. Eight antibiotic discs representing the commonly available antibiotic classes were dispensed onto the plate using a disc dispenser to ensure even distribution and sterility. The antibiotics used for the tests included penicillin (10 IU), gentamicin (10 µg), oxacillin (1 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), and colistin (50 µg) [25] [26]. The plates were then incubated in an aerobic incubator at 37˚C for 24 h. The diameter of the zone of clearance for each antibiotic was measured using a ruler and recorded in centimeters after 24 h of incubation. The recorded diameter for the zone of clearance was compared with the standard value ranges provided by the manufacturer of the antibiotic discs to categorize the response of a given bacterial isolate to a specific antibiotic as susceptible, intermediate or resistant [26].

2.6. Data Analysis

Data from the CMT tests, culture, and sensitivity tests were entered into Microsoft Excel version 2013 and sorted. Descriptive statistics were generated using SPSS software version 23 and presented as frequencies and percentages in a tabular format.

2.7. Ethical Considerations

This study was approved by the institutional review board (No. VAB/REC/15/104) at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University. Informed consent was obtained from the farm owners, and their personal data were kept confidential. Milk samples were collected from cows following strict guidelines on humane handling of animals and respect for animal welfare.

3. Results

3.1. Comparison of Herd Prevalence of SCM in HARA and LARA Regions

The overall SCM herd prevalence was higher in farms located in HARA (66.3%) than in those located in LARA (28.2%). The herd prevalence of SCM in HARA ranged from 60% to 80%, whereas in LARA it ranged from 8% to 65% (Table 1).

3.2. Comparison of SCM Prevalence at Individual Cow Level in the Two Regions

In total, 748 teats from 187 cattle from HARA and 404 teats from 101 cows from LARA were tested for SCM. Overall, the prevalence of SCM at the individual cow level was 71.5% and 27.7% for HARA and LARA cows, respectively. On the SCM positivity score scale, the majority of cattle teats from the HARA region had high CMT-positive scores of 2+ and 3+, indicating severe SCM infections (Table 2). However, the majority (72.3%) of the udder quarters tested in the LARA region were normal compared to those in the HARA region (28.5%).

3.3. Prevalence of SCM at Udder Quarter Level in HARA and LARA Regions

A total of 187 and 101 milking cows and udders were tested for SCM using the CMT method in HARA and LARA, respectively. Overall, the prevalence of SCM at the udder-quarter level was higher in HARA (85.2%) than in LARA (44.6%). (Table 3). In addition, many cows (67.5%) in HARA had at least three out of four udder quarters infected with SCM, while very few (20.8%) cows had more than two out of four udder quarters infected with SCM in LARA.

Table 1. Herd prevalence of sub-clinical mastitis in HARA and LARA regions.

HARA		LARA	
Farm codes (sample size)	**Frequency (%)**	**Farm codes (sample size)**	**Frequency (%)**
A (14)	10.0 (70.0)	K (10)	1.3 (13.0)
B (23)	15.0 (60.0)	L (10)	1.0 (10.0)
C (18)	12.0 (60.0)	M (10)	2.0 (20.0)
D (19)	14.3 (70.0)	N (10)	3.3 (33.0)
E (28)	22.0 (70.0)	O (10)	5.3 (53.0)
F (23)	19.0 (80.0)	P (10)	6.5 (65.0)
G (19)	12.8 (60.0)	Q (10)	0.0 (0.0)
H (10)	6.3 (63.0)	R (10)	0.8 (8.0)
I (19)	12.0 (60.0)	S (10)	3.0 (30.0)
J (14)	10.5 (70.0)	T (10)	5.0 (50.0)
N = 187	13.4 (66.3)§	N = 100	2.82 (28.2)§

N = total number of cattle per region; n = number of cattle sampled per herd/farm; % = prevalence; § = average prevalence per herd/farm in respective region.

Table 2. The severity of sub-clinical mastitis in cattle from HARA and LARA using CMT Positive scoring.

Area	Number of udder Quarters (%)			
	Normal udder quarters	1+	2+	3+
HARA (n = 748)	213 (28.5%)	172 (23.0%)	167 (22.3%)	186 (24.9%)
LARA (n = 404)	292 (72.3%)	50 (12.4%)	40 (10%)	22 (5.4%)

CMT—California Mastitis Test; Scores range from 1 to 3; "1+"—distinct thickening, no gel formation); "2+"—distinct thickening, slight gel formation; "3+"—gel is fully formed.

Table 3. Prevalence of sub-clinical mastitis infection at Udder quarter level in HARA and LARA regions.

Area	Number of Herds/farms	Number (%) of cattle affected*				
		1/4 of Udder	2/4 of udder	3/4 of udder	Whole Udder	Normal Udder
HARA	10	12 (6.6)	20 (11)	29 (15.9)	94 (51.6)	27 (14.8)
LARA	10	11 (10.9)	13 (12.8)	9 (8.9)	12 (11.9)	56 (55.4)
Total	20	23 (17.5)	33 (23.8)	38 (24.8)	106 (63.5)	83 (70.2)

*The proportion of the udder affected by sub-clinical mastitis.

3.4. Prevalence of SCM Causing Bacteria Recovered from the Two Regions

Bacterial isolates, such as *Staphylococcus aureus*, CNS, coliforms, *Corynebacterium bovis*, *Streptococcus agalactae* and other *Streptococcus* species, were recovered from CMT-positive cows from HARA. *Staphylococcus aureus*, CNS, coliforms, and *Pseudomonas auroginosa* were isolated from CMT-positive cow milk obtained from LARA. *Staphylococcus aureus* was the most prevalent causative agent of SCM (44.2%) isolated from CMT-positive cows at HARA. In LARA, *Staphylococcus aureus* was the second most prevalent (19.0%) bacterium causing SCM. Coagulase-negative *Staphylococcus* was the most common (47.6%) bacterium isolated from CMT-positive milk from LARA. Similarly, it was also found to be high (37.9%) in the HARA group (Table 4).

3.5. Antibiotic Sensitivity Results for Mastitis Causing Bacterial Isolates Recovered from CMT Positive Cattle from the Two Regions

A total of 83 and 43 bacterial isolates from HARA and LARA, respectively, were subjected to antibiotic susceptibility test (AST) involving eight different antibiotics (Figure 2). These included penicillin (10 IU), gentamicin (10 µg), oxacillin (1 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), and colistin (50 µg). *Staphylococcus aureus* (58), coagulase-negative *Staphylococcus* (15), *Streptococcus agalactae* (2), and other *Streptococcus* (8) were the main bacterial isolates tested from HARA,

whereas coagulase-negative *Staphylococcus* (25), *Staphylococcus aureus* (12), coliforms (8), and *Pseudomonas auroginosa* (1) were the main bacteria tested from LARA (Table 5).

Generally, the bacterial isolates from HARA exhibited resistance to colistin (100%), penicillin (84.3%), and tetracycline (77.1%), whereas those from LARA exhibited resistance to colistin (97.8%), penicillin (95.6%), and oxacillin (76.1%). The percentages of susceptible bacterial isolates from HARA to various antibiotics were 15.7%, 13.2%, 14.4%, 23%, 6%, 29%, 37.3%, and 0% against penicillin, gentamicin, oxacillin, trimethoprim/sulfamethoxazole, tetracycline, ciprofloxacin, erythromycin, and colistin, respectively. On the other hand, the percentage of susceptible bacterial isolates from LARA to various antibiotics was found to be 4.4%, 89.1%, 23.9%, 60.8%, 54.3%, 80.4%, 28.2%, and 2.2% against penicillin, gentamicin, oxacillin, trimethoprim/sulfamethoxazole, tetracycline, ciprofloxacin, erythromycin, and colistin, respectively (Table 5 and Table 6).

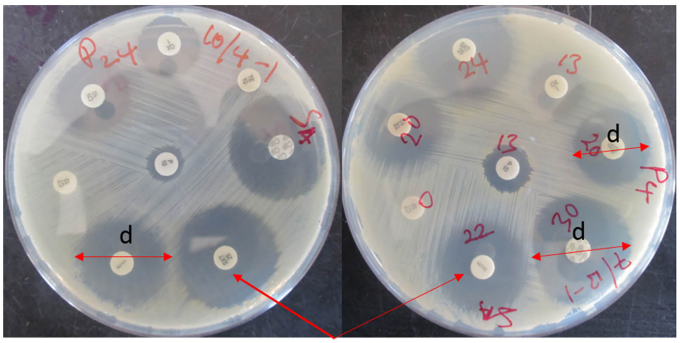

Figure 2. Antibiotic sensitivity test results. Key: d = diameter of zone of clearance.

Table 4. Prevalence of sub-clinical Mastitis bacteria recovered from HARA and LARA regions, Uganda.

Type of bacteria	HARA Number (%)*	LARA Number (%)
Staphylococcus aureus	84 (44.2)	12 (19.0)
Coagulase Negative *Staphylococcus* (CNS)	72 (37.9)	30 (47.6)
Coliforms	10 (5.2)	17 (27.0)
Corynebacterium bovis	03 (1.6)	0 (0)
Streptococcus agalactae	03 (1.6)	0 (0)
Pseudomonas auroginosa	0 (0)	4 (6.3)
Other *Streptococcus* spp.	18 (9.4)	0 (0)
Total no. of isolates	190	63

*Number (%) of bacteria isolated from CMT positive milk samples.

Table 5. Antibiotic sensitivity results for sub-clinical mastitis causing bacteria isolated from CMT positive cattle from HARA.

Antibiotic	Response	Number of confirmed bacteria isolates*				Number (%)
		S. aureus	CNS	Other *Streptococcus*	*Streptococcus agalactae*	
Penicillin	R	56	13	0	0	69 (84.3)
	I	0	0	0	0	0 (0)
	S	2	2	8	2	14 (15.7)
Gentamicin	R	4	2	3	2	11 (13.2)
	I	49	12	0	0	61 (73.2)
	S	5	1	5	0	11 (13.2)
Oxacillin	R	3	12	4	2	21 (25.3)
	I	50	0	0	0	50 (60.2)
	S	5	3	4	0	12 (14.4)
Trimethoprim/ sulfamethoxazole	R	3	4	2	1	10 (12)
	I	54	0	0	0	54 (65)
	S	1	11	6	1	19 (23)
Tetracycline	R	47	9	7	1	64 (77.1)
	I	11	1	1	1	14 (16.8)
	S	0	5	0	0	5 (6)
Ciprofloxacillin	R	1	0	0	0	1 (1.2)
	I	55	1	0	2	58 (69.8)
	S	2	14	8	0	24 (29)
Erythromycin	R	0	1	0	0	1 (1.2)
	I	40	9	1	1	51 (61.4)
	S	18	5	7	1	31 (37.3%)
Colistin	R	58	15	8	2	83 (100)
	I	0	0	0	0	0 (0)
	S	0	0	0	0	0 (0)

*Total number of isolates were 83; R = Resistant; I = Intermediate; S = Susceptible; CNS = Coagulase negative *Staphylococcus*.

Table 6. Antibiotic sensitivity results for sub-clinical mastitis causing bacteria isolated from CMT positive cattle from LARA.

Antibiotic	Response	Number of confirmed bacteria isolates*				Number (%)
		CNS	*S. aureus*	Coliforms	*P. auroginosa*	
Penicillin	R	25	12	6	1	44 (95.6)
	I	0	0	0	0	0 (0)
	S	0	0	2	0	2 (4.4)

Continued

Gentamicin	R	1	2	0	0	3 (6.5)
	I	0	2	0	0	2 (4.4)
	S	24	8	8	1	41 (89.1)
Oxacillin	R	22	4	8	1	35 (76.1)
	I	0	0	0	0	0 (0)
	S	3	8	0	0	11 (23.9)
Trimethoprim/ sulfamethoxazole	R	5	1	5	0	11 (23.9)
	I	5	2	0	0	7 (15.2)
	S	15	9	3	1	28 (60.8)
Tetracycline	R	1	10	5	0	16 (34.7)
	I	5	0	0	0	5 (10.8)
	S	19	2	3	1	25 (54.3)
Ciprofloxacillin	R	1	1	1	0	3 (6.5)
	I	5	0	1	0	6 (13.0)
	S	19	11	6	1	37 (80.4)
Erythromycin	R	7	5	4	1	17 (37.0)
	I	9	5	2	0	16 (34.8)
	S	9	2	2	0	13 (28.2)
Colistin	R	24	12	8	1	45 (97.8)
	I	0	0	0	0	0 (0.0)
	S	1	0	0	0	1 (2.2)

*Total number of isolates/samples is 46; R = Resistant; I = Intermediate; S = Susceptible; CNS = Coagulase negative *Staphylococcus*.

The majority of the isolates from HARA were found to be at the second stage in the process of antibiotic resistance development (intermediate) to the antibiotics used in this study whereas majority isolates from LARA were either susceptible or intermediately susceptible to antibiotics considered in this study. The majority (77.1%) of the isolates from HARA exhibited significantly high resistance to tetracycline, whereas the majority (54.3%) of isolates from LARA was highly susceptible to tetracycline. The number of bacterial isolates exhibiting intermediate responses to antibiotics was higher among the isolates recovered from samples collected from HARA than in very few isolates obtained from LARA, which showed an intermediate response to antibiotics. Although the majority (73.2%) of the bacterial isolates from HARA showed an intermediate response to gentamicin, the majority (89.1%) of the isolates from LARA were susceptible to gentamicin. Notably, the majority (60.2%) of HARA isolates showed

an intermediate response to oxacillin, whereas the majority (76.1%) of LARA isolates showed resistance to the same antibiotic. Similar results were observed for erythromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole. The AST tests for the bacterial isolates from HARA and LARA were similar for penicillin and colistin, with the majority of isolates from both regions resisting the above antibiotics (Table 7).

Table 7. Comparison of the efficacy of antibiotics against SCM-causing bacteria isolated from CMT-positive dairy cattle from HARA and LARA regions.

Antibiotic	Bacterial response to antibiotic	Number (%)	
		HARA	LARA
Penicillin	R	69 (84.3)	44 (95.6)
	I	0 (0)	0 (0)
	S	14 (15.7)	2 (4.4)
Gentamicin	R	11 (13.2)	3 (6.5)
	I	61 (73.2)	2 (4.4)
	S	11 (13.2)	41 (89.1)
Oxacillin	R	21 (25.3)	35 (76.1)
	I	50 (60.2)	0 (0)
	S	12 (14.4)	11 (23.9)
Trimethoprim sulfadiazole	R	10 (12)	11 (23.9)
	I	54 (65)	7 (15.2)
	S	19 (23)	28 (60.8)
Tetracycline	R	64 (77.1)	16 (34.7)
	I	14 (16.8)	5 (10.8)
	S	5 (6)	25 (54.3)
Ciprofloxacillin	R	1 (1.2)	3 (6.5)
	I	58 (69.8)	6 (13.0)
	S	24 (29)	37 (80.4)
Erythromycin	R	1 (1.2)	17 (37.0)
	I	51 (61.4)	16 (34.8)
	S	31 (37.3)	13 (28.2)
Colistin	R	83 (100)	45 (97.8)
	I	0 (0)	0 (0.0)
	S	0 (0)	1 (2.2)

R = Resistant; I = Intermediate; S = Susceptible; high acaricide resistance areas (HARA) and low acaricide resistance areas (LARA).

It is worth noting that different bacterial isolates respond differently to various antibiotics. However, *Staphylococcus aureus* and coagulase-negative *S. aureus* from both regions exhibited high resistance to penicillin, tetracycline, and oxacillin. Although nearly all bacterial isolates from both regions exhibited resistance to colistin, surprisingly, we were able to confirm one CNS isolate from LARA that was naïve to colistin and was highly susceptible. *Streptococcus agalactae* recovered from samples collected from HARA were resistant to nearly all antibiotics except penicillin and, to a certain extent, ciprofloxacin (intermediate). Other *Streptococcus* isolates were susceptible to penicillin, erythromycin, and ciprofloxacin, and the majority of the isolates were susceptible to gentamicin and trimethoprim/sulfamethoxazole. They also exhibited high resistance to tetracycline, colistin, and cloxacillin. The majority of coliforms isolated from samples collected from LARA were resistant to penicillin, oxacillin, trimethoprim/sulfamethoxazole, colistin, tetracycline, and erythromycin, and susceptible to gentamicin and ciprofloxacin antibiotics. *Pseudomonas auroginosa* isolates from LARA showed resistance to penicillin, colistin, oxacillin, and erythromycin, but were fully susceptible to the other antibiotics considered in this study.

4. Discussion

This study revealed that cattle kept in HARA had a higher prevalence of SCM both at the cow and udder levels than those kept in LARA. The prevalence of SCM reported in this study is comparable to that reported by several scholars in Uganda [1] [4] [5] [9] [12] [27]. A recent study by Miyama *et al.* (2020), conducted in the neighboring district of Mbarara, which lies within the HARA region, reported a SCM prevalence of 68.6% and 39.2% at the cow and udder levels, respectively. The findings of this study are statistically similar to those of this study. This may indicate that the SCM burden is widespread within the HARA region. The severity of SCM, in terms of somatic cell counts, increased with the CMT-positive score. Comparing the CMT positivity scores of the teats tested in both HARA and LARA, it was noted that dairy cattle in HARA experienced much more severe SCM, as indicated by the results of CMT positive scores than the cattle kept in LARA.

The difference in the prevalence of SCM observed in the two regions may be partly attributed to the differences in the breeds of cattle maintained in the two regions. Cattle from HARA have been reported to be better milk producers than their counterparts, and this factor predisposes the cattle in HARA to SCM. Factors such as incomplete milking, bucket feeding of calves, and unhygienic milking practices may have contributed to the high prevalence of SCM recorded in this study. On the other hand, indigenous cattle kept in LARA [15] are inherently low milk producers, further reducing their chances of developing SCM. In addition, the farmers in LARA allow the calves to suckle, which ensures complete milking of the udders and further reduces the chances of SCM in those animals. The high CMT-positive score observed in cattle in HARA may be indicative of a high burden of both SCM and clinical mastitis in the region. This

may indirectly indicate that dairy farmers from HARA may incur substantial production losses associated with the high burden of SCM.

The SCM-causing bacteria isolated from CMT-positive cows are similar to those reported by several scholars both in Uganda and the region at large, with very minor differences [1] [5] [6] [7] [8] [9] [11] [12] [14] [28]. However, it should be noted that *Staphylococcus aureus* and CNS were the most prevalent SCM-causing bacteria isolated from CMT-positive cattle in HARA and LARA, respectively. *Staphylococcus aureus* (30.8%) was identified as the most prevalent bacterium in a previous study conducted in the same study area [9]. Other studies also found *Staphylococcus aureus* to be the most common bacteria implicated in the cause of SCM [29]. In contrast, the findings from LARA indicated that the CNS was the most prevalent. Although this finding contradicts some reports [8] [9] [29], it agrees with some previous studies that reported similar findings in Uganda [1] [8]. Other reports have found *Streptococcus* species and corynebacteria species to be the most prevalent SCM-causing pathogens in both Uganda and Ethiopia [14] [30]. A study conducted in Ethiopia considered mastitis causing pathogens in general, combining both clinical and subclinical mastitis, which may explain the high prevalence of *Streptococcus* species, especially *S. agalactae* which is more associated with clinical mastitis. However, this study only considered SCM cases, which may explain the observed differences in terms of the most prevalent SCM-causing bacteria.

Generally, the majority of the bacterial isolates from HARA were found to be more resistant to the classes of antibiotics used in this study than those recovered from LARA. The majority of the isolates from HARA were found to be in the second stage (intermediate) of development of antibiotic resistance to the antibiotics used in this study. The findings, especially those from HARA, agree with those of previous studies conducted in other districts within this region [9] [27]. The general trend observed in the antibiotic response of SCM-causing bacteria from HARA is indicative of the development of antimicrobial resistance. This may be partly explained by the reported increase in TBD incidence at the farm level [18] which warrants routine use of antibiotics on farms in HARA to manage increased TBD cases, especially East Coast fever [31] related to the challenge of tick acaricide resistance. The findings from HARA may be further explained by previous reports of irresponsible and indiscriminate use of antibiotics in livestock in Uganda and the region [20] [32]. In addition, a high incidence of clinical mastitis among cattle in HARA has been reported [2], which may further encourage farmers to use antibiotics indiscriminately, leading to the observed increasing trend of antibiotic resistance in that region. The findings of this study highlight pertinent concerns, especially the extent to which acaricide resistance contributes to the observed trend of antibiotic resistance in the HARA region. Overall, the challenge of antibiotic resistance is not just a local problem but a global challenge. Globally, the World Organization for Animal Health (OIE) has raised concerns regarding the rapidly growing challenge of antimicrobial resistance in the veterinary sector. The rational use of veterinary antibiotics has been

advocated to delay the development of this problem [33].

However, the response of SCM-causing bacteria to antibiotics from LARA indicates that the majority of bacteria in that region are still fairly susceptible to the major antibiotic classes available on the market. This may be due to farmers using fewer antibiotics in the routine management of animal diseases or may be due to lower disease incidence, especially TBDs and mastitis [20]. This is further supported by the CMT test results of this study, which revealed a low prevalence of SCM and, ultimately, low incidences of clinical mastitis.

The observed response of specific SCM-causing bacteria, especially *Staphylococcus aureus* is quite similar to that previously reported in Uganda, although many more isolates of *Staphylococcus aureus* exhibited resistance to routinely used antibiotics in this particular study. Wide-spread tetracycline and penicillin resistance was confirmed in HARA, which is consistent with previous reports [1] [8] [9] [12].

5. Conclusion

This study revealed that dairy farmers in HARA are faced with the challenge of high prevalence of SCM at both herd and udder levels compared to their counterparts in LARA. *Staphylococcus aureus* and CNS are the most prevalent SCM-causing bacteria isolated from CMT-positive cattle in the HARA and LARA regions, respectively. The observed general trend in the antibiotic response of SCM-causing bacteria from HARA indicates the growing challenge of antimicrobial resistance. In addition to facing the challenge of tick acaricide resistance, dairy farmers from HARA are likely to face another challenge: failure to effectively treat and manage mastitis in their herds due to the growing resistance of mastitis-causing bacteria to antibiotics. Tick acaricide resistance may be an indirect driver of veterinary antimicrobial resistance, especially in dairy farms in Uganda. The vibrant dairy sector depends directly on the good udder health of individual dairy cows. Therefore, a situation in which antibiotics fail to treat mastitis should be avoided as it may jeopardize the growth of the dairy industry. The findings of this study should awaken authorities to put in place measures to fight and prevent veterinary antimicrobial resistance in Uganda by carefully understanding the role of tick acaricide resistance in the development of antimicrobial resistance in the veterinary sector.

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Authors' Contribution

Conception and design of the study; JB, IR, VP: Performed sample collection

and analysis in the laboratory; JB, YS: Performed data entry and data analysis; JB, YS: wrote the first draft of the manuscript; JB, YS, IR: Provided guidance, advice, and review of the draft manuscript; VP, IR. All the authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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