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Mastitis Incidences and Antibiotic Resistance in Bovines in Uasin Gishu County, Kenya

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Abstract

Mastitis is an inflammatory reaction induced by a bacterial infection of the udder tissue. Bovine mastitis is one of the terrible diseases that causes enormous losses to the global dairy business. Antimicrobial-resistant (AMR) bacteria on farms in developing countries, where the bulk of livestock is kept on smallholdings, are poorly understood. There is a need to understand the factors that contribute to the increased occurrence of mastitis in order to control it. Consequently, this study evaluated mastitis incidences and antibiotic resistance in Bovines in Uasin Gishu County, Kenyan. A random sample consisting of one hundred and fifty lactating dairy cows; fifty each from three purposively chosen study sites, from several dairy farms across Uasin Gishu County- Kenya were tested in a study of mastitis. Causative microbes were identified both by cultural morphology and biochemical tests. Culture and sensitivity testing using the disc diffusion method were done to determine their in vitro resistance to various antimicrobial agents. The Draminski Mastitis Detector was used to screen udder quarters for subclinical mastitis while a strip cup and visual examination were used to detect visible changes to the udder and /or the milk for clinical mastitis. Out of the 76 positive samples obtained at screening, 72 had bacterial growth while 4 had none. The bacterial or fungal genus isolated were Staphylococcus sp 31.6%, followed by Escherichia sp 22.4%, Klebsiella sp 18.4%, Streptococcus sp 17.1%, Corynebacterium sp 2.6%, Pseudomonas sp 1.3% and Candida sp 1.3%. Resistance by the isolated microbes was greatest to the two sulphonamides; sulphamethoxazole 17.3% and cotrimoxazole 17.3%, followed by chloramphenicol 16.6%, nalidixic acid 15.9%, ampicillin 15.2%, tetracycline 11.2%, streptomycin 5.1%, kanamycin 2.8% and gentamicin 2.2% in that decreasing order. In order to alleviate this problem of increase in antimicrobial resistance, the study recommended that cases detected are promptly and vigilantly treated with suitable antimicrobials after culture and sensitivity tests have been carried out.

Keywords: Mastitis, Antimicrobial-resistant Bacteria, Milk, Bovine

INTRODUCTION

Mastitis in dairy cattle is inflammation of the udder tissue. It occurs when white blood cells (leucocytes) are released into the mammary gland usually in response to an invasion by bacteria of the teat canal (Blood et al., 2006). Affected milk secreting tissue and ducts in the mammary gland are damaged due to toxins produced by bacteria. Mastitis can also occur as a result of chemical, mechanical or thermal injury. The mammary gland with mastitis produces little or no milk. The udder sac or affected quarter may be hot to the touch, painful, swollen, hard, tight and usually firm (Blowey & Edmondson, 2010).

The mammary infections are described as being sub clinical or clinical mastitis (DaRong et al., 2010; Memon et al., 2012).

Sub clinical mastitis is the presence of an infection without apparent signs of local inflammation or systemic involvement that can be detected by visual examination or by a strip cup (Islam et al., 2012; Lakew et al., 2009). Although transient episodes of abnormal milk or udder inflammation may appear, these infections are for the most part asymptomatic and if the infection persists for at least two months then they are termed as being chronic. Once established, many of these infections persist for the entire lactation period or the life of the cow (Kirk, 2010). Mastitis is a major cause of economic losses to the dairy industry. Detection is best done by examination of milk for somatic cell counts (predominantly neutrophils) using the California Mastitis Test (CMT) or the automated methods such as the Draminski electronic mastitis detector (National Mastitis Council 1996).

Somatic cell counts (SCC) are positively correlated with the presence of infection. Although variable (especially if determined on a single analysis), cows with a SCC of $\geq$ 280,000 cells/ml (> a linear score of 5) have a >80% chance of being infected. Likewise, the higher the SCC in a herd bulk tank, the higher the prevalence of infection in the herd. Causative agents are best identified by bacterial culture of milk. Clinical mastitis is an inflammatory response to infection causing visibly abnormal milk (e.g. colour, fibrin clots) (Webster, 2017; Brezovan et al., 2010). As the extent of the inflammation increases, changes in the udder (swelling, heat, pain, and redness) also become more apparent (Argaw, 2016; Peters et al., 2015). The most common cause of mastitis in dairy cattle is bacterial infections especially *Streptococcus agalactiae, Staphylococcus aureus* and *Escherichia coli*. *E. coli* is especially important in housed or confined cattle (Gilbert et al., 2013; Zhao & Lacasse, 2008; Riekerink et al., 2007). Many other bacterial species can cause mastitis in cattle (Zadoks et al., 2011). This potentially fatal mammary gland infection is the most common disease in dairy cattle in the Uasin Gishu County accounting for 77.5% of all reported diseases of large animals during the three-year period (GOK- V.I.L. - Eldoret Annual Reports 2008-2011).

It is thus a major problem to dairy farmers in the area under study as clinical mastitis causes economic loss due to treatment costs, lost quarters, perhaps dying cows and most importantly, discarded milk (Halasa et al., 2007; Hillerton & Berry, 2005). Sub clinical mastitis on the other hand reduces milk production and quality but is not noticeable until detected with a somatic cell count or by instruments that detect changes in electrical resistance of milk (Kirk, 2010).

The mainstay of treating bacterial mastitis is the use of antibiotics administered parenterally (injected into the body), or more commonly as an intra-mammary infusion directly into the affected gland or quarter (Krishnamoorthy et al., 2013). For those infused into the gland their success in treating mastitis depends on the degree of binding of the drug to mammary tissues and secretions, its ability to pass through the lipid phase of milk and the degree of ionization. For antibiotics administered parenterally the rate of diffusion into the udder tissue from the bloodstream is greater in damaged than in normal tissue (Blood et al., 2006).

In most countries surveys of the incidence of mastitis, irrespective of cause, show comparable figures of about 40% morbidity amongst dairy cows and an udder quarter infection rate of about 25% (Lakew et al., 2009; Ibrahim, 2017). A major survey of dairy herds in Britain revealed an udder quarter infection rate, in terms of positive cell count, of 27%, but an actual quarter infection rate, as indicated by infection with a significant pathogen, of only 9.6% (Blood et al., 2006). Mastitis is one of the most common and costly diseases of dairy cattle (Rodernberg, 2012). Annual reports of 2011-2014 from the regional Veterinary Investigation Laboratory (VIL) - Eldoret indicates that confirmed cases of mastitis are 77.5% of all diagnosed diseases of large animals during the period. In recent years there has been an increase in the occurrence

of antimicrobial resistance (AMR) to the standard antibiotics and sulphonamides commonly used for mastitis treatment (Regional V.I.L. Annual Report, (2008-2011) Call et al. (2008). Antimicrobial resistance (AMR) is a major concern to physicians, veterinarians, farmers and consumers worldwide because resistance can render some diseases untreatable. This is because whenever we treat an animal or human with an antimicrobial drug, a certain selection pressure is placed on the microbial population that could ultimately select for AMR. From a public health perspective, because animal products become food, there is concern about AMR pathogens disseminating from the livestock sector into the human population (Oliver et al., 2011). This could occur by direct contact with animals, through environmental contamination or through the food chain. This public concern has led to increased pressure to reduce antimicrobial usage in livestock throughout the world. Understanding AMR and the prudent usage of antimicrobials in livestock is therefore important for everyone involved in the industry (Waller et al., 2011).

Dairy cattle mastitis is important because it affects the udder which is the organ that synthesizes milk (the raw material for the whole dairy industry). It has also become the most commonly reported disease of dairy cattle in the area according to the VIL Eldoret reports of 2008-2011. An understanding of its occurrence, prevalence, etiology, risk factors, antimicrobial resistance, treatment and control is therefore of great importance to many a stakeholder especially in Uasin Gishu county.

METHODOLOGY

The Study Area and Location of the Study Site

The study was carried out in Turbo and Soy sub-counties of Uasin Gishu County in Kenya. With reference to the National and UGD Maps, the region lies between longitudes 34º 50’ E and 35º 37’ E and latitudes 0º 03’ S and 0º 55’ N (Waweru & Jebotip, 2016; Ansari et al., 2016). The majority of farmers in the area have cultural attachment to cattle and almost every household keeps some livestock particularly dairy cattle (Cherogony, 2013). Milk is a very important food to the people in the study area and is also a source of ready income from its sales.

Three study sites were purposively selected as study sites based on the density of dairy farmers. The indication for this was the presence of a centre for milk collection, cooling and bulk transporting to processors. Sugoi centre is a milk bulking and cooling plant (an International Fund for Agricultural

Development (I.F.A.D). funded dairy commercialization unit with an estimated dairy cattle population of 1,350 cows in milk at any one time; Ziwa Sirikwa is another milk bulking and cooling plant (a Bill and Melinda Gates funded project via the East African Dairy Development (E.A.D.D.) Project) with an estimated dairy cattle population of 16,875 cows. Moisbridge dairies, with a cattle population estimated to be approximately 20,000 was the third collection centre selected for study. It covers Moisbridge and Matunda locations, Kaplelai, Cherangani and parts of Trans-Nzoia County.

Sample Size

One hundred and fifty cows, fifty each location, were screened for both clinical and subclinical mastitis throughout the three study sites. All lactating animals on each visited farm were examined. At the same time, information regarding each cow's breed, parity/age, stage of lactation, and average daily milk supply was collected. Forty respondent farmers were given a questionnaire regarding mastitis testing and control techniques.

Experimental Design

The study covered the three study blocks/sites; i.e. Sugoi, Ziwa Machine and Moisbridge dairy blocks. The survey was based on a Randomized Block Design (RBD). All the lactating cows from randomly

chosen dairy farms in each block were screened for mastitis. At least 15 ml of milk from each individual quarter of every cow sampled was squirted into the Draminski Electronic Mastitis Detector and the readings recorded. The electrical resistance readings of the respective milk samples were taken and interpreted on the spot. Any milk from a quarter with a reading below 300 units was considered a positive reaction. The positive samples were taken to the V.I.L- Eldoret for bacterial culture and in vitro antimicrobial sensitivity testing. Any cow whose milk (using a strip cup) and udder showed visible changes (clots or colour changes) was recorded as having clinical mastitis. Negative reactors at farm level formed statistics for calculation of prevalence of mastitis among the sampled lactating dairy cows. The prevalence of mastitis was computed and expressed as a percentage of the number of positive reactors (infected cows) divided by the total number of cows that were screened in all the three study sites as follows;

Prevalence = Number of cows whose milk showed reading <300units / Total number of cows screened

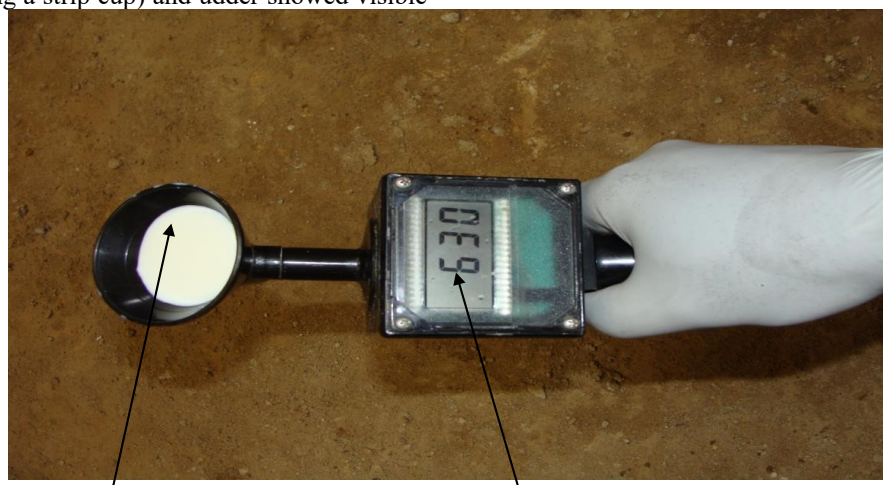


Figure 1: The Draminski Mastitis Detector showing a reading from milk obtained from one udder quarter.

Detection of the Presence of Antimicrobial Resistant Mastitis Causing Organisms

This was done using the agar diffusion method as described by Silva et al. (2010). It is based on the determination of diameters of growth inhibition zone around a paper disc that is impregnated with a defined amount of antimicrobial agent.

The microbial inoculums were evenly spread on a blood agar plate for growth. Nine different types of antimicrobial discs were then applied and the agar incubated for a period of 12 hours at 37^{o}C. During this time period, the antimicrobial agent diffused from the disc into the agar and suppressed the growth of the bacteria depending on the susceptibility level of the corresponding bacteria. After this incubation period, the zone diameter around each disc was measured in millimeters and compared with the zone diameter break points given in the respective AST manual. The antimicrobial discs that were used are shown in Table 1.

Table 1: Types and strengths of antimicrobial discs used in culture and sensitivity tests

Disc Type	Strength of the active Ingredient per disc	Class of antimicrobial
Ampicillin (AMP)	25 µg	B-lactam antibiotic
Nalidixic acid (NA)	25 mg	Quinolone
Tetracycline (TE)	25 µg	Tetracycline
Co-trimoxazole (COT)	25 µg	Potentiated sulphonamide
Streptomycin (S)	10 µg	Aminoglycoside
Kanamycin (K)	30 µg	Aminoglycoside
Gentamicin (GEN)	10 µg	Aminoglycoside
Sulfamethoxazole (SX)	200 µg	Sulphonamide
Chloramphenicol (C)	30 µg	Chloramphenicol

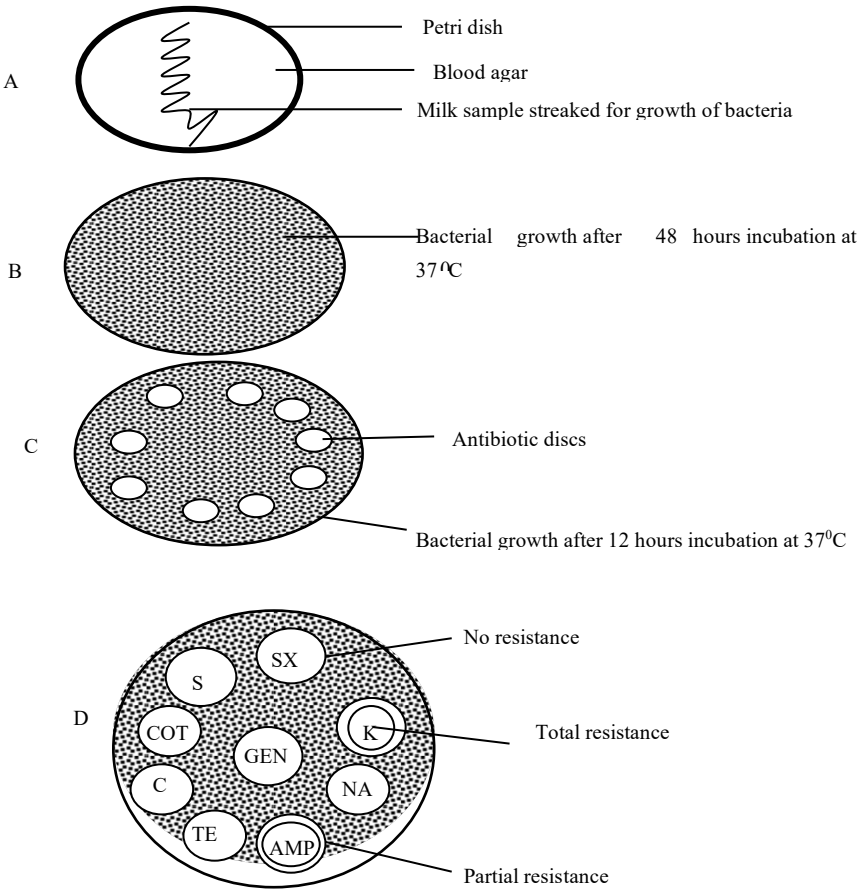


Figure 2: Sketch illustrating the use of petri dishes for microbial in vitro culture and sensitivity/resistance tests.

The presence of bacterial growth around a disc after 48 hours incubation indicated bacterial resistance to that antimicrobial as shown in the discs (Fig 6). A clear area around a disc after the same period indicated sensitivity of the bacteria to the antimicrobial present in the disc. For example, the figure 6 D above would indicate that the bacteria are

resistant to streptomycin (S), gentamicin (GEN), sulfamethoxazole (SX) and chloramphenicol (C) while they are partially sensitive to ampicillin (AMP) and totally resistant to kanamycin (K). The diameters of the circular zone or bacterial growth clearance were measured and compared with published standards to determine susceptibility or resistance. The effect of the various classes of antibacterial agents on the types of bacteria (Gram+ or Gram-) was also observed and recorded as follows:

Table 2: Results of *in vitro* culture and antimicrobial resistance tests

Class of antimicrobial agent	Effects on bacteria	
	Resistant	**Sensitive**
Penicillins		
Tetracyclines		
Sulphonamides		
Aminoglycosides		
Macrolides		
Chloramphenicol		

Statistical Data Analysis

All relevant data was analysed using descriptive statistics and ANOVA. SPSS was used to calculate percentages, arithmetic means, standard deviation, and coefficients of variation. Means were separated using ANOVA and tested at $p < 0.05$. Also, proportions of antimicrobial resistant mastitis (ARM) organism's vs susceptible bacteria were calculated. After data collection, PROC GLM was used to express mastitis prevalence as a percentage of infected vs. uninfected cattle.

RESULTS

Experiment One Results: Prevalence of Mastitis in the Study Area

Table 3 and Figure 3 show the prevalence of mastitis at the total sampled cow population level was 50.7% (76/150). Out of these, clinical mastitis was 24.7% (37/150, sub-clinical mastitis was 17.3% (26/150) and cows with both clinical and sub-clinical mastitis were 8.7% (13/150). The udder quarter prevalence was 21.8% (131/600). Out of this 11.5% (69/600) were clinical mastitis while 10.3% (62/600) were subclinical mastitis (Table 8). Of the 150 lactating cows sampled, Moisbridge had the highest incidence of mastitis (both clinical and subclinical) at 43.4% (33/76) followed by Sugoi at 28.9% (22/76) and lastly Ziwa Machine area 27.6% (21/76). However, there were no statistically significant differences in prevalence between the three study areas. The prevalence of clinical mastitis in Sugoi and Moisbridge was higher than that of subclinical mastitis. In Ziwa the opposite was true.

Table 3: Prevalence of the three forms of mastitis in the study areas

Study site	No. of cows sampled	Clinical mastitis	Subclinical mastitis	Mixed infections (both clinical and sub-clinical)	Total positive reactors
Sugoi	50	10	6	6	22
Moisbridge	50	18	10	5	33
Ziwa Machine	50	9	10	2	21
Total	150	37	26	13	76
Percent prevalence among cows	**100**	**24.7**	**17.3**	**8.7**	**50.7**

The overall percent prevalence within the sites/ blocks and among the cows is 50.7%.

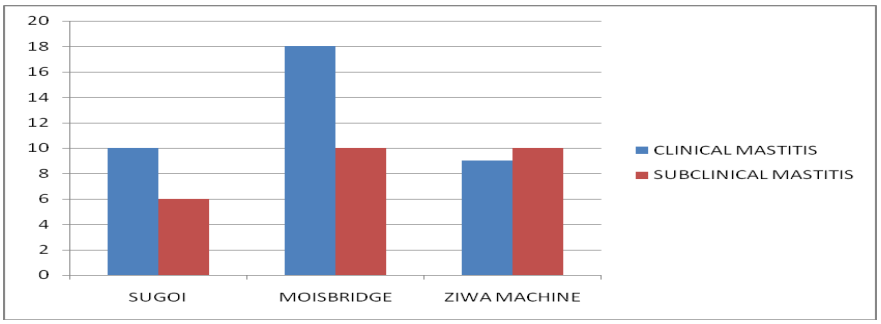


Figure 4: A comparison of the prevalence of clinical and subclinical mastitis within the sites.

Table 4: The prevalence of mastitis by udder quarters

Quarter prevalence of mastitis	Out of 600 quarters	% Prevalence
Clinical mastitis	69/600	11.5
Subclinical mastitis	62/600	10.3
Total infected	131/600	21.8

Identification of the Genera of Mastitis Causing Microorganisms Isolated from Laboratory Culture of Milk Samples

Out of the 76 samples tested, 72 cultures had isolates while 4 cultures had none. Of the 72 cultures with isolates 66 (91.7%) grew one type of bacterium or yeast while 6 (8.3%) grew mixed infections. Six genera of bacteria and 1 of yeast (*Candida*) were isolated and identified. The microbe genera identified in decreasing order of prevalence were *Staphylococcus* 24 (31.6%), *Escherichia* 17 (22.4%), *Klebsiella* 14 (18.4%), *Streptococcus* 13 (17.1%), *Corynebacterium* 2 (2.6%), *Pseudomonas* 1 (1.3%) and *Candida* 1 (1.3%) as shown in table 5.

Table 5: Types of mastitis causing microorganisms isolated and identified in milk samples from different study areas

Microorganism genus	Sugoi	Mois bridge	Ziwa machine	Total samples of that isolate	Prevalence %	Order of ranking in frequency
Staphylococcus sp.	6	12	6	24	31.6	1
Escherichia sp.	5	7	5	17	22.4	2
Klebsiella sp.	3	7	4	14	18.4	3
Streptococcus sp.	4	6	3	13	17.1	4
Corynebacterium sp.	1	1	0	2	2.6	5
Pseudomonas sp	0	0	1	1	1.3	6
Candida sp.	1	0	0	1	1.3	6
No isolate	3	1	0	4	5.3	
Total	23	34	19	76		

Antimicrobial Resistance by the Isolated Bacteria

The increasing order of *in vitro* resistance to the antimicrobials by the microbe samples was gentamicin (2.2%; 3 samples), kanamycin (2.8%; 8 samples), streptomycin (5.1%; 22 samples), tetracycline (11.2%; 39 samples), ampicillin (15.2%; 48 samples),

nalidixic acid (15.9%; 52 samples), chloramphenicol (16.6%; 54 samples), sulphamethoxazole (17.3%; 58 samples) and cotrimoxazole (17.3%; 58 samples) (Tables 6 and 7). The three aminoglycosides (gentamicin, kanamycin and streptomycin) as a group had the least number of samples resistant to them with a combined total of 33 (mean of 9.8%). They were followed in increasing order of resistance by the tetracyclines 39 (11.3%), the penicillins 48(15.2%), the quinolones 53 (15.9%), the chloramphenicols 54(16.6%) and lastly the sulphonamides 116 (34.6%) (Table 8 and table 7). Only two samples of *E. coli* and one of *Streptococcus sp*. were found to be resistant to gentamicin (Table 6). The effectiveness of the sulphonamides (combined total of 1.2% for sulphamethoxazole and cotrimoxazole) as a group was found to be very low. The percentage of resistance of the isolates to the antimicrobials agents tested across all the types of antimicrobials was found to be *Pseudomonas* sp. (20% mean), *Corynebacterium* sp. (14.3%), *Staphylococcus* sp (12.5%), *Klebsiella* sp. (12.4%), *Streptococcus* sp (11.1%) and *E. coli* (11.1%) as indicated in Table 9.

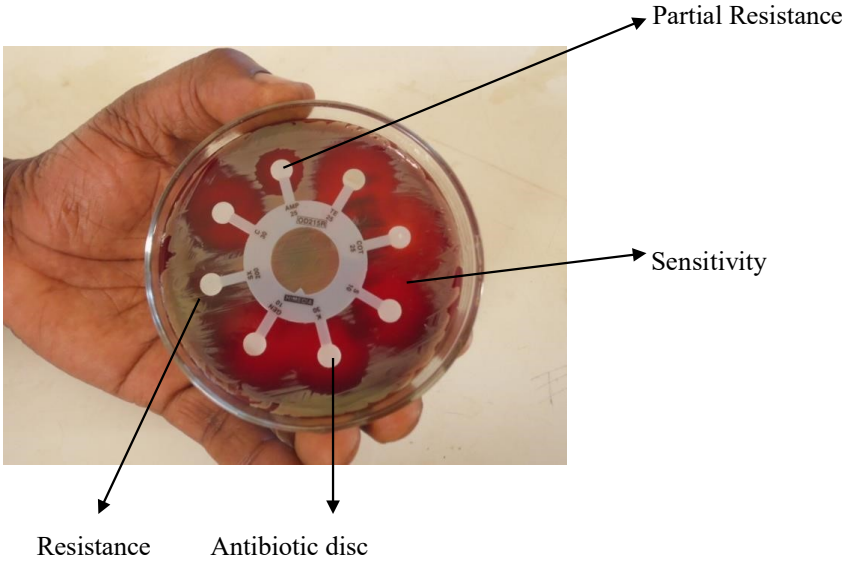


Figure 5: Results of culture showing resistance and sensitivity tests in blood agar.

Table 6: Resistance of the isolated genera to antimicrobial agents

	Number of microbe samples resistant to the individual antimicrobial types									Total No. of samples showing cross resistance	Mean cross resistance
Type of microorganism isolated	**AMP**	**NA**	**TE**	**C**	**SX**	**COT**	**S**	**K**	**GEN**		
Staphylococcus sp	11	18	7	16	18	18	5	2	0	95	11.87
E. coli	16	12	15	15	17	17	6	2	2	102	11.33
Klebsiella sp	13	12	11	13	13	13	8	2	0	85	10.62
Streptococcus. Sp	5	7	5	7	7	7	2	2	1	43	4.78
Corynebacterium. Sp	2	2	1	2	2	2	1	0	0	12	1.71
Pseudomonas sp	1	1	0	1	1	1	0	0	0	5	1.00
Total	48	52	39	54	58	58	22	8	3	342	

Table 7: Percentage of samples resistant to individual antimicrobials

Type of microorganism isolated	AMP	NA	TE	C	SX	COT	S	K	GEN	Mean % Resistance by microbes to all the antimicrobials
Staphylococcus sp.	11.6	18.9	7.4	16.8	18.9	18.9	5.3	2.1	0	12.5
Escherichia sp.	15.7	11.8	14.7	14.7	16.7	16.7	5.9	2	2	11.1
Klebsiella sp.	15.3	11.8	14.1	15.3	15.3	15.3	9.4	2.4	0	12.4
Streptococcus Sp.	11.6	16.3	11.6	16.3	16.3	16.3	4.7	4.7	2.3	11.1
Corynebacterium Sp.	16.7	16.7	8.3	16.7	16.7	16.7	8.3	0	0	14.3
Pseudomonas sp.	20	20	0	20	20	20	0	0	0	20
Mean % cross resistance to each antimicrobial agent	15.2	15.9	11.2	16.6	17.3	17.3	5.1	2.8	2.2	

NB: The means indicate the overall resistance to individual antimicrobials across bacterial genera.

An analysis of variance (ANOVA) of this data and comparison of the means of the antimicrobials is given in Table 8 below.

Table 8: The means of resistance to antimicrobials across micro-organisms

Antimicrobial	Mean
Ampicillin	15.15a
Nalidixic acid	15.92a
Tetracycline	9.35b
Chloramphenicol	16.63a
Sulphamethoxazole	17.32a
Cotrimoxazole	17.32a
Streptomycin	5.60b
Kanamycin	1.87c
Gentamicin	0.72c

S.E.M. ± 3.10 Means not sharing the same letter are significantly different ($p < 0.05$).

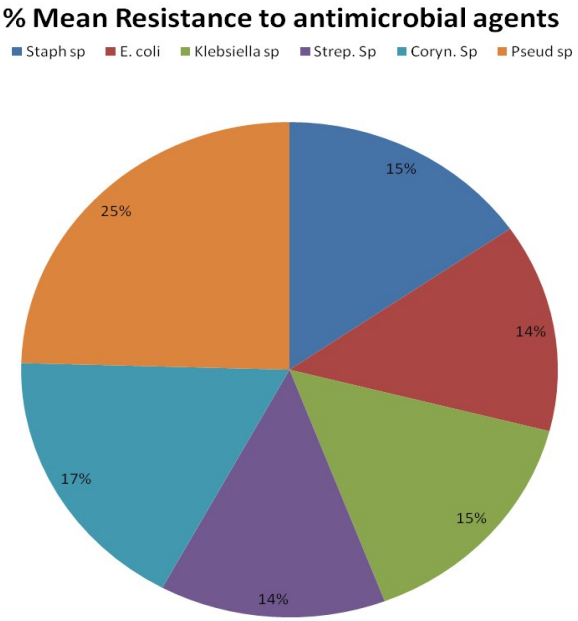


Figure 6: Mean % Resistance by microbes to antimicrobials.

Table 9: Number of microorganism samples resistant to each antimicrobial group

	Group1	**Group2**	**Group3**	**Group4**	**Group5**	**Group6**	**Summary as mean number resistance across groups**
Staphylococcus Sp	11	18	7	16	36	7	15.83
Escherichia sp.	16	13	15	15	34	10	17.16
Klebsiella sp.	13	12	11	13	26	10	14.16
Streptococcus sp.	5	7	5	7	14	5	7.16
Corynebacterium Sp.	2	2	1	2	4	1	2.00
Pseudomonas sp.	1	1	0	1	2	0	0.83
Mean resistance to each antimicrobial group	8.00	8.83	6.50	9.00	19.33	5.50	

KEY: Group 1 = Penicillin's (Ampicillin)
Group 2= Quinolones (Nalidixic acid)
Group 3= Tetracyclines (Tetracycline)
Group 4= Chloramphenicol (Chloramphenicol)
Group 5= Sulphonamides (sulphamethoxazole and cotrimoxazole)
Group6 = Aminoglycosides (streptomycin, kanamycin and gentamicin)

The order of least resistance is 5.50 (aminoglycosides, 6.50 (tetracyclines), 8.00 (penicillins), 8.83 (quinolones), 9.00 (chloramphenicol), 19.33 (sulphonamides)

Table 10: Summary of % resistance to each antimicrobial group by the isolated microorganisms

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group6**	**Mean resistance of each bacterial specie across antimicrobial groups**
Staphylococcus Sp	11.6	18.7	7.4	16.8	37.9	7.4	16.63
E.coli	15.7	11.8	14.7	14.7	33.3	9.8	16.67
Klebsiella sp	15.3	11.8	14.7	15.3	30.6	11.8	16.58
*Streptococcu*s Sp	11.6	16.3	11.6	16.3	32.6	11.6	16.67
*Corynebacterium*Sp	16.7	16.7	8.3	16.7	33.3	8.3	16.67
Pseudomonas sp	20	20	0	20	40	0	16.67
Mean % resistance within each group	15.2	15.9	11.3	16.6	34.6	9.8	

KEY: Group 1 = Penicillins
Group 2 = Quinolones
Group 3 = Tetracyclines
Group 4 = Chloramphenicol
Group 5 = Sulphonamides
Group 6 = Aminoglycosides

NB: The means indicate the overall resistance to the group of antimicrobial by the micro-organisms.

An analysis of variance (ANOVA) of this data and comparison of the means of the antimicrobial group is given below in Table 11.

Table 11: The means of resistance to antimicrobial groups by the microorganisms

Antimicrobial group	**Means**
Group 1 (Penicillins)	15.15b
Group 2 (Quinolones)	15.88b
Group 3 (Tetracyclines)	9.45c
Group 4 (Chloramphenicol)	16.63b
Group 5 (Sulphonamides)	34.55a
Group 6 (Aminoglycosides)	8.15c

S.E.M. ± 4.18

Means not sharing the same letter are significantly different ($p < 0.05$).

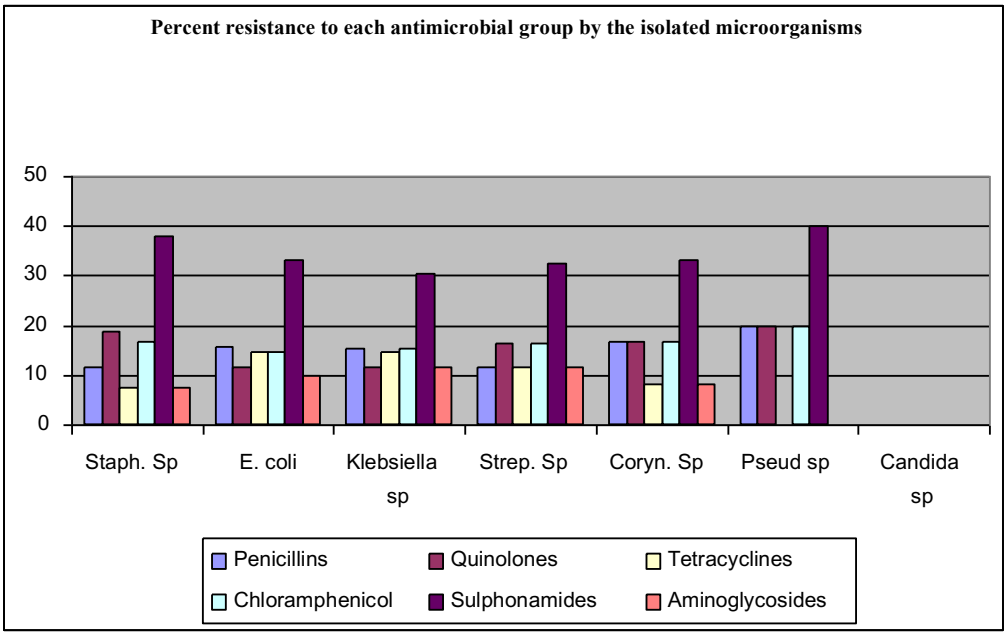
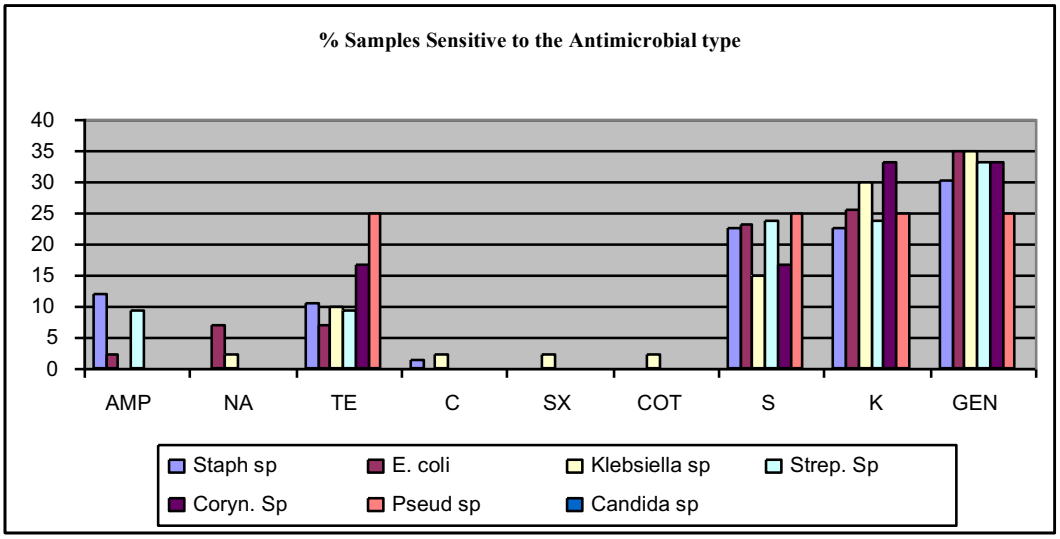


Figure 7: Percent resistance of the isolated microorganism genera to different antimicrobial groups.



KEY: AMP = Ampicillin; NA = Nalidixic acid; TE = Tetracycline; C = Chloramphenicol; SX = Sulfamethoxazole; COT= Cotrimoxazole; S = Streptomycin; K = Kanamycin; GEN= Gentamicin

Figure 14: Percentage of samples sensitive to various antimicrobial agents.

DISCUSSION

The combined mean prevalence of both clinical and subclinical mastitis in the three study sites was 50.67% with an udder quarter infection rate of 21.8%. This is lower than 68.8% reported by Bishi et al. (2003) on prevalence of mastitis around Addis Ababa in Ethiopia and 82.9% found by Ondiek et. al. (2013) at Tatton farm of Egerton University, Njoro in Kenya. According to

Blood et al. (2006), a major survey of dairy herds in the United Kingdom showed a mastitis prevalence of about 40%, which is lower than the findings, and a quarter infection rate of 27% which was higher than ours of 21.8%. The overall udder quarter prevalence of clinical mastitis and subclinical mastitis was 11.5% and 10.3% respectively. However, the udder quarter prevalence of subclinical mastitis in all the three areas which averaged 10.3% is high and is, therefore, an area of concern as it portends a silent reduction in milk yield from the affected cows, and it could expose healthy animals to contagious pathogens, by acting as a reservoir within the herd, which may then progress to become clinical mastitis. Worse still, subclinical mastitis can progress further to chronic infection that is unresponsive to antibiotic treatment (Hortet and Seegers, (1998). Barlow et al. (2009) found that approximately 25-30% of cows with chronic cases of subclinical mastitis may exhibit clinical symptoms that require antibiotic treatment and withholding of milk with loss of income to the farmer. Hence there is a need for improvement of the detection and management of subclinical mastitis on the dairy farms.

Several mastitis causing pathogens were isolated and identified up to the genus level. The order of prevalence of the pathogens so isolated was *Staphylococcus sp* (31.6%), Escherichia *sp* (22.4%), *Klebsiella sp* (18.4%), *Streptococcus sp* (17.1%), *Corynebacterium sp* (2.6%), *Pseudomonas sp* (1.3%), *Candida sp* (1.3%). The prevalence of *Staphylococcus sp* in this study was similar (37.6%) to the findings of a study by Odongo et al. (2013) conducted around Kabete area of Kiambu County. It is however much lower than the 58.8% realized by Ondiek et al. (2013) in Njoro. In all the three different studies *Staphylococcus* species was the most prevalent cause of mastitis. The prevalence of *E. coli* (22.4%) and *Klebsiella sp* (18.4%) on the other hand is much higher compared to 17.2% and 9.7% of Ondieki et al. (2013) respectively probably suggesting a lower effort on farm hygiene in the area of study given that these two coliforms are environmental pathogens. The three coliforms; *Escherichia sp*, *Klebsiella sp* and *Pseudomonas sp*, had a combined total prevalence of 42.1%. This is quite high and suggests a high incidence of poor hygiene in and around the milking parlors since they are environmental agents that cause mastitis. *Staphylococcus sp* were high at 31.6% in prevalence. This is indicative of a high rate of spread of mastitis by contact since all *Staphylococcus sp* (except coagulase –ve *Staphylococci*) are contagious agents.

There were different degrees of resistance to the various antimicrobials by the isolated microbes. In general, the three aminoglycosides were the antimicrobials to which the majority of the isolated pathogens had the least resistance with gentamicin having the least at 2.2% followed by kanamycin at 2.8% and streptomycin at 5.1%. There were only two *Escherichia sp* and one *Streptococcus sp* sample isolates that were resistant to gentamicin. This is very encouraging since gentamicin is a last line drug for the treatment of mastitis and especially that caused by coliforms.

The other aminoglycosides also need to be used with caution to avoid development of resistance to them by microbes. The widespread resistance to the two sulphonamides (cotrimoxazole 17.3% and sulphamethoxazole 17.3%) at the other extreme is reason for worry since it suggests a possible long term or indiscriminate use of antimicrobial preparations containing them as the active ingredients in the areas studied allowing the pathogens to develop resistance to them. Hence there is need for caution in their use to avoid further development of resistance or their use without success in treating mastitis. An Analysis of Variance (ANOVA) shows that there is no significant difference ($p < 0.05$) between ampicillin, nalidixic acid, chloramphenicol, sulphamethoxazole and cotrimoxazole in terms of resistance to them across the bacterial genera. The same is true for tetracycline and streptomycin and forf

kanamycin and gentamicin. In terms of total resistance by the microbes, *Pseudomonas sp* was the highest at 20%. The two coliforms *Klebsiella sp* and Escherichia *sp.* are Gram - bacteria that, are now largely resistant to the sulphonamides, penicillins and tetracyclines. They are quite sensitive to the aminoglycosides especially to gentamicin and kanamycin both of which have preparations available in our market. Ondiek et al. (2013) listed the best three drugs, in decreasing order of effectiveness at treating mastitis among dairy cows at Tatton farm of Njoro, as Augmentin® (a combination of amoxicillin and clavulanic acid), gentamicin and cotrimoxazole. In our study the order was found to be gentamicin, kanamycin and streptomycin all aminoglycosides. Cotrimoxazole was the least effective. However, this study did not investigate Augmentin®.

CONCLUSION

The study identified the following factors as important contributors to the prevalence of mastitis in the areas studied; A high overall prevalence of mastitis (50.7%) among the dairy farms and failure to detect and recognize subclinical mastitis cases that then act as reservoirs for mastitis- causing bacteria. A low frequency of routine testing for mastitis at milking among the farmers and lack of awareness about the advantages of pre and post dipping of teats in suitable germicides at milking as control measures. Widespread resistance by some of the microbes to some antimicrobials such as sulphamethoxazole (17.3%) and co-trimoxazole (17.3) that are among the most commonly used around here to treat mastitis.

RECOMMENDATIONS

The following measures were therefore recommended to alleviate the problem:

1. Regular routine testing for mastitis at milking by all the farmers to detect subclinical mastitis using a strip cup or by such electronic devices as the Draminski subclinical mastitis detector.
2. Prompt and vigilant treatment of any mastitis cases found using antimicrobials to which there has not been much resistance by the microbes such as gentamicin (2.2% resistance), kanamycin (2.8%) and streptomycin (5.1%) to avoid subclinical maturing to clinical mastitis and to minimize development of resistance by the microbes to the antimicrobials used in mastitis treatment.
3. Avoidance of routine use of the sulphonamide-based antimicrobials especially cotrimoxazole and sulphamethoxozole to treat mastitis in the areas unless culture and sensitivity tests have proved them useful in each case.
4. Improvement of hygiene of the farm environment especially at the milking parlor to minimize the presence of mastitis causing agents that are environmental in origin such as *Escherichia sp* .and *Klebsiella sp*.
5. Studies on the mechanisms of antimicrobial resistance by the microbes and other risk factors associated with the development of mastitis among dairy cattle in the area.

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