

Prevalence of bovine mastitis and antimicrobial sensitivities of the bacterial causes in smallholder farms of Kisumu County, Kenya

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Abstract

Prevalence of bovine mastitis in Kisumu County, risk factors and antibiotic sensitivities of the causative bacteria were determined in this cross-sectional study. Sub-clinical mastitis (SCM) was diagnosed using California Mastitis Test (CMT). Risk factors were identified through the administration of 64 questionnaires and assessment of 134 lactating cows. Bacteria were identified by culturing 72 CMT-positive udder quarter milk samples and their sensitivities to antibiotics investigated using Kirby-Bauer disc diffusion test. Only SCM was detected and had cow level prevalence of 33% (44/134). Prevalence of SCM was significantly ($p < 0.05$) higher in cows under complete and semi-zero grazing systems, at mid lactation, those pregnant and with parity of 1–3. *Staphylococcus* species was the most common (63.8%, $n = 58$) isolate. Other isolates were *E. coli* (13.8%), *Streptococcus* species (12.1%) and *Pseudomonas* (5.2%). *Staphylococcus* and *Streptococcus* isolates were 100% sensitive to streptomycin, kanamycin, gentamycin and chloramphenicol. Additionally, *Streptococcus* species were 100% sensitive to ampicillin, tetracycline and cotrimoxazole. *Staphylococcus* species had developed varying levels of resistance against sulfamethoxazole, cotrimoxazole, ampicillin and tetracycline. *Streptococcus* species was 100% resistant to sulfamethoxazole. A significantly high SCM prevalence was reported in this study thus an appropriate control strategy is needed that consists of awareness creation, good milking hygiene practices, teat disinfection, regular screening for SCM and preventing spread of mastitis in the herd by milking infected cow(s) last.

Keywords: Bacteria, California Mastitis Test, dairy cattle, risk factors, sub-clinical mastitis

1 Introduction

Mastitis, refers to inflammation of mammary glands and is the most economically important disease in dairy milk production globally (Viguier *et al.*, 2009; Ndirangu *et al.*, 2019), yet current data on the disease is less readily available (DaRong *et al.*, 2010). Dairy cattle provide milk for families and are a source of income, especially in smallholder farms which form majority of dairy farms in developing countries like Kenya (Van Leeuwen *et al.*, 2012). Losses associated with mastitis include reduced milk production and lowered milk quality, veterinary costs, milk rejection at collection points and shortening of cows' productive life (Mungube

et al., 2005; Hogeveen *et al.*, 2011). In addition, mastitis poses a public health risk through transmission of zoonotic diseases to humans (Radostits *et al.*, 2000).

Mastitis is mainly caused by infectious pathogens such as bacteria, viruses, mycoplasma, yeasts and algae (Chaneton *et al.*, 2008). Bacteria cause majority of mastitis infections although only a few species are incriminated namely *Staphylococcus* spp, *Streptococcus* spp, *Escherichia coli* and *Corynebacterium bovis* (Gitau *et al.*, 2014; Ndirangu *et al.*, 2017). The disease has been reported among dairy cattle in different parts of Kenya (Ondieki *et al.*, 2013; Ndirangu *et al.*, 2019). However, its prevalence varies from one geographical area to another depending on climatic conditions, prevailing risk factors and management practices. Kisumu

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County is an emerging dairy zone compared to the high dairy potential zones of central Kenya and the Rift-Valley. The county has varying dairy cattle production systems consisting of intensive, semi-intensive and extensive systems. It was important to identify mastitis risk factors since the factors vary with climatic conditions and production systems. It was also important to identify bacterial causes of mastitis in the county and the drugs of choice since this is a pre-requisite in developing an effective mastitis control program for an area. Indeed, effective therapy and prophylaxis of mastitis are mainly hindered by having inadequate current epidemiological data in an area, lack of information on the prevailing mastitis causative agents and drugs of choice to be used in mastitis therapy. Additionally, routine *in-vitro* antibiotic sensitivity testing of milk prior to commencement of treatment regime is recommended as a way of preventing indiscriminate use of antibiotics, accumulation of drug residues in milk and guarding against development of antimicrobial resistance. This study was meant to provide an insight into the status of mastitis problem in dairy cattle herds, a current profile of the prevailing mastitis causing organisms and the most effective antimicrobial agents for therapy in Kisumu County.

2 Materials and methods

2.1 Study area and sampling

The study was undertaken in Kisumu County, western Kenya. Out of the six sub-counties four were purposively selected depending on proximity to Kisumu city, urbanisation level, and dairy cattle population and management system to represent varying dairy production systems. Selected sub-counties included Kisumu East and Kisumu West representing urban and peri-urban production systems, and Nyando and Nyakach representing rural and extensive dairy production systems, respectively.

Multi-stage sampling was employed to select study farms. Dairy farms were randomly selected from the list of farmers generated by sub-county extension officers and list of active members of dairy farmers' co-operatives. The number of smallholder dairy farms included in the study was 64.

2.2 Study design

The study was cross-sectional where data were collected at a single point in time (Thrusfield, 2005), thus during the month of April, 2018, each farm was visited once. Farms to be sampled were randomly selected from a list of dairy farms generated by the respective sub-county extension officers completed by a list of active members of dairy co-operatives. During the farm visit cow examination and milk



Fig. 1: Map of Kisumu County showing the study sub-counties.

sample collection was done on the same day as the questionnaire was administered.

A smallholder dairy farm was defined as one having one cow being milked or a minimum of 3 cows and a maximum of 16 adult dairy cows (Aleri *et al.*, 2012). In farms with 5 or less lactating cows, all were examined; while in farms with more than 5 milking cows simple systematic sampling was used where every second cow was examined. Within farms cow examination and milk sampling was done as per the routine milking practice in the particular farm following the established order in which the cows follow each other at milking time. Within the 64 farms, a total of 134 lactating cows were examined.

2.3 Questionnaire survey

The risk factors of mastitis associated with management and socio-cultural issues were identified through administration of questionnaires. Factors captured in the questionnaire included attributes of herd owners like gender, age and level of education. The management factors captured were grazing system, mastitis control practices like on-farm mastitis testing, washing of udder prior to milking, use of towels, milking jelly and teat dips, order of milking mastitic cow(s) in the herd, and handling of mastitic milk. Additionally, the status of milking hygiene was scored as good (where the

recommended hygienic milking was practiced), fair (where only part of hygienic milking practices were applied) or poor (where hygienic milking practices were not at all in use).

2.4 Clinical mastitis

Physical examination of the lactating cow culminated in detailed examination of the udder. Milk from each quarter was observed for change in colour and consistency. Each cow examined had her characteristics captured in an individual cow's sample record form. Details included breed, lactation stage, parity, pregnancy status and milking hygiene.

2.5 Sub-clinical mastitis

Screening for sub-clinical mastitis (SCM) was performed using California Mastitis Test (CMT) as described by Quinn *et al.* (1999). About 2 ml of milk from each quarter was put in each of the four wells of a CMT-paddle. An equal amount of commercial CMT reagent was added and the mixture swirled for 15 seconds. The CMT results were read and scored based on gel formation as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). A cow was defined as having SCM when at least one of its udder quarters had a CMT score of 1.

2.6 Bacterial growth

Individual CMT positive quarter milk samples were collected aseptically just before milking [National Mastitis Council (NMC) (2004)] for establishing causes of mastitis. Just before collecting milk the udder and milker's hands were washed with clean water and teats swabbed with cotton dipped in 70 % ethyl alcohol. The first 2 squirts of milk were discarded before samples were collected into sterile bi-jou bottles and appropriately labelled. In the field and during transportation to the laboratory milk samples were preserved in an ice-packed cool box. In total, 72 CMT positive milk samples were analysed in the bacteriology laboratory of KALRO-Veterinary research Centre Muguga, within one week after collection. In the laboratory, samples were stored under refrigeration at 4 °C. Bacterial culture and isolation was performed according to the standard microbiological procedures described by NMC (2004). The petri-dishes were examined for growth after 24 hours. Growth was examined visually and scored 1-4 depending on the number of streaking lines where growth was evident. Bacteria genera were identified through growth characteristics and microscopically examination after gram-staining (Gitau *et al.*, 2014). Biochemical tests such as rapid slide catalase technique (Cheesbrough, 1985) and indole test were the confirmatory tests undertaken.

2.7 Antibiotic sensitivity

Bacterial cultures selected for sensitivity testing were those yielding pure colonies and with a growth score of three and four. Using this criterion 11 *Staphylococcus* and four *Streptococcus* isolates were selected. The isolates were tested against eight antibiotics using the Kirby-Bauer disc diffusion method as described by Quinn *et al.* (1999). The effectiveness of each drug was determined based on the ability of the test antibiotic to inhibit growth indicated by the diameter of zone of inhibition around the disc; the larger the diameter the more effective the drug. A scale of 0–3 as described by Gitau *et al.* (2011) was used to score the relative sensitivity of a particular bacterium into resistant, slightly sensitive, sensitive and very sensitive to the test antibiotic.

2.8 Data entry and analysis

Field and laboratory data were entered into MS-excel 2007 worksheets where descriptive statistics were generated. Exploration of data was done in form of numerical summaries and tabulation. Prevalence was calculated as the number of cows diagnosed with mastitis divided by the total number of cows examined and expressed as a percentage (Thrusfield, 2005). Cows with mastitis were stratified by stage of lactation into early (0-3 months), mid (> 3-9 months) and late (> 9 months) as described by Ndirangu *et al.* (2017). Similarly cows were stratified on the basis of parity into few (1-3 times), moderate (4-6 times) and many (> 6 times). The association between mastitis prevalence and risk factors was determined using Pearson Chi-square tests for independence with statistical significance set at $p < 0.05$ (95 % CI). In addition, the data were subjected to logistic regression using R-program version 4.1.0 in order generate the odds ratio on the cow level and some management risk factors. The isolates were first classified by genus and the isolation rate calculated as number of isolates of each genus divided by total number of milk samples cultured and expressed as a percentage. Similarly, sensitivity was calculated as the number of isolates that were sensitive to each of the eight antibiotics (those with diameter of zone of inhibition of > 8 mm) divided by the total number of isolates tested. The sensitive isolates were further classified based on diameter of zone of inhibition into slightly sensitive (9-15 mm), sensitive (16-22 mm) and very sensitive (> 22 mm).

3 Results

All 64 farmers interviewed responded to the questionnaire. Age of farmers ranged from 22 to 84 years. Socio-cultural characteristics of farmers and mastitis management practices varied as depicted in Table 1.

Table 1: Demographic characteristics of farmers and mastitis management practices in smallholder dairy farms of Kisumu County.

Variable, n = 64	Type/response	% (95% CI)
Gender	Male	58 (45.5-69.4)
	Female	42 (30.6-54.5)
Education level	No formal education	11 (4.9-20.4)
	Primary	23 (14.3-35)
	Secondary	30 (19.5-41.7)
	College	6 (2-14.4)
	University	30 (19.5-41.7)
Milking method	Hand	98.5 (87-99.5)
	Machine	1.5 (0.7-2.3)
Wiping of cows with towel	Yes	75 (63.3-84.4)
	No	25 (15.6-36.7)
Separate towel per cow	Yes	30 (19.5-41.7)
	No	70 (58.3-80.5)
Use of milking jelly	Yes	84.4 (73.9-91.8)
	No	15.6 (8.2-26.1)
Milking jelly used	Medicated	45 (33.5-57.6)
	Non-medicated	52 (39.4-63.6)
	Both types	3 (0.5-9.9)
Post-milking teat dipping	Yes	16 (8.2-26.1)
	No	84 (73.9-91.8)
Order of milking mastitic cow(s)	Last	30 (19.5-41.7)
	No order	12.5 (6-23.2)
	First	1.6 (0.1-7.5)
	No response	56.3 (44-68)
Discarding of mastitic milk	Given to dogs and calves	21.9 (13-33.2)
	On the ground	20.3 (11.8-31.5)
	Humans consume	1.6 (0.1-7.5)
	No response	56.3 (44-68)

Cows examined during the study had only SCM with a cow level prevalence of 33% (44/134). The prevalence was highest in exotic cattle (Ayrshire and Friesian), cows at mid stage of lactation, with few parity and those pregnant (Table 2). The effect of management related risk factors namely, grazing system and milking hygiene, on prevalence of SCM varied (Table 2).

Out of 72 CMT positive udder quarter milk samples subjected to bacterial culture 80.6% (58/72) showed positive bacterial growth. *Staphylococcus* species was most isolated at 63.8% (37/58), followed by *E. coli* 13.8% (8/58), *Streptococcus* species 12.1% (7/58), then *Pseudomonas* and other coliforms at 5.2% (3/58) each.

Antimicrobial sensitivity profiles of 11 *Staphylococcus* species and four *Streptococcus* species isolates varied as shown in Table 3, where *Streptococcus* species were fully sensitive to all antimicrobials except sulfamethoxazole which had 100% resistance. All *Staphylococcus* isolates

were sensitive to streptomycin, kanamycin, gentamycin and chloramphenicol.

4 Discussion

Findings of this study revealed that bovine mastitis is present among lactating cattle reared in smallholder dairy farms of Kisumu County. Although the disease has two forms, in the study area only sub-clinical mastitis was diagnosed. This is consistent with the findings of a previous study in a dairy herd at a research farm in Naivasha that found only SCM (Ndirangu et al., 2017). The Naivasha herd had a mastitis cow level prevalence of 36% which is close to the 33% prevalence reported in the current study. This finding can be attributed to the fact that SCM in most instances is occult and most efforts are directed to treating clinical case(s). In the current study absence of clinical form of mastitis may be attributed to the limited or small sample of cows

Table 2: Cow level and management risk factors of sub-clinical mastitis among cows in Kisumu County, Kenya (N = 134)

Risk factor	Variable	n	% prev (95% CI)	Odds ratio	p-value
Breed	Local zebu	23	8.7 (1.5-24.5)	0.1	
	Crossbreeds	22	40.9 (13.0-83.4)	6.2**	0.021**
	Ayrshire	44	38.6 (13.6-81.0)	6.6**	0.018**
	Friesian	43	37.2 (12.8-80.0)	7.3**	0.023**
	Guernsey	2	0	0	
Stage of lactation	Early (0-3 months)	31	10.0 (2.6-23.9)	0.1	
	Mid (> 3-9 months)	56	36.2 (14.8-72.0)	5.1**	0.015**
	Late (> 9 months)	47	43.5 (18.7-78.0)	6.9**	0.004**
Parity	= 1	54	19.3 (10.2-31.8)	0.2	
	> 1	80	24.7 (11.0-45.8)	1.4**	0.018**
Pregnancy status	Non-pregnant	86	20.0 (12.5-29.3)	0.3	
	Pregnant	48	55.1 (24.6-81.8)	4.9**	0.000**
Grazing system	Extensive	25	12.0 (3.1-28.2)	0.1	
	Semi-zero	62	33.9 (3.5-87.1)	3.8**	0.049**
	Complete zero	47	42.6 (4.9-90.8)	5.4**	0.013**
Milking hygiene status	Poor	48	36.7 (24.2-50.7)	0.6	
	Fair	48	32.0 (10.1-65.6)	0.8	0.620
	Good	38	28.6 (7.8-64.1)	0.7	0.435

** Odds ratio > 1 and is significant (p < 0.05); prev. denotes prevalence; (95%CI is the 95% confidence interval); Parity = 1 means cow has given birth once (uniparous) and > 1 means cow has given birth more than once (multiparous).

Table 3: Antimicrobial sensitivities of Staphylococcus and Streptococcus isolates from mastitic cattle milk samples in Kisumu County.

Antimicrobial drug	Resistant (0-8 mm)	Slightly sensitive (9-15 mm)	Sensitive (16-22 mm)	Very sensitive (> 22 mm)	Overall sensitivity
Staphylococcus species, n = 11					
Ampicillin	2 (18%)	2 (18%)	2 (18%)	5 (46%)	9 (82%)
Tetracycline	1 (9%)	6 (55%)	2 (18%)	2 (18%)	10 (91%)
Cotrimoxazole	7 (64%)	0	4 (36%)	0	4 (36%)
Streptomycin	0	1 (9%)	6 (55%)	4 (36%)	11 (100%)
Kanamycin	0	3 (27%)	6 (55%)	2 (18%)	11 (100%)
Gentamycin	0	0	2 (18%)	9 (82%)	11 (100%)
Sulfamethoxazole	9 (82%)	2 (18%)	0	0	2 (18%)
Chloramphenicol	0	0	8 (73%)	3 (27%)	11 (100%)
Streptococcus species, n = 4					
Ampicillin	0	2 (50%)	1 (25%)	1 (25%)	4 (100%)
Tetracycline	0	0	3 (75%)	1 (25%)	4 (100%)
Cotrimoxazole	0	1 (25%)	2 (50%)	1 (25%)	4 (100%)
Streptomycin	0	1 (25%)	2 (50%)	1 (25%)	4 (100%)
Kanamycin	0	1 (25%)	3 (75%)	0	4 (100%)
Gentamycin	0	0	0	4 (100%)	4 (100%)
Sulfamethoxazole	4 (100%)	0	0	0	0
Chloramphenicol	0	0	4 (100%)	0	4 (100%)

examined, which usually happens in most cross-sectional studies. However, some authors have reported presence of both clinical and sub-clinical forms of mastitis. For ex-

ample Ondieki *et al.* (2013) reported that a cattle herd in Nakuru County had both forms of mastitis with sub-clinical mastitis being more prevalent than clinical mastitis. Simi-

lar findings were also reported by Ndirangu *et al.* (2019) for cows in Kajiado County. Usually the form of mastitis is determined by the degree of inflammation which depends on many factors such as nature of causative pathogen, breed, age, health and lactation cycle of the animal (Radostits *et al.*, 2000). Indeed, since sub-clinical mastitis is not visible to the naked eyes, farmers cannot easily decide on what order to milk mastitic animals. This can lead to the spread of mastitis within the herd and ensuing severe economic losses including progressing to clinical mastitis.

The 33 % SCM prevalence reported in the current study is lower than the 64 % prevalence of SCM for cows in Thika (Mureithi & Njuguna, 2016) and 56 % among cows in Kajiado (Ndirangu *et al.*, 2019), both in Kenya. The low prevalence found may be attributed to some management and mastitis control practices observed among the farms visited, although these factors were not subjected to statistical analysis. Most of the previous reports (Mureithi & Njuguna, 2016; Ndirangu *et al.*, 2017) have indicated very low on-farm application of such practices. Additionally, variability in the prevalence of bovine mastitis between research studies is suggestive of the complex nature of mastitis. The disease has several etiological pathogens and an array of risk factors differences in farm management, breeds, prevailing environmental conditions, variations in veterinary service provision and farmers level of awareness towards the disease.

Among the cow-level risk factors of mastitis investigated there were significant differences in prevalence of mastitis between different breeds where crossbreeds and exotic breeds (Ayrshire & Friesian) had higher odds of getting infected with sub-clinical mastitis with a risk of 6-7 times higher than local Zebu breeds ($p < 0.05$). Similarly cows in mid and late stage of lactation have risk of 5 to 6.9 times higher than those in early lactation. Mureithi & Njuguna (2016) reported similar findings for cows in Thika sub-County where SCM prevalence was highest in Ayrshire breed. This can be attributed to the higher milk yield among exotic breeds as compared to indigenous cattle. High yielding cows are generally more prone to mastitis for several reasons such as not completely emptying the udder, especially where hand milking is used which was the case for most (98.5 %) of the farms in the current study. Radostits *et al.* (2000) reported that higher-yielding cows are more susceptible to mastitis due to the position of teat and udder, and anatomy of teat canal, that make them prone to injury. They also associated this to less efficacy of phagocytes in such cows which is associated with a dilution effect.

Mureithi & Njuguna (2016) similarly reported that SCM was most prevalent during mid stage of lactation as reported in this study. This was also the case for the Sahiwal herd at

a research centre in Naivasha (Ndirangu *et al.*, 2017). This is probably associated with more milk production since in most cases cows attain peak milk production at this stage which increases susceptibility of the mammary tissue to infection (Radostits *et al.*, 2007). Another plausible explanation for this finding is that most cows at mid-lactation are usually pregnant again. Pregnant cows tend to get stressed and may spend a lot of time laying down hence predisposing themselves to mastitis, especially in an environment of poor hygiene. This could actually have been the situation in this study since it was also found in this study that SCM prevalence was significantly higher among pregnant cows than in non-pregnant ones.

The study further revealed that SCM was most prevalent in cows with a low parity of 1-3 times. Similar results were reported by Ndirangu *et al.* (2017) for cows in Naivasha, Kenya, as well as by Tekle & Barihe (2016) for cattle in Ethiopia. However, this differed with results of some previous studies that prevalence of mastitis increased with age and parity (Abrahmsen *et al.*, 2014; Mureithi & Njuguna, 2016). The reason for the difference is unknown and this requires further research.

Study results also showed that the form of grazing system had some influence on the prevalence of SCM in cattle where the highest proportion of cows with SCM were being reared under complete zero and semi-zero grazing systems, with only a very low proportion being kept under free grazing. This concurs with previous reports (Abrahmsen *et al.*, 2014; Nkoroi & Maitho, 2014; Mureithi & Njuguna, 2016). A possible explanation for this is the increased contamination of cattle houses with animal discharges such as urine, leaked milk and dung. Frequent cleaning and drying of animal houses and open enclosures is of paramount importance in order to reduce intra-mammary infections.

Bacterial culture of CMT-positive milk samples had a high bacterial growth rate of 80.6 %. Similar results were reported (Ondieki *et al.*, 2013; Ndirangu *et al.*, 2017) and this is an indication that CMT is a good test for diagnosing SCM. Bacterial culture and antimicrobial sensitivity testing is only applied as a confirmatory test and to assist in identifying the most effective therapy. Failure to recover bacteria from a few CMT-positive milk samples observed in this study can be attributed to presence of very low concentration of bacteria in milk, intermittent shedding, intracellular location of some bacteria and presence of inhibitory substances in milk (Radostits *et al.*, 2007). There is also a possibility that this was due to the method used where milk samples stored at refrigeration temperature instead of freezing, such that the mastitis causing pathogen could not compete for growth with the spoilage microbes.

Staphylococcus spp. was the main bacterial etiological agent of bovine mastitis in Kisumu County. This is consistent with what was reported by Mureithi & Njuguna (2016) for cattle in Thika and by Ndirangu et al. (2017) for cows in Naivasha. This high isolation rate of *Staphylococcus* spp. may be a result of hand milking and the generally poor to fair milking hygiene standards witnessed in the majority of the farms. This bacterium is transmitted between cows and udder quarters directly from infected ones during the milking process through the milkers' hands and wiping towels (Nkoroi & Maitho, 2014). This is supported by the finding that only 30% of farmers interviewed used separate towels for each lactating cow. The spread of *Staphylococcus* in the present scenario may further have been enhanced by the finding that none of the farmers was using on-farm sub-clinical mastitis test, thus they were not in a position to identify infected animal(s). Moreover, even if this was possible only 30% of the farmers were aware that infected cows should be milked last as a way of controlling mastitis. As such cows with SCM were not necessarily milked last as recommended, therefore assisting in spread of the bacterium as well as mastitis within the herd unknowingly and faster. Other bacteria identified were *E. coli*, followed by *Streptococcus*, *Pseudomonas* and other coliforms, which have also been reported previously as important mastitis causing bacteria in Kenya (Ondieki et al., 2013; Ndirangu et al., 2017) and elsewhere (Tekle & Barihe, 2016). The high number of environmental mastitis bacteria isolated in this study can be due to poor housing facilities observed especially under complete zero and semi-zero grazing systems, which favours accumulation of dung on cows thus increasing the rate of exposure of teats and udder to pathogens (Radostits et al., 2007). Actually, results of this survey showed that prevalence of SCM was higher in cows kept under poor and fair hygiene conditions compared to those under good hygiene. This corroborates with what was reported by Mureithi & Njuguna (2016) who further incriminated dirty floors as a potential source of mastitis organisms.

Staphylococcus and *Streptococcus* isolates tested for antibiotic susceptibility were both fully (100%) sensitive to streptomycin, kanamycin and gentamycin hence these are the drugs of choice for treatment of mastitis caused by either bacterium. However, chloramphenicol though effective is not recommended for use in livestock since this is a 'last resort' antibiotic for humans. This antibiotic was tested in this study since it was one of the antibiotics included in the commercially available antibiotic discs. Ndirangu et al. (2017) reported similar findings where bacterial isolates from mastitic cows in Naivasha were fully sensitive to gentamycin and ampicillin. Ondieki et al. (2013) reported gentamycin to be the most effective drug. Additionally, results of this study showed that *Streptococcus* had 100% sensitivity to ampicillin, tetracycline and cotrimoxazole thus these drugs are effective in therapy of mastitis cases confirmed to be caused by *Streptococcus* and can be applied without prior in-vitro antibiotic susceptibility testing. These results showed that most of the antibiotics available for mastitis therapy are effective in Kisumu County. This is particularly so considering that this County is an up-coming dairy production zone compared to other high potential dairy production regions like parts of Rift Valley and Central Kenya.

However, the current finding further revealed that *Staphylococcus* had developed varying levels of resistance against sulphamethoxazole, followed by cotrimoxazole, ampicillin and tetracycline, while *Streptococcus* showed 100% resistance against sulphamethoxazole. These variations in antimicrobial sensitivities among the two bacterial species may partly be explained by the fact that majority of mastitis cases in the area are of staphylococcal origin. This means that most of the antibiotics are directed to this bacterium as compared to *Streptococcus*, hence the reason for higher resistance in *Staphylococcus* as a result of extensive use or even any misuse. In Kenya, some of the most commonly used antibiotics for mastitis therapy include penicillin, streptomycin, tetracycline, gentamycin, ampicillin and sulphonamides (Ndirangu et al., 2013). Some of the drugs like tetracycline and ampicillin to which there is resistance, have been available and extensively used in Kenya for many years as opposed to some effective drugs like gentamycin and kanamycin which were recently introduced. Indeed, resistance may also have been contributed by the fact that intramammary antibiotic tubes for mastitis therapy are readily available to farmers who often use it without prior sensitivity testing. This is compounded by the fact that mastitis testing laboratories are not available in most parts of the country.

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5 Conclusion

1. Bovine mastitis in Kisumu County was occurring in the sub-clinical form thus farmers are not able to detect the disease visually thus requiring use of diagnostic tests which is an impediment to mastitis control.
2. *Staphylococcus* species is the main bacterial cause of bovine mastitis in Kisumu County.
3. Streptomycin, kanamycin and gentamycin are the drugs of choice for treatment of mastitis caused by either *Staphylococcus* species or *Streptococcus* species while ampicillin, tetracycline and cotrimoxazole are ef-

fective in therapy of mastitis cases caused by *Streptococcus* species.

6 Recommendations

1. Cattle farmers in Kisumu County need to be empowered with on-farm diagnostic methods for detecting sub-clinical mastitis.
2. In an effort to reduce bovine mastitis burden in Kisumu County, there is need for application of an appropriate control strategy consisting of awareness creation through extension, practicing good milking hygiene, regular screening for SCM, milking infected cow(s) last, and post-milking teat dipping.
3. Bacterial culture and antibiotic susceptibility testing for drugs that were not having 100 % sensitivity is recommended prior to treatment. This will guard against indiscriminate use of these drugs further cushioning them against development of antibiotic resistance.

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Conflict of interest

The author declares that they have no conflict of interest.

References

- Abrahmsen, M., Person, Y., Kanyima, B. M., & Bage, R. (2014). Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Tropical Animal Health and Production*, 46(1), 99–105.
- Aleri, J. W., Nguhiu-Mwangi, J., Mogoa, E. M., & Mulei, C. M. (2012). Welfare of dairy cattle in the smallholder (zero-grazing) production systems in Nairobi and its environs. *Livestock Research for Rural Development*, 24, article No. 159.
- Chaneton, L., Tirante, L., Maito, J., Chaves, J., & Bussmann, L. E. (2008). Relationship between milk lactoferrin and etiological agent in the mastitic bovine mammary gland. *Journal of Dairy Science*, 91, 1865–1873.
- Cheesbrough, M. (1985). Medical laboratory manual for tropical countries, vol. II: Microbiology. *Journal of livestock production*, 79, 273–283.
- DaRong, C., Shan, Y. Z., ZhaoHua, Y., WenWei, D., Zhixia, M., ZhiRui, S., & HuaiChang, S. (2010). Prevalence of bacterial infection responsible for bovine mastitis. *African Journal of Microbiology Research*, 4(11), 1110–1116.
- Gitau, G. K., Wabacha, J. K., Mulei, C. M., Ndurumo, S., & Nduhiu, J. M. (2011). Isolation rates and antimicrobial sensitivity patterns of bovine mastitis pathogens in peri-urban area of Nairobi, Kabete, Kenya. *Ethiopian Veterinary Journal*, 15(1), 1–13.
- Gitau, G. K., Bundi, R. M., Vanleeuwen, J., & Mulei, C. M. (2014). Mastitogenic bacteria isolated from dairy cows in Kenya and their antimicrobial sensitivities. *Journal of the South African Veterinary Association*, 85(1), 01–08.
- Hogeveen, H., Huijps, K., & Lam, T.J. (2011). Economic aspects of mastitis: new developments. *New Zealand Veterinary Journal*, 59, 16–23.
- Mungube, E. O., Tenhagen, B. A., Regassa, F., Kyule, M. N., Sheferaw, Y., Kassa, T., & Baumann, M. P. O. (2005). Reduced milk production in udders with sub-clinical mastitis and associated economic losses in crossbred dairy cows in Ethiopia. *Tropical Animal Health and Production*, 37(6), 503–512.
- Mureithi, D. K., & Njuguna, M. N. (2016). Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub-County, Kenya. *Livestock Research for Rural Development*, 28(2).
- National Mastitis Council (NMC) (2004). *Microbiological procedures for the diagnosis of udder infection*. 3rd ed. Arlington, National Mastitis Council Inc.
- Ndirangu, P. N., Wesonga, H. O., Siamba, D., Mugambi, J. M., & Nginyi, J. M. (2013). Mastitis therapy in western and central parts of Kenya: veterinary service providers' perspective. Eastern Africa Agricultural Productivity Project (EAAPP) mini-conference held at KARI-Naivasha, Kenya on 12th-15th November, 2013. Book of Abstracts, pp. 39.
- Ndirangu, P. N., Siamba, D., Wesonga, H. O., Mungube, E. O., Maichomo, M. W., & Mugambi, J. M. (2017). Prevalence of bovine mastitis and multi-antibiotic resistant *Staphylococcus* and *Streptococcus* species in a research centre farm at Naivasha, Kenya. *Bulletin of Animal Health and Production in Africa*, 65, 289–301.
- Ndirangu, P. N., Mungube, E. O., Maichomo, M. W., Nyongesa, P. K., Wamae, D., On'gala, J. O., Gicheru, N. W., Keya, G., Wesonga, H. O., & Siamba, D. (2019). A novel pH-based pen-side test for detection of sub-clinical mastitis: validation in cattle and camels, Kenya. *Livestock Research for Rural Development*. Volume 31, Article #7. [urlhttp://www.lrrd.org/lrrd31/1/erick31007.html](http://www.lrrd.org/lrrd31/1/erick31007.html)

- Nkoroi, J. M., & Maitho, T. (2014). Prevalence of mastitis and effectiveness of mastitis control in dairy cattle in Mathira constituency, Nyeri County, Kenya. *The Kenya Veterinary Journal*, 38(1), 24–31.
- Ondieki, J. O., Ogore, P. B., Shakala, E. K., & Kaburu, G. M. (2013). Prevalence of bovine mastitis, its therapeutics and control in Tatton Agriculture Park, Egerton University, Njoro District of Kenya. *Basic Research Journal of Agricultural Science and Review*, 2(1), 15–20.
- Quinn, P. J., Carter, M. E., Markey, B., & Carter, G. R. (1999). *Clinical Veterinary Microbiology*. 1st ed. London: Elsevier Ltd. pp. 237–242.
- Radostits, O. M., Blood, D. C., Gay, C. C., Hinchiff, K. W., & Handerson, J. A. (2000). Mastitis. In: *Veterinary Medicine*. 9th Ed. WB Saunders Company, London, UK. pp. 603–700.
- Radostits, O. M., Gay, C. C., Hinchliff, K. W., Kelton, D. F., & Scholl, T. (2007). *Veterinary Medicine*. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th ed., Saunders Elsevier, Spain. pp. 1045–1046.
- Tekle, Y., & Barihe, T. (2016). Bovine mastitis: Prevalence, risk factors and major pathogens in the Sidamo zone SNNPRS, Ethiopia. *European Journal of Biology and Medical Science Research*, 4(5): 27–43.
- Thrusfield, M. V. (2005). *Veterinary Epidemiology*, 3rd ed, pp 234–238. Blackwell Science, Oxford, London, UK.
- Van Leeuwen, J. A., Mellish, T., Walton, C., Kanaru, A., Gitau, R., Mellish, K., Maina, B., & Wichtel, J. (2012). Management, productivity and livelihood effects on Kenyan smallholder dairy farms from interventions addressing animal health and nutrition and milk quality. *Tropical Animal Health and Production*, 44(2), 231–238.
- Viguier, C., Arora, S., Gilmartin, N., Wellbeck K., & Kennedy, R. (2009). Mastitis detection: current trends and future perspectives. *Trends in Biotechnology*, 27, 486–493.