PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF MASTITIS CAUSING BACTERIA FROM COWS ON FARMS IN UASIN-GISHU COUNTY, KENYA.

D. Ounah[1,3#], G. Kikuvi[1] and P. Gatongi[2]

[1]Department of Public Health, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, 00202 Nairobi, Kenya. [2]Department of Public Health and Epidemiology, Moi University, P.O. Box 100, 30100 Eldoret, Kenya. [3]State Department of Livestock, Ministry of Agriculture, Livestock, Fisheries & Cooperatives, P.O. Box 29053-00625, Nairobi, Kenya

ABSTRACT

Bovine mastitis is potentially zoonotic with global distribution, and its increasing prevalence is attributed to the rising microbial resistance to frequently used antimicrobial drugs. The disease is attributable to more than 135 microbial pathogens, including bacteria, mycoplasma and fungi. The objective of this study was to establish the prevalence and antimicrobial susceptibility patterns of mastitis-causing pathogens from dairy cows in Uasin Gishu County of Kenya. This was a prospective cohort study in which 216 cows on 81 smallholder farms were recruited. The cows were first screened using the microbiological culture method, and only those free from mastitis were followed up for study. A multi-stage sampling of pooled mid-stream milk for bacterial isolation, identification and antimicrobial sensitivity testing was done every 21 days for ten months. Among the bacterial isolates from cows that developed mastitis, 104 (48.2%) were identified through biochemical tests and antimicrobial susceptibility testing using the Bauer Kirby disc diffusion method. A total of 10 bacterial species were isolated: - *Staphylococcus epidermidis* (21.3%), *S. aureus* (9.3%), *Escherichia coli* (5.1%), *Citrobacter freundii* (2.8%), *Micrococcus* species (2.3%), *Streptococcus* species (2.3%) with *Klebsiella pneumoniae, Pseudomonas aeruginosa* and other species occurring at (<1.9%). The cumulative antimicrobial resistance rates were high, ranging from Ampicillin (81.7%), Penicillin (80.8%) and Sulfamethoxazole (60.6%). At the same time, Gentamicin (94.2%) and Kanamycin (66.3%) were the most sensitive antimicrobials (P<0.0004), which can be recommended for the treatment of mastitis. *Staphylococcus* species and *E. coli* were the main causative agents of mastitis. The antimicrobial susceptibility laboratory testing for mastitis pathogens prior to treatment is recommended.

[#]Corresponding author:davidounah1@gmail.com

KEYWORDS: Antimicrobials, bovine, mastitis, productivity and susceptibility

INTRODUCTION

Mastitis is a multi-etiologic disease with global distribution (Bradley, 2002, Mbindyo *et al.,* 2020). On the American continent, for instance, the dairy sub-sector is struggling largely because of the high prevalence of mastitis (Swinkels *et al.*, 2005). In USA, annual losses in the dairy sub-sector due to mastitis were approximately 526 million dollars in 2003 (Varshney and Naresh, 2004). The Asian continent also experienced high incidences of mastitis, with China and India worst hit by bovine mastitis, causing reduced milk production (Jian-Ping *et al.,* 2009). African countries also bore the brunt of mastitis. The disease was rampant in West Africa, Northern Africa and the Horn of Africa; in Ethiopia, mastitis prevalence was 62.6% at the cow level (Rahmeto *et al.*, 2016). The East African region as well experienced many cases of bovine mastitis. In Uganda, the prevalence rate of mastitis was 61.3% (Byarugaba *et al.*, 2008), while in Rwanda, it was 50.4% (Mpatswenumugabo *et al.*, 2017). In Kenya, bovine mastitis is rampant, with prevalence in Kiambu County at 93% mainly from *Staphylococcal* mastitis at 31.7% (Odongo *et al.,* 2012). In Nakuru county and Mukurweini region, mastitis prevalence was 58.7% (Gitau *et al.,* 2012).

Mastitis is attributable to more than 135 different types of microbial pathogens, including bacteria, mycoplasma and fungi (Hawari and Fowzi, 2008; Mbindyo *et al.,* 2020). Previous research studies have generated microbial profiles of bacteria, mycoplasma and fungi as the main causative pathogens for mastitis. These included *Staphylococcus species, Streptococcus species, Klebsiella species, Escherichia coli, Proteus species, Pseudomonas aeruginosa, Corynebacterium species, Clostridium species, Aerobacter species, Pasteurella multocida, P. haemolytica, Mycobacterium species, Bacillus cereus,*

Serratia marcescens, Brucella species, Enterobacter species, Citrobacter species, Micrococcus species, Salmonella species, Shigella species while fungi included *Candida albicans, Cryptococcus species* (Ruth *et al.,* 2011; Uhlemann *et al.,* 2014; Edilu and Getachew, 2017). Other research findings established bacterial profiles in livestock with mastitis as follows: - *Staphylococcus aureus* (45.0%), *Streptococcus species* (17.5%), E*scherichia coli* (17.5%), *Klebsiella species* (7.2%), *Serratia* species (1.0%) and *Streptococcus species.* (1.0%) (Joanna *et al.,* 2013). Johanna *et al.* (2017), in their cross-sectional cohort study, enumerated the following bacterial pathogens as the main cause of mastitis; *Staphylococcus aureus* (21%), *Streptococcus dysgalactiae* (8%) and *Escherichia coli* (5%). In Zimbabwe, *Staphylococcus* species prevalence was 43.9%, *Escherichia coli* at 21.2% and *Klebsiella pneumonia* at 15.5% (Simbarashe *et al.,* 2013). Tesfaheywet and Gerema (2017), in their epidemiological study, predominantly isolated *Staphylococcus aureus* (24.2%), *Streptococcus agalactiae* (17.1%) and *Micrococcus species* at (2.1%) as the main causes of bovine mastitis. Moreover, in southern Ethiopia, Adane *et al*. (2012) reported the prevalence of *Staphylococcus* species at 29.2%, *Streptococcus* species *at* 12.5% and *Escherichia coli* at 11.4%. Studies in Rwanda documented *Staphylococcal* species occurrence at 51.5% and *Streptococcus* species at 10.3% (Mpatswenumugabo *et al.*, 2017). In Kajiado, Kenya, Mbindyo *et al.* (2020) enumerated *Staphylococcus aureus* prevalence of 15.7%, *Streptococcus* species 22.2% and *Pseudomonas aeruginosa* at 5.1% while in Kabete Kenya, Odongo *et al.* (2012) reported prevalence of mastitis-causing microbial pathogens as; *Staphylococcus species* (31.7%), *Escherichia coli* (17.2%), *Streptococcus* species (10.3%), *Klebsiella species* (9.7%), *Pseudomonas aeruginosa* (7.6%), *Candida albicans* (6.3%), *Proteus species* (0.4%), and *Citrobacter species* (0.6%). In many of the incidences reported*, Staphylococcus* species were the most occurring aetiologic agent of mastitis (Jully *et al.*, 2017).

In sustained efforts to manage mastitis in dairy cows, antimicrobial medicines have been overwhelmingly used (Tiwari *et al*., 2013). In China, a study by Jian-Ping *et al.* (2009) on antimicrobial susceptibility testing showed that 90.7% of the microbial pathogens were resistant to at least one antimicrobial agent; resistance to both penicillin and Ampicillin was recorded as (77.3%) and tetracycline (60.0%). In another study in Brazil by Freitas *et al.* (2018), thirty *Staphylococcal* bacterial isolates were subjected to an antimicrobial panel, where all the isolates (100.0%) were resistant to trimethoprim and 96.7% to tetracycline. The implication of these results demonstrates a therapeutic challenge in mastitis management (Freitas *et al.,* 2018). Nevertheless, many other studies show that indigenous cows are hardy and resistant to mastitis compared to exotic breeds (Edilu and Getachew, 2017; Heriazon *et al.,* 2009). In Ethiopia, prevalence of *Escherichia coli* was 7.1%, and when these isolates were subjected to a panel of antibiotics, antimicrobial resistance against Ampicillin was recorded as 68.7%, sulfamethoxazole-trimethoprim 50% and streptomycin 25% (Messele *et al*., 2019). In another study by Mahlangu *et al.* (2018), antimicrobial resistance against Gentamicin was less than 25.0% for *Escherichia coli*; further, he documented antimicrobial resistance of 100.0% against Penicillin for *Klebsiella pneumoniae, Escherichia coli* and *Citrobacter freundii* bacteria. In yet a different study by Omwenga *et al.* (2021), low *Staphylococcus aureus* resistance against Ampicillin was reported at 37.0%, tetracycline at 51.0% and Kanamycin at 16.0%. In the same study in Isiolo, Omwenga *et al.* (2021), reported *Staphylococcus aureus* resistance against Ampicillin at 64.0% and Kanamycin at 5.0%. These persistent episodes of antimicrobial resistance point to the importance of identifying microbial pathogens implicated in the aetiology of mastitis and *in-vitro* susceptibility testing of frequently used antimicrobial medicines before their applications *in-vivo* to guard against the development of antimicrobial resistance (Health Canada, 2003; Silva *et al.,* 2005).

METHODS AND MATERIALS

Study area

The study was conducted in the Moiben and Kapseret sub-counties of Uasin-Gishu County. The two sub-counties have similar weather patterns and conditions. The sub-counties lie in the same geographical zone on the western lower region of North Rift and the eastern side of the Lake Victoria basin. The area has a conducive environment and climate for livestock production. Over 90% of the dairy cows kept here were exotic breeds and crossbreeds (between exotic cows and indigenous zebu cows). Rainfall is bimodal, ranging from 500mm to 1500mm, with an average temperature of 18^0C. In 2021, the sub-counties received long rains between March to May. However, during the short rains (normally occurring in September-October), the two sub-counties experienced

heavy downpours that started in July, extending to November 2021.

Study population

The total study population of dairy cows was 211,020 of which 130,911 were in Moiben sub-county while 80,109 were in the Kapseret sub-county. Out of these; 147,714 (70%) were pedigree exotic breeds, 42,204 (20%) crossbreeds and 21,102 (10%) Indigenous breeds. Each sub-county had five wards and the cow population was as follows: Karuna 53,732, Moiben 32,239, Tembelio 22,470, Sergoit 15,631, Kimumu 6,839, Kapseret 26,378, Ngeria 23,447, Kipkenyo 11,723, Langas 10,746 and Megun 7,816 cows. This information was as per the records of the County Directorate of Livestock production and County Integrated Development Plan (CIDP, 2013).

Study design and sample size determination

This study adopted a prospective cohort study design. The study cows were recruited at the farm level to determine the baseline results. All cows that tested negative for mastitis using the microbiological culture method were recruited into the study and followed up between Jan.-Oct. 2021 to determine bovine mastitis infections. The follow-up was done every 21 days or earlier should the farmer report a sick cow. A total of 14 follow-up visits were done. A total sample size N was 216 lactating dairy cows recruited on 81 farms with smallholder herds of between 3-10 cows. The sample size was determined using Kasiulevicius formula, on determination of sample size in epidemiological studies (Kasiulevicius *et al.,* 2006); Significance level = the cut-off below which to reject or fail to reject the null hypothesis P = 0.05 (5% margin of error and 95% confidence level), $n = \underline{P_1(1-P_1)} + \underline{P_2(1-P_2)} * f(a,b)/ (P_2-P_1)^2$; n = 0.613(1-0.613) + 0.387(1-0.387) *10.5074/(0.387 – 0.613)^2 Where: n = sample size, f(a,b) = 10.5074 (@ a = 0.05 (2 tail), b = 0.1), P_1 = prevalence effect in exposed population, P_2 = prevalence effect in non-exposed population, N = n + 10% attrition level = 98 + (10/100)*98*2(two study sites, Moiben & Kapseret) = 216.

Sampling design

The study employed a multi-stage sampling design. This type of sampling comprised stratified, cluster and simple random sampling methods. Out of five sub-counties in Uasin-Gishu County, two sub-counties were randomly selected – Moiben and Kapseret. All the administrative wards and locations in each sub-county were purposively sampled. Farms with small herds were identified with the help of local Animal Health Officers. They were sampled and interviewed using a semi-structured questionnaire. A single cow was sampled from each herd in a ratio of 1:3 (i.e., for a herd of three cows, one cow was randomly sampled; for a herd of six cows, two cows were randomly sampled; and for a herd of nine cows three cows were randomly sampled). The farmer milked the first few drops of milk and then milked the mid-stream milk into sterile sampling vials (Bourabah *et al.,* 2013). The Milk samples that were not processed immediately within eight hours were moved to the laboratory and stored at 4-8 ^oC for one week or frozen at −20 ^oC until they were ready for culture.

Microbiological culture and antibiotic Susceptibility Testing

The microbiological culture method was adopted according to Mahlangu *et al. (*2018) and Mureithi and Njuguna (2016). Five percent (5%) Sheep Blood Agar, MacConkey Agar and various Biochemical testing media were prepared according to the manufacturer's manual (Himedia, India). The process of microbiological culture was carried out in a biosafety cabinet level II. Fresh milk was cultured onto two Blood agar plates and one MacConkey agar plate alongside American Type Culture Collection (ATCC) standard control micro-organisms by streaking a loop full of milk. One blood agar plate was incubated anaerobically at 37^0C for 18-24/72 hours with regular checks every 18-24 hours. While the second blood agar and MacConkey agar plates were incubated aerobically at 37 ^oC for 18-24/48 hours, further incubation for up to 48 hours was allowed to rule out negative growth. Colonial characteristics were scored as in colony morphology - shape, colour, texture, odour and size. Gram stain was performed to distinguish the colony shape of gram-negative and gram-positive micro-organisms. Gram-positive cocci organisms were subjected to a catalase and coagulase tests to distinguish between *Staphylococcus* species (catalase-positive) and *Streptococcus* species (catalase-negative). Colonies were subjected to coagulase and mannitol salt agar testing to identify different Staphylococcus species. Gram-negative rods were identified using biochemical tests and media. Motility Indole and oxidase tests were used to identify *Escherichia coli* and *Pseudomonas aeruginosa*. Other gram-negative micro-organisms were inoculated on Triple

Sugar Iron agar, Urea agar, citrate Simon's agar, Eosin-Methylene-Blue (EMB) agar, Methyl red broth, Voges Proskauer agar and identified accordingly (Cheesbrough, 2006; Mahlangu *et al.,* 2018).

The bacterial pathogens identified were subjected to antimicrobial sensitivity testing using Bauer-Kirby disc diffusion method according to Byarugaba *et al.* (2008). The Mueller-Hinton agar culture plate was taken from the refrigerator to acquire room temperature on the working bench. The Mueller-Hinton agar plate was labelled with a unique code number of the sample & date. Using a sterile wire loop, distinct colonies from the primary culture plate were picked and emulsified in a 5ml test tube containing 3ml physiological saline. They were gently shaken and mixed well to form homogeneous turbidity of test micro-organisms that were equivalent to the turbidity of 0.5 McFarland standard of micro-organisms (i.e., turbidity of 0.5 on a scale of McFarland). ATCC standard control micro-organisms were run alongside the test sample micro-organisms. Using a sterile swab, the swab was dipped into a test micro-organisms tube for 30 seconds. The swab was then withdrawn while pressing and rotating it against the test tube wall to tap off the excess micro-organism suspension, avoiding excess inoculum. The surface of Mueller-Hinton agar was inoculated gently by swabbing the entire surface of the media; the plate was swabbed in three dimensions by rotating it at 60^{o}C until it was uniformly covered. The Mueller-Hinton agar was left on the working bench for a few seconds for the surface of the media to air dry. Once the surface was dry, flame sterilised forceps were used to pick an impregnated antibiotic susceptibility octo-disc and place it at the Centre of the plate. The disc was gently but firmly pressed using the forceps to secure it so that it does not fall off during inverted incubation. The susceptibility culture plates with sensitivity discs were incubated at 37 ^{o}C for 18-24 hours, and the susceptibility test reactions were read. The zones of inhibition were measured using a Vanier-clippers, and the interpretations were done in reference to the minimum inhibition zones (MIZs) diameters of Clinical and Laboratory Standards Institute (CLSI) standards (Byarugaba *et al.,* 2008; Mbindyo *et al.,* 2020; Omwenga *et al.,* 2021)

Ethical approval

The study obtained requisite approvals before the commencement of sample and data collection on smallholder dairy farms in Moiben and Kapseret sub-counties between January and October 2021. Approval to collect, process, and analyse animal samples in Veterinary Laboratories was obtained from the Directorate of Veterinary Services Reference number (MOALF&I/SDL/DVS/GEN/VOL.1/57), Masinde Muliro University of Science and Technology ethics review committee approval number (MMUST/IERC/155/2021), National Commission for Science, Technology and Innovation (NACOSTI) research License number (NACOSTI/P/21/9459) and County Government of Uasin-Gishu Reference number (CDVS/UG/TRAINING/VOL.1/16). Informed consent was obtained from farmers who acted as respondents. The right to participate and withdraw from the study was free and voluntary.

Statistical analysis

The microbiological culture data on microbial isolates and antimicrobial susceptibility testing was obtained from the microbiology laboratory. The data was coded and entered into a Microsoft Excel (Microsoft, USA) spreadsheet. The data was then exported into SPSS version 20 (Microsoft, USA) software and processed. Z-Scores were computed for antimicrobial susceptibility testing to establish statistical significance at P-value less than 0.05 and 95% CI. The information was then summarised in tables (Tables I and II).

RESULTS

A total of 104 (48.2%) animals from the 216 cows that were sampled were positive for mastitis. Ten different bacterial pathogens were identified from the infected samples. *Staphylococcus epidermidis* was the most predominant bacteria isolated at 21.3%, followed by *Staphylococcus aureus* at 9.3% and *Escherichia coli* at 5.1%. The least isolated bacteria were *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at 0.9% each. The gram-positive bacteria attributable to causing contagious mastitis in this study were predominant at 35.2%, unlike most gram-negative coliforms implicated in the causation of environmental mastitis at 13.0% (Table I)

TABLE I - MICROBIAL PATHOGENS ISOLATED FROM COWS ON SMALL HOLDER DAIRY FARMS IN MOIBEN AND KAPSERET SUB-COUNTIES, UASIN-GISHU COUNTY.

Bacterial pathogen isolated	**Sub-county**		Total n**umber examined n=216**	**Total percentage**
	Moiben n=134	Kapseretn=82		
Gram positive				
Staphylococcus epidermidis	23	23	46	21.3
Staphylococcus aureus	15	5	20	9.3
Streptococcus species	4	1	5	2.3
Micrococcus species	3	2	5	2.3
Total	45	31	76	35.2
Gram negative				
Escherichia coli	9	2	11	5.1
Citrobacter freundii	6	0	6	2.8
Serratia marcescens	4	0	4	1.9
Proteus vulgaris	1	2	3	1.4
Pseudomonas aeruginosa	1	1	2	0.9
Klebsiella pneumoniae	2	0	2	0.9
Total	23	5	28	13.0
Total bacterial isolates	68	36	104	48.2
No Growth obtained	66	46	112	51.8
Grand Total	134	82	216	100.0

Antimicrobial susceptibility patterns

Overall, the results obtained in this study demonstrate that cumulative resistance of all bacterial isolates against antimicrobial agents was high (>51.9%) for Ampicillin, Tetracycline, Cotrimoxazole, Streptomycin, Sulfamethoxazole and Penicillin except for Kanamycin and Gentamicin which posted low resistance (<33.7%). The findings on Kanamycin and Gentamicin were statistically significant (P<0.0004), meaning they were sensitive to most isolated bacterial pathogens. All gram-negative bacterial isolates were (100.0%) resistant to Ampicillin. Similarly, *Serratia marcescens* and *Proteus vulgaris* recorded (100.0%) resistance against Streptomycin and Penicillin. *Klebsiella pneumoniae* exhibited multiple drug resistance recording (100.0%) resistance against Cotrimoxazole, Sulfamethoxazole and Penicillin, as shown in Table II.

DISCUSSION

Of the 104 bacterial isolates implicated in the causation of mastitis in the Moiben and Kapseret study area, *Staphylococcus epidermidis* was predominant at 46 (21.3%), followed by *Staphylococcus aureus* at 20 (9.3%), *Escherichia coli* at 11 (5.1%), *Citrobacter freundii* at 6 (2.8%), *Streptococcus* species and *Micrococcus* species at 5 (2.3%) each. Other types of bacterial pathogens had occurrence rates of less than 2.0%. These results were comparable to those by Johanna *et al. (*2017), who enumerated the prevalence of *Staphylococcus aureus* at 21.0% and *Escherichia coli* at 5.0%. Tesfaheywet and Gerema (2017), in an epidemiological study in Ethiopia, as well isolated *Micrococcus* species at 2.1%. In the three studies, the results were comparable due to similarity in cow environmental conditions and microbiological culture method used to determine the prevalence of mastitis-causing bacterial pathogens. However, high prevalence of *Staphylococcus* species at 43.9% was reported in Zimbabwe, Escherichia coli followed this at 21.2% and *Klebsiella pneumoniae* at 15.5% (Simbarashe *et al.,* 2013). In Rwanda *Staphylococcal* species were equally high at 51.5% and *Streptococcus* species at 10.3% (Mpatswenumugabo *et al.,* 2017). In southern Ethiopia *Staphylococcus* species was 29.2%, *Streptococcus* species was 12.5%, and *Escherichia coli* 11.4% (Adane *et al.,* 2012). In Kajiado Kenya Mbindyo *et.al.* (2020) reported *Streptococcus species* 22.2% and *Pseudomonas aeruginosa* at 5.1%. In another study in the Kabete area of Kiambu county Kenya, Odongo *et al.* (2012) reported still high prevalence of mastitis causing microbial pathogens; *Staphylococcus aureus* 31.7%, *Escherichia coli* 17.2%, *Streptococcus* species 10.3%, *Klebsiella* species 9.7%, *and Pseudomonas aeruginosa* 7.6%. These results were higher than our findings probably because of low udder hygiene and lack of teat therapy in these studies as compared to our case. In sharp contrast, the low prevalence was recorded in Iowa State, USA, where *Serratia* species and *Streptococcus* species were 1.0% each (Joanna *et al.,* 2013). Another low prevalence was also documented by Simbarashe *et al.* (2013) in Zimbabwe, where environmental *Streptococcus* species was 1.6%, and in

TABLE II - OVERALL RESULTS OF ANTIMICROBIAL SUSCEPTIBILITY TESTING AGAINST ALL ISOLATED MICROBIAL PATHOGENS IN MILK FROM SMALL HOLDER DAIRY FARMS IN MOIBEN AND KAPSERET SUB-COUNTIES, UASIN-GISHU COUNTY.

Bacterial isolates	**Number isolated**	**Antimicrobial Resistance**							
		Amp. (%)	Te. (%)	Cot. (%)	Strep. (%)	Kan. (%)	Gen. (%)	Sx. (%)	Pen. (%)
Gram positive									
Staphylococcus Epidermidis	46	35 (76.1)	19 (41.3)	17 (37.0)	21(45.7)	15 (32.6)	2 (4.4)	21(45.7)	37(80.4)
Staphylococcus aureus	20	16 (80.0)	11 (55.0)	18 90.0)	14 (70.0)	7 (35.0)	1(5.0)	16(80.0)	18(90.0)
Streptococcus species	5	4 (80.0)	3 (60.0)	3 (60.0)	3 (60.0)	2(40.0)	0(0.0)	4(80.0)	5(100.0)
Micrococcus species	5	2 (40.0)	0 (0.0)	1(20.0)	1(20.0)	1(20.0)	0(0.0)	1(20.0)	2(40.0)
Gram negative									
Escherichia coli	11	11 (100.0)	10 (90.9)	11(100.0)	8 (72.7)	3 (27.3)	1(9.1)	9(81.8)	7(63.6)
Citrobacter freundii	6	6 (100.0)	3 (50.0)	2 (33.3)	5 (83.3)	3 (50.0)	1(16.7)	4(66.7)	4(66.7)
Serratia marcescens	4	4 (100.0)	3 (75.0)	0 (0.0)	4 (100.0)	1 (25.0)	1(25.0)	3(75.0)	4(100.0)
Proteus vulgaris	3	3 (100.0)	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	0(0.0)	3(100.0)	3(100.0)
Klebsiella pneumoniae	2	2 (100.0)	1(50.0)	2(100.0)	1(50.0)	1(50.0)	0(0.0)	2(100.0)	2(100.0)
Pseudomonas aeruginosa	2	2 (100.0)	1(50.0)	1(50.0)	1(50.0)	2 (100.0)	0(0.0)	0(0.0)	2(100.0)
Cumulative resistance		85 (81.7)	54(51.9)	55(52.9)	61(58.7)	35 (33.7)	6(5.8)	63(60.6)	84(80.8)
Cumulative sensitivity		19 (18.3)	50(48.1)	49(47.1)	43(41.3)	69 (66.3)	98(94.2)	41(39.4)	20(19.2)
Z-Score		-6.4718	-0.3922	-0.5883	-1.7651	3.334	9.021	-2.1573	-6.2757
***P*-Value**		1.0000	0.6526	0.7219	0.9612	0.0004	0.0001	0.9845	1.0000

Key: Amp. – Ampicillin, Te. – Tetracycline, Cot. -Cotrimoxazole, Strep. – Streptomycin, Kan. - Kanamycin, Gen. – Gentamicin, Sx. – Sulfamethoxazole and Pen. – Penicillin.

Thika, Kenya, in a study by Mahlangu *et al.* (2018), where *Streptococcus* species was 2%, and *Micrococcus* species was as low as 0.6%. The variance in animal husbandry brings about the difference in prevalence on these farms. In the present study, farms reported sporadic low hygiene and sanitation compared to these farms.

Microbial pathogens demonstrated increased antimicrobial resistance to commonly used antibiotics. The cumulative antimicrobial resistance for Ampicillin was scored at 85 (81.7%), Tetracycline 54 (51.9%), Cotrimoxazole 55 (52.9%), and Streptomycin 61 (58.7%), Sulfamethoxazole 63 (60.6%) and Penicillin 84 (80.8%). Kanamycin and

Gentamicin produced high cumulative sensitivity of 69 (66.3%) and 98 (94.2%), respectively. The sensitivity results were statistically significant (P<0.0004). These findings were in concurrence with the findings of similar studies by Jian-Ping in China and Freitas in Brazil. In China, that antimicrobial resistance to penicillin and Ampicillin were 77.3% and tetracycline was 60.0% (Jian-Ping *et al.,* 2009) while in Brazil, Ampicillin was 100.0% resistant, tetracycline 96.7% and streptomycin 80.0% (Freitas *et al.,* 2018). These high antimicrobial resistance were attributed to the haphazard and indiscriminate use of antimicrobial agents. In essence, this underscores the importance of *in-vitro* susceptibility testing of frequently used antimicrobial medicines before their applications *in-vivo* to guard against the development of antimicrobial resistance and achieve effective antimicrobial therapy (Health Canada, 2003; Silva *et al.,* 2005).

Specific bacterial isolates also registered high antimicrobial resistance. For instance, resistance against Ampicillin was (100.0%) for all gram-negative isolates vis *Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae, pseudomonas aeruginosa* and *Proteus vulgaris.* Further, *E. coli and K. pneumoniae* were as well (100.0%) resistant to cotrimoxazole while *K. pneumoniae* and *P. vulgaris* registered (100.0%) multiple drug resistance against sulfamethoxazole and penicillin. However, all microbial isolates showed low resistance of less than (<50.0%) against Kanamycin and Gentamicin, with a statistical significance of (P <0.0004), meaning the two antibiotics were more sensitive against bacterial pathogens implicated in the causation of mastitis.

Micrococcus species – produced a low antimicrobial resistance of <40.0% to all antibiotics. Similar findings were reported by Mahlangu *et al.* (2018) in Thika where antimicrobial resistance was 100.0% against Penicillin for *K. pneumoniae, . coli* and *Citrobacter freundii;* In this same study, antimicrobial resistance against Gentamicin was less than 25.0% for the above microbial pathogens. Other studies reported comparably lower microbial resistance against antimicrobial agents than in the current study, where *Escherichia coli* antimicrobial resistance against Ampicillin was 68.7%, sulfamethoxazole-trimethoprim 50% and streptomycin 25% (Messele *et al.,* 2019).

Similarly, Omwenga *et al.* (2021) in Marsabit also reported low *S. aureus* resistance against Ampicillin at 37.0%, tetracycline at 51.0% and Kanamycin at 16.0%. In the same Omwenga *et al*. (2021) study in Isiolo, *S. aureus* was noted to be resistant to Ampicillin at 64.0% and Kanamycin at 5.0%. The low resistance was attributable to the judicious use of antimicrobial therapy in the management of bovine mastitis on these farms. In the current study, farmers reportedly were not strict in following the drug administration regimen recommended by animal health officers. As such the high antimicrobial resistance in the present study was attributable to prolonged and haphazard use of antimicrobials and by the fact that over 90.0% of study cows were exotic and crossbred cows. In the Messele *et al*. (2019) and Omwenga *et al.* (2021) studies, all the cows were indigenous (Borana and Zebu) cows. Indigenous cows are hardy and resistant to mastitis compared to exotic breeds, which are more vulnerable (Heriazon *et al.,* 2009; Edilu and Getachew, 2017). These findings imply that mastitis can be highly prevalent unless farmers in smallholder farms in Uasin-Gishu county practice improved animal husbandry by maintaining good cow udder hygiene and judicious use of antimicrobials informed by laboratory results and regular teat dipping.

CONCLUSIONS

Staphylococcus species and *Escherichia coli* were the main causative agents of mastitis in milk on smallholder dairy farms in Moiben and Kapseret sub-counties of Uasin-Gishu County. They exhibited varying degrees of resistance to frequently used antimicrobial agents.

Antimicrobial Resistance against bacterial pathogens isolated in milk from dairy farms in Moiben and Kapseret study area demonstrated that Ampicillin, Streptomycin, Cotrimoxazole, Tetracycline, Sulfamethoxazole and Penicillin were highly resistant. Subsequently, Gentamicin and Kanamycin were found to be sensitive against bacterial pathogens implicated in the aetiology of mastitis by exhibiting low antimicrobial resistance and effective in the treatment of mastitis.

RECOMMENDATIONS

Intervention strategies are recommended to prevent and control predominant *Staphylococcus* species and *Escherichia coli* pathogens. These strategies could include maintaining good hygiene and sanitation of cow housing and milking crushes, cleaning cow teats and udder before and after milking by using a single towel for each cow. These are sure strategies that could immensely minimise contagious transmission of mastitis on smallholder dairy

farms.

Gentamicin and Kanamycin are recommended for mastitis treatment, especially *Staphylococcal* and Coliform mastitis attributable to *Staphylococcus species* and *E. coli* since the two antimicrobials were shown to be sensitive to these micro-organisms. Subsequently, Ampicillin, Streptomycin, Cotrimoxazole, Tetracycline, Sulfamethoxazole and Penicillin produced high Antimicrobial Resistance, and we recommend their gradual withdrawal from the list of animal health essential drugs.

The microbiological culture method used in this study was appropriate but on overall we recommend the molecular sequencing method, which is superior to be used for the identification of bacterial isolates and genotyping for antimicrobial-resistant genes.

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