Asian Journal of Research in Animal and Veterinary Sciences

9(3): 36-45, 2022; Article no.AJRAVS.87397

Occurrence of Antibiotic Resistance in Bacteria Isolated from Milk of Dairy Cows in Small-Holder Farms in Juja Sub-County, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/87397

Original Research Article

Received 22 March 2022
Accepted 01 June 2022
Published 04 June 2022

ABSTRACT

Sub-clinical mastitis (SCM) remains a serious hindrance to small-holder dairy farms in Kenya. A cross-sectional study was conducted to determine the risk factors associated with occurrence of SCM and antibiotic resistance in smallholder dairy farms in a peri-urban set-up area of Juja Sub-county, Kenya. Milk samples was obtained from 120 lactating dairy cows in 60 farms and screened for SCM using California mastitis test (CMT). Positive CMT samples were cultured and bacteria identification was done using standard methods. A questionnaire survey was administered at the household level to assess the risk factors associated with SCM. Sensitivity of the *Staphylococcus aureus* and *Escherichia coli* isolated from milk against commonly used antibiotics was determined using disc diffusion method. The prevalence of SCM at cow and udder level was found to be 66.7% and 61.3%, respectively. The highest prevalence of SCM was in Kalimoni (88.8%), Murera (82.7%) and Witeithe (71.4%) wards. The least affected area being Juja (60%) and Theta (44.4%) wards. Sub-clinical mastitis was significantly higher (p=0.007) in Friesian (74.1%) and Guernsey (66.7%) breeds as compared to the indigenous breed (53.8%). Cows with parity of four and above had higher (p=0.001) prevalence of SCM (73.7%) as compared to those of lower parity stage.

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Additionally, the prevalence (81%) of SCM in cows at late lactation was higher (P=0.002) compared to early (61.5%) and mid-lactation (56.4%), respectively. Higher prevalence (59%) of SCM was found in cows kept in less frequently cleaned housing compared to those more frequently cleaned (10%). The bacterial organisms isolated from the milk were *Staphylococcus* spp. (41.7%), *Klebsiella* spp. (24.5%), *Pseudomonas* spp. (22.1%), *Escherichia coli* (6.8%), *Shigella* spp. (1.8%) and *Salmonella* spp. (3.1%). The isolated *Staphylococcus* spp. and *E. coli* were most resistant to Oxytetracycline (79%, 100%, respectively) and Streptomycin (44%, 90%, respectively). Multidrug resistance (MDR) involving a combination of oxytetracycline, tetracycline, streptomycin and chloramphenicol and gentamycin was observed amongst *Staphylococcus* spp. (29.4%) and *E. coli* (45.5%) isolates. In conclusion, the study showed that large proportion of dairy cows was affected by SCM and the antibiotic resistance (AR) was high. This calls for animal health extension experts and other relevant stakeholders in the industry to train farmers on efficient control of SCM and emerging cases of AR.

Keywords: Sub-clinical mastitis; milk; risk factors; peri-urban; antibiotic resistance; Kenya.

1. INTRODUCTION

In Kenya, the current number of dairy cattle populations stands at 4.3 million out of an estimated 18 million cattle population in the country [1]. Most of the dairy cattle are reared in smallholder farms (with the average farm size of five cows) for household milk consumption and generation of income through sales. Thus, the dairy sector in Kenya contributes significantly to poverty alleviation and enhance nutrition. However, the production per cow (4,575kg/cow/year in high potential areas) falls way behind the international standards [2]. Further, the quality is a great concern to stakeholders due to the presence of zoonotic pathogens and antimicrobial drug residues [3]. In Kenya, there is a national drive to address the existing food deficit by devising different strategies including improvements of the productivity of livestock sector by controlling major causes of this deficit which includes infectious diseases such as mastitis [4].

Mastitis is one of the most complex diseases of dairy cows that generally involve interplay between management practices and infectious agents, having various causes and degrees of intensity [4,5]. Studies in Kenya have shown that prevalence of the disease ranges from 6% to 87.5% and is intricately associated with management practices in the herd [6]. Transmission occurs mainly at milking time through contaminated milking machines, udder-washing clothes as well as hands of workers and machine operators. In Kenya subclinical mastitis, has received little or no attention perhaps because farmers and animal health practitioners are focused on diagnosis of acute diseases and subsequent treatment of clinical cases. Mastitis results in financial losses as a result of reduced milk yield and low quality. This may lead to milk rejection at quality control level discarding of milk following antibiotic therapy, high treatment costs related to veterinary care and culling of cows chronically infected with mastitis [7].

The overall objective of the present study was to determine the prevalence of mastitis in cows and their predisposing risk factors within small-holder dairy farms in a peri-urban setting in Kenya. Further the study determined the sensitivity of isolated bacteria to common antibiotics

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was conducted in Juja Sub-county, Kenya, which is located about 22km north-east of Nairobi. It lies between latitude 1^0 02' 60.00"S and longitude 37^0 04' 60.00"E of the equator. It has a human population of 139,853 which is growing rapidly due to proximity to Nairobi City and Thika Town which provide ready market for farm produce. Juja Sub-county has five administrative wards including Juja, Witeithe, Kalimoni, Theta and Murera. The sub-county has a tropical type of climate with an average annual rainfall of 840 mm (range from 468 mm to 900 mm) and average temperatures of 19.8°C (ranging between 14°C - 26ºC). Despite the study area being residential, peri-urban livestock farming is common with recent Kenya's census showing that the sub-county had 136,251 cattle, 17,300 pigs, 63,031 chicken, 32,000 sheep and 12,699 goats [8].

2.2 Study Design and Sample Size Determination

A cross-sectional study design was used for the study. According to a recent study [10], the prevalence of mastitis in a similar peri-urban set-up of Nakuru County, Kenya was 36%. Using this prevalence, the study sample size was adjusted for small population like one of the lactating cows in Juja Sub-county (<10,000 lactating cows), using the formula by Thrushfield [9]. The minimum sample size was 101 dairy cows. In this study therefore a total of 120 lactating cows were sampled.

The cows were sampled using a multistage random sampling technique to select study wards, households and animals. From each ward (Kalimoni, Juja, Witeithe, Murera and Theta), three villages were selected randomly. Further, from the sampling frame provided by the local veterinarians, respective herds were randomly selected and sampling of a maximum three lactating cows in the herd was done.

2.3 Sample Collection and California Mastitis Test

Milk samples were collected as previously described [11]. The milked cows were restrained, and the udder and teats were cleaned with warm water and disinfected using cotton wool soaked with 70% ethanol. The teat was then dried using disposable towels. The first three streams of milk from each teat were discarded. A sample of milk from individual quarters of each udder from the selected cows were screened. This was done using the commercial kits (CMT Kit, ImmuCell Corporation Company) of California Mastitis Test (CMT) where the consistency of the fluid in each well was observed, and the amount of gel reaction was recorded after 20 minutes [12]. The results were read on a score of 0-3, where a score of 0, trace and 1 was considered negative while a score of 2 and 3 was considered positive.

Thereafter, 10 ml of milk was then collected aseptically from lactating cows into sterile test tubes. The samples were transported in ice at 4°C to Jomo Kenyatta University of Agriculture & Technology (JKUAT) Microbiology Laboratory. The samples were processed immediately or stored at 4°C until processed as described below.

2.4 Culture and Bacterial Identification

In the laboratory, bacteriological cultures were performed on the milk samples according to the method described earlier [11]. Identification of the bacterial species was done on the basis of plating on selective media, Gram Stain reaction, colony morphology and biochemical tests [11,13].

2.5 Antibiotic Susceptibility

Antibacterial sensitivity was determined by the disk diffusion assay [14]. The isolated bacteria were tested for antibiotic sensitivity using a panel of antimicrobial drugs on the disks. The panel of 5 antibiotics commonly used for treatment of mastitis in Kenya were Oxytetracycline, Gentamycin, Tetracycline, Streptomycin and Chloramphenicol. The discs were applied onto the surface of Nutrient agar that were inoculated uniformly with the either *Staphylococcu*s spp. or *E. coli* (isolated as described above) and then incubated overnight at 37^0 C. The effectiveness of a drug was determined by measuring the diameter of the zone of inhibition around the disk [14]. The following standard criteria [14] were used to summarize the various sensitivity classes for each of the antimicrobial used: a zone diameter of 0 mm to 8 mm scored 0 or 'R' for resistance, a zone diameter of 9 mm to 15 mm scored + or slightly sensitive, a zone diameter of 16 mm to 22 mm scored ++ or sensitive and a zone diameter of 23 mm and above scored +++ or very sensitive.

2.6 Risk Factor Determination

A questionnaire was administered to 60 dairy farmers to determine the age, parity, lactation stages and management of the sampled dairy cows. Age of the study cows were determined by the information from the owner and dentition characteristic. Parity was categorized using a scale of 1-4, with one being the least parity and 4 (and above) being the highest parity recorded. Lactation stage of the sampled cow was also documented.

2.7 Data Analysis

The collected data was entered into MS Excel (Microsoft 2013, USA) and thereafter exported to a statistical package SPSS (Statistical Package for the Social Science, (Microsoft, USA) for the statistical analysis. Descriptive statistics were presented as tables and figures. A Chi-square test was used to evaluate relationship between the risk factors and occurrence of sub-clinical mastitis. Significance level was set at $p<0.05$.

3. RESULTS

3.1 Characteristics of Sampled Farms

A total of 60 farmers were selected randomly from Kalimoni [12], Juja [10] Theta [14], Murera [12] and Witeithe [12**]** wards of Juja Sub-county. The characteristics of the farms and the sampled cattle are shown in Table 1. Majority of the farmers had tertiary level of education (54%) while intensive system (58%) of production was the most practiced method of cattle farming. It was noted that majority of farmers kept other domestic animals (90%). The farmers also indicated that the structures where cattle were housed and milked were cleaned daily (48%), and few were cleaned twice in a month (4%). Most farmers (98%) indicated that they performed udder washing procedures before milking while others (48%) reported occurrence of persistent mastitis in a herd. Further, most (84%) of farmers administered drugs without submitting samples to the laboratory for culture and sensitivity.

Table 1. Dairy farm structures and features cattle in Juja Sub County, Kenya

Variable	Frequency	Percentage
Education level		
Non formal	6	10
Primary	10	16
Secondary	12	20
Tertiary	32	54
Production system		
Intensive	32	58
Semi intensive	12	20
Extensive	16	22
Frequency of cleaning structure		
Daily	29	48
Once per week	23	38
Twice a month	2	4
Never	6	10
Keeping of other domestic animals		
Yes	54	90
No	6	10
Dairy cattle breed kept		
Exotic	35	58
Indigenous	7	12
Crosses	18	30
Pre and post milking hand hygiene		
Yes	54	90
No	6	10
Towel used to clean the udder		
Disposable	4	6
Reusable	55	92
Others	1	2
Occurrence of persistent mastitis		
Yes	29	48
No	25	42
Not know	6	10
Submission of sample for culture and sensitivity		
Yes	12	20
No	48	80

3.2 Prevalence of Sub-clinical Mastitis and Identification of Bacteria

California Mastitis Test was used to examine the prevalence of sub-clinical mastitis in the study area. A total of 80 (66.7%) of the sampled cows were CMT positive, while the prevalence at udder level was found to be 43.3%. The prevalence of sub-clinical mastitis by wards were as follows: Kalimoni (88.8%), Murera (82.7%), Witeithe (71.4%), Juja (60%) and Theta (44.4%) in the order prevalence.

A total of 163 isolates of bacteria were obtained from the culture. In descending order, the isolates were *Staphylococcus* spp. (42%), *Klebsiella* spp. (25%) *Pseudomonas* spp. (22%), *E. coli*. (7%), *Salmonella* spp. (3%) and *Shigella* spp. (2%). The bacteria with the highest prevalence in the 120 sampled dairy cattle was *Staphylococcus* spp. 56.7%, while the least was *Shigella* spp. 2.5% (Table 2).

3.3 Relationship between Prevalence of sub-clinical Mastitis and Risk Factors

The results of CMT were used to evaluate the relationship between risk factors and prevalence of sub-clinical mastitis. The prevalence of mastitis was found to be highest (p=0.007) in Friesian breed (74.1%), followed by Guernsey (66.7%), Ayrshire (63.2%), crosses (61.1%), Jersey (57%) and indigenous (53.8%) breeds in that order (Table 3).

Cows in late lactation stage were more (81%) affected by SCM. Lactation stage was significantly (p=0.002) associated with SCM (Table 3).

The study showed that those cows with parity of more than four had significantly higher (P=0.001) predisposition to sub-clinical mastitis compared to those in first to third parity. Cows with greater than a parity of four had the highest prevalence of SCM (73.7%) (Table 3).

The highest (59%) prevalence of sub-clinical mastitis was found among the dairy cattle whose farm structures had low frequency of cleaning. The cleaning strategy was significantly (p=0.019) associated with prevalence of SCM. Further, the results also showed that hand washing during milking and washing of hands between cows during milking reduced (p=0.01) the occurrence of mastitis (Table 3). The findings of the study indicate that farms in which the farmers do not consult health workers in administering the drug was significantly and positively associated with mastitis (p=0.003).

3.4 Antibiotic Susceptibility

Antibiotic susceptibility was only tested against *Staphylococcus* spp. and *E. coli* (Table 4). In general, *Staphylococcus* species that were isolated from the milk were most sensitive to gentamycin (81%) and least sensitive to oxytetracycline (13%). However, *Staphylococcus* spp*.* isolates were resistant to oxytetracycline (79%), streptomycin (44%) and tetracycline (37%). *E. coli* were most sensitive to chloramphenicol (73%) but had high resistance to oxytetracycline (100%), streptomycin (90%), tetracycline (82%) (Table 4).

The occurrence of multidrug resistance (MDR) was observed amongst the *Staphylococcus* spp. and *E. coli* isolates. Most of the MDR involved oxytetracycline in combination with other

Table 2. Prevalence of specific pathogens causing subclinical mastitis in small holder farms in Juja

Bacteria species	Ward					
	Witeithe (%)	Theta (%)	Murera (%)	Kalimoni (%)	Juja (%)	Total (%)
Staphylococcus spp.	14.7	17.6	25.0	27.9	14.7	68 (56.7%)
Klebsiella spp.	20.0	15.0	25.0	22.5	17.5	40 (33.3%)
Pseudomonas spp.	16.7	11.1	41.7	11.1	19.4	36 (30%)
Escherichia coli	9.1	63.6	0.0	9.1	18.2	11 (9.2%)
Salmonella spp.	20.0	40.0	20.0	0.0	20.0	5 (4.2%)
Shigella spp.	0.0	33.3	33.3	0.0	33.3	3 (2.5%)

n= number of positive cows

Table 3. Association of potential risk factors with subclinical mastitis at cow level as identified by CMT in Juja Sub-county, Kenya

Variable	N	Number of positive cows	Proportion (%)
Breed			
Friesian	54	40	74.07
Guernsey	9	6	66.70
Ayrshire	19	12	63.16
Jersey	7	4	57.00
Crosses	18	11	61.11
Indigenous	13	7	53.84
Lactation stage			
Early	39	24	61.54
Mid	39	22	56.41
Late	42	34	81.00
Parity			
1	13	5	38.46
2	28	17	60.71
3	41	29	70.73
4 and above	38	28	73.63
Hand washing			
Yes	50	46	76
No	70	17	24
Cleaning schedule			
Daily	46	5	10.9
Once/week	47	14	30.4
Twice/month	27	15	58.7

N= Number of sampled cattle

Table 4 Antibiotic susceptibility pattern of bacterial isolates from milk samples of cows in smallholder dairy farms in Juja Sub-county, Kenya

Isolate	Antibiotic	No of isolates	Sensitive	Intermediate	Resistant
Staphylococcus spp.	Tetracycline	68	33 (49%)	10 (15%)	25 (37%)
	Chloramphenicol	68	35 (51%)	20 (29%)	13 (19%)
	Oxytetracycline	68	9 (13%)	5 (7%)	54 (79%)
	Streptomycin	68	16 (24%)	22 (32%)	30 (44%)
	Gentamycin	68	55 (81%)	9 (14%)	4 (6%)
Escherichia coli	Tetracycline	11	2 (18%)	0 (0%)	9 (82%)
	Chloramphenicol	11	8 (73%)	1 (9%)	2 (18%)
	Oxytetracycline	11	0 (0%)	0 (0%)	11 (100%)
	Streptomycin	11	1 (10%)	0 (0%)	10 (90%)
	Gentamycin	11	6 (55%)	3 (27%)	2 (18%)

antibiotics. For *Staphylococcus* spp., the MDR was observed to involve oxytetracycline and tetracycline (34/68 isolates, 50%), oxytetracycline and chloramphenicol (45/68 isolates, 66.2%), oxytetracycline and streptomycin (47/68 isolates, 69.1%), and oxytetracycline and gentamycin (44/68 isolates, 64.7%), oxytetracycline, tetracycline, streptomycin and chloramphenicol (27/68 isolates, 40%) and oxytetracycline, tetracycline, streptomycin and chloramphenicol and gentamycin (20/68, 29.4%). For *E. coli*, the MDR involved oxytetracycline and tetracycline (8/11 isolates, 72.7%), oxytetracycline and streptomycin (8/11 isolates, 72.7%), oxytetracycline, tetracycline and chloramphenicol (9/11 isolates, 81.8%), and oxytetracycline, tetracycline, chloramphenicol and streptomycin (7/11 isolates, 64.7%) and oxytetracycline, tetracycline.

4. DISCUSSION

The current study evaluated the risk factors associated with occurrence of sub-clinical mastitis in dairy cows in Juja Sub-county, a peri-urban farming area in Kenya. The prevalence of sub-clinical mastitis (66.7%) was found to be higher compared to studies done in Ethiopia (52.3% - 34.9%) [15,16], Kenya (59.5%) [17], Egypt (40%) [18], Uganda (37%) [19] and Tanzania (53.2%) [20]. The results were comparable to the ones found in Ethiopia [21] which found a prevalence of 61.1%. However, the results were lower than those reported in Nigeria (85.5%) [22], Ethiopia (72.7%) [23]. The reason for high SCM prevalence in the present study may be due to lack of proper mastitis control practices in the study area. Intensive production system which is practiced in the study area is associated with low hygiene standards and low farmers awareness on SCM [6,24]. However, it should be noted that with CMT as the selection criteria for SCM the prevalence might be underestimated due to the fact that the sensitivity of CMT for S. aureus SCM is very low: This has to be elucidated because it influences the prevalence. In the current study, only those cows with CMT score of +2 and above were considered as having mastitis. A previous study [24] noted that milk of cows with CMT score of +2 and above was at least five times as likely to come from infected quarters.

The current study showed that exotic breed of cows such as Friesians were more vulnerable to mastitis as compared to the indigenous cattle breed. In exotic breeds, such as the Friesians, this could be due to anatomy of udder and teat, position of udder and teat, high milking yield and sucking of teat and udder making them prone to trauma and this can introduce pathogenic microorganisms to the udder**.** Similar to other studies [25,26,16], the present study showed that SCM cases were higher in late lactation stage compared to other stages and this could be due to the fact that as lactation progresses, cows are exposed to mastitis because of daily contact of cows during milking process [27,28]. The study also showed increased mastitis cases in higher parity cows which may also be due several exposures to mastitis pathogen during the lifetime of the cow. According to Suleiman *et al*., [24], poor integrity of the teat canal due to aging leads to easy ingress of bacteria to teats after milking and pendulous udder may be prone to injury in older cows compared to younger cows while decreased immunity in older cows may lead to increased susceptibility to mastitis.

The present study also showed that there were increased SCM cases in herds where there was earthen floor compared to those housed in pens having concrete floor. It is possible that concrete floors washing can maintain better hygiene in cows. The earthen floor on the other hand could create microenvironments favorable for multiplication and survival of bacteria pathogens [29,30]. In the current study, prevalence of mastitis was associated with poor teat and udder hygiene which has also been reported earlier [31]. The predominant source of infection is from the udder of infected cows transmitted through milker's hands, towels, milking utensils and the environment in which the cows are kept.

In the current study, *Staphylococcus* species was the main causative agent of sub-clinical mastitis while other bacteria included *Klebsiella* spp., *Pseudomonas* spp., *E. coli*, *Salmonella* spp. and *Shigella* spp. The ubiquitousness and chronic nature of *Staphylococcus* spp. could explain the high prevalence observed in this study and others [32]. *Staphylococcus* spp. is a contagious pathogen which can spread from one cow to the other or by personal contact with cows during unhygienic milking procedures. On the other hand, *E. coli* infections could be due to fecal contamination on the udder and teats [33].

Majority of the bacteria were sensitive to Chloramphenicol and Gentamicin and could be explained by their infrequent use in treatment of mastitis in the area of study. This is due to its high cost and un-availability of the drugs in the area (County Veterinary Officer, personal communication). However, the isolated bacteria were less susceptible to Tetracycline and Oxytetracycline. Similar profiles of resistance have been reported elsewhere in Uganda [34,35] and Tanzania [36]. High resistance of bacterial isolates to Oxytetracycline has been reported in Nigeria [37], Kenya [38], Tanzania [20] and Uganda [39]. Oxytetracycline, owing to its low cost of acquisition and ease of availability for mastitis treatment can explain for the high levels of resistance as observed in the study [40].

Further, the present study showed that some isolates exhibited multidrug resistance pattern to a combination of oxytetracycline, tetracycline, streptomycin, chloramphenicol and gentamycin. This could be attributed to the erratic and extensive use of antibacterial drugs without prior antimicrobial susceptibility testing. Most farmers in the current study indicated that they did not submit samples to the laboratory for culture and sensitivity, while previous studies have shown there is extensive use of antibiotics by farmers and animal health provider, with oxytetracycline being the most used [11]. AR can pose serious health related hazards to animals and human beings [41,42,43]. Where there is transmission from animals to humans this can result in community transmission and inability to treat these infections at hospital level. Thus, strategies for proper and regulated use of antibiotics in the animal health sector should be urgently addressed.

5. CONCLUSIONS

In the present study, breed, lactation stage, parity, teat and udder hygiene were found to be risk factors significantly associated with SCM cases. It is recommended that extension workers such as the animal health practitioners should train farmers on effective strategies for control of mastitis as well proper use of antibiotics in treatment and control of SCM. There was a high level of Oxytetracycline and streptomycin drug resistance in the isolated *Staphylococcus* spp. and *E. coli* and this imply that interventions to limit spread of resistant pathogens should be advocated.

ACKNOWLEDGEMENTS

The Grand Challenges Africa programme (GCA/AMR/rnd2/079) financially supported the work. Grand Challenges Africa is a programme of the African Academy of Sciences (AAS) implemented through the Alliance for Accelerating Excellence in Science in Africa (AESA) platform, an initiative of the AAS and the African Union Development Agency (AUDA-NEPAD). GC Africa is supported by the Bill & Melinda Gates Foundation (BMGF) and The African Academy of Sciences and partners. The views expressed herein are those of the author(s) and not necessarily those of the AAS and her partners. The authors are also grateful to farmers and the technical assistance provided by JKUAT technical staff.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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    DOI: 10.1016/j.prevetmed.2006.10.011

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/87397