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Research Article

Antimicrobial Susceptibility Profiles of Bacteria Isolated from Traditionally Fermented Bovine Milk from Selected Farms of Kajiado- Central Sub-County, Kenya

Jane Kiarie[1], John M Kagira[2], Maina Ngotho[2], Naomi Maina[3], Peter Achoki[2], and J. Maingi[4]

[1] Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200, Nairobi, Kenya
[2] Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200, Nairobi, Kenya
[3] Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200, Nairobi, Kenya
[4] Department of Biochemistry and Microbiology, Kenyatta University, P.O. Box 00100-43844, Nairobi, Kenya

* ***Corresponding author:*** John M Kagira, Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200, Nairobi, Kenya. Email: jkagira@jkuat.ac.ke

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ABSTRACT

Introduction: Fermented bovine milk provides a conducive environment for the growth of bacteria some of which could be of zoonotic importance. These bacteria can develop antimicrobial resistance (AMR) due to the regular use of antibiotics in animals.
Materials and methods: This cross-sectional study was undertaken to determine the antibiotic sensitivity of bacteria isolated from traditionally fermented milk obtained and processed from 114 indigenous cows kept by Maasai pastoralists in Kajiado County, Kenya. The Kirby-Bauer Disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolated *Staphylococcus* spp., *Escherichia coli, Klebsiella* spp., and *Salmonella typhi*.
Results: The susceptibility of the isolated bacteria was determined using nine antibiotics namely chloramphenicol (10µg), kanamycin (30µg), penicillin G (10µg), streptomycin (10µg), oxytetracycline (30µ) tetracycline (30µg), vancomycin (30µg), gentamycin (10µg) and Ampicillin (10µg). The *Staphylococcus (S.) aureus* isolates exhibited diverse resistance patterns to the antibiotics with the greatest resistance observed against oxytetracycline (69.2%), streptomycin (69.2%), and kanamycin (61.5%). However, a significant proportion of the *S. aureus* strains demonstrated a 100% susceptibility rate to gentamycin and vancomycin. *Escherichia coli* isolates exhibited resistance to vancomycin (100%), tetracycline (80%), oxytetracycline (80 %), and ampicillin (60 %) and were highly (100%) sensitive to the other antibiotics. *Salmonella typhi* isolates were resistant to vancomycin (88.8%) and highly (100%) sensitive to chloramphenicol, penicillin G, gentamycin, and streptomycin. *Klebsiella* spp. were highly resistant to vancomycin (100%) and were sensitive to gentamycin (100%) and streptomycin (100%).
Conclusion: The study showed a high prevalence of AMR in bacteria isolated from traditional milk consumed by the pastoralists and thus there is a high risk of zoonotic spread of the pathogenic bacteria. There is a need to educate the local households on strategies to minimize the occurrence of AMR in animals and also improve hygiene practices in the preparation of traditionally fermented milk.

1. Introduction

Globally, in both human and veterinary medicine, the efficacy of antibiotics is diminishing, posing a global challenge due to the widespread emergence of resistance, resulting in infections that are challenging to treat. The

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World Health Organization estimates that currently close to 5 million deaths annually are associated with antimicrobial resistance (AMR) and the United Nations (UN) predicts that by 2050, up to 10 million deaths could be caused by microbes having AMR[1,2]. The cost of AMR to national and international economies and their health systems is significant as it affects the productivity of the patients and their caretakers through prolonged hospital stays and the need for more expensive and intensive care[3]. Several studies project that AMR would cost more than $1 trillion annually by 2050 worldwide[1,2,4]. Thus, AMR is a key priority area identified by both national and international agencies and is mushrooming as a silent pandemic[5]. While poorly quantified, the burden of AMR is comparably higher in low-income countries including Kenya and the wider Sub-Saharan Africa[6].

Although antibiotic use in livestock has largely contributed to improving their health and productivity, it has also played a significant role in the evolution of resistant bacterial strains[5]. Regarding zoonotic-resistant strains, they can end up getting transmitted from animals to human beings through products such as milk[7]. The burden of AMR has not been well studied amongst pastoralists, who are people who keep herds of animals under nomadic settings and consume products such as milk emanating from these animals. Bovine milk has over a long time been considered nature's single most complete food and is one of the most valuable and regularly consumed foods in many households[7]. Amongst the milk products consumed, traditional fermented milk is popular amongst pastoralists in Africa. The improper handling of raw milk and milk products including consumption of the same without proper formal or informal education on milk hygiene serves as a threat to the rising challenge[8]. Due to the poor storage facilities in the traditional setup, proliferation of the milk pathogens may ensue. In a recent study[9], amongst the Maasai pastoralists in Kajiado Kenya, the traditional fermented milk was found to be contaminated with bacterial species including *Klebsiella* spp. (15.79%), Coagulase Negative *Staphylococcus* (14.91%), *Pseudomonas* spp. (14.04%), *Staphylococcus aureus* (11.4%), *Salmonella (S.) typhi* (7.89%), and *Escherichia coli* (4.39%). Studies conducted in Brazil, Egypt, Nigeria, and Zambia in traditionally fermented dairy products revealed that *Staphylococcus* spp., *Listeria monocytogenes*, *Streptococcus* spp., *Pseudomonas* spp., *Escherichia coli* (*E. coli*), *Klebsiella* spp., and *Salmonella* spp., among other bacteria, are prevalent contaminants in milk[10-13].

This contamination of milk coupled with the use and misuse of antibiotics in the treatment of various diseases in the livestock have contributed to the build-up of antibiotic-resistant strains of some of the known zoonotic pathogens[14]. To date, only a handful of studies on the burden of AMR in bacterial isolates from traditional milk consumed by communities in Africa[15,16]. This study therefore aimed at determining the antimicrobial susceptibility profiles of bacteria isolated from traditionally fermented Maasai milk against commercial antibiotics and the risk factors that could be associated with the problem of AMR.

2. Materials and Methods

2.1. Ethical approval

The ethical approval was obtained from the JKUAT Institutional Ethical Review Committee (JKU/2/4/896B). The livestock farmers consented to participate in the study.

2.2. Study area

We did the study between May and October 2021

The study was conducted between October and December 2020 in Kajiado Central Sub-County, Kenya, which is characterized as semi-arid with an average annual temperature of 18.9°C. The region experiences an annual rainfall of 500 mm, following a bimodal pattern with short rains occurring from October to December and long rains from March to May. The population of the sub-county stands at 161,862 individuals and has an area of 4,239.50km^2. The predominant population is Maasai pastoralists keeping various livestock such as cattle, sheep goats, and donkeys. The latest census shows that the number of cattle in Kajiado Central Sub County was 95,534[17].

2.3. Study design and sample size determination

A cross-sectional study design was implemented, collecting fermented indigenous cow milk from households randomly selected for participation. The sample size, determined using Cochran's formula, was set at 114 for the study of small population samples[19]. The farmers were randomly selected from the list provided by extension officers from the Directorate of Veterinary Services, Kajiado County, Kenya.

2.4. Fermented milk sample collection

At the household level, the fermented milk samples were stored in traditional milk guards locally known as *Enkukuri.* Each guard contained milk from one cow. The milk samples were collected aseptically and aliquoted in sterile 20ml vials, tightly closed, labeled, and transported into the JKUAT Microbiology laboratory in a cool box. In the laboratory, the milk was stored at -4°C until it was analyzed.

2.5. Determination of pathogenic bacteria

The samples were cultured in selective media for the determination of bacteria present in the milk as described previously[9]. After incubation, individual pure colonies were identified and characterized using standard bacteriological methods. The isolates were frozen at -20°C until further analyzed.

2.6. Antibiotic sensitivity testing

The present study employed the Kirby-Bauer Disk diffusion susceptibility test method[20]. Antimicrobial susceptibility testing was specifically conducted for the isolated *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., and *S. typhi*. which were randomly chosen and were assumed to have zoonotic potential. Muller Hilton Agar plates were inoculated with pure culture isolates of the isolated suspensions with their concentration determined using 0.5% McFarland's standard. These bacteria were selected because they were assumed to have pathogenic potential. Inoculation was done by the spread plate method. The antibiotic disks were placed on the inoculated agar surface and the culture plates were then incubated while inverted at 37°C for 24 hours[20]. The antibiotic disks (HI Media Ltd, India) used were tetracycline (30ug), gentamycin(10ug), chloramphenicol (50µg), ampicillin (10µg), vancomycin (20 ug), streptomycin (10ug), penicillin G (10IU) and kanamycin (30ug). The areas where the growth of bacteria was inhibited during susceptibility testing were measured with a ruler and interpreted based on the 2019 guidelines from the Clinical and Laboratory Standards Institute (CLSI)[21]. The outcomes were documented as either resistant or susceptible to the particular antibiotics.

2.7. Data analysis

The data was entered into a Microsoft Excel (Microsoft, USA) spreadsheet and subsequently transferred to Statistical Package for the Social Sciences (IBM, USA) for comprehensive statistical analysis. The analyzed data were presented in the form of percentages and frequencies, and the results were organized into tables. The proportion of bacterial isolates resistant to antibiotics was calculated as the number of isolates resistant to at least one antibiotic divided by the total number of bacterial isolates.

3. Results

A total of 6 pathogenic bacteria species were isolated from the milk and these included *Streptococcus* spp. (43.86%), *Klebsiella* spp. (15.79%), Coagulase Negative *Staphylococcus* (14.91%), *Pseudomonas* spp. (14.04%), *Staphylococcus aureus* (11.4%), *S. typhi* (7.89%) and *E. coli* (4.39%). These results are described in a previous publication[20] Among these bacteria, an evaluation of the antimicrobial susceptibility profiles was conducted specifically for the isolated *S. aureus*, Coagulase Negative *Staphylococcus*, *E. coli, Klebsiella* spp., and *S. typhi*. These bacteria, known to be prevalent in milk, were presumed to have zoonotic potential.

3.1. Antibiotic susceptibility of the isolated bacteria

Staphylococcus aureus isolates showed diverse resistance to the tested antibiotics (Table 1) with the highest resistance being against oxytetracycline (69.2%), streptomycin (69.2%), and kanamycin (61.5%). The majority of the *S. aureus* were highly sensitive to gentamycin (100%), vancomycin (100%), chloramphenicol (84.6%), penicillin G (84.6%), and ampicillin (76.92%). Coagulase Negative *Staphylococcus* isolates were resistant to kanamycin (94.1%) while being sensitive to chloramphenicol (100%), penicillin G (100%), gentamycin (100%), streptomycin (100%), vancomycin (100%), ampicillin (100%) and tetracycline (88.2%).

The *E. coli* isolates were shown to be resistant (Table 1) against vancomycin (100%), tetracycline (80%), oxytetracycline (80 %), and ampicillin (60 %) while being highly sensitive to kanamycin, (100%), penicillin G (100%), gentamycin (100%) and streptomycin (100%). *S. typhi* isolates exhibited more resistance to vancomycin (88.8%) while being sensitive to chloramphenicol (100%), penicillin G, (100%), gentamycin (100%), and streptomycin (100%) (Table 2). *Klebsiella* spp. were highly resistant to vancomycin (100%), while being sensitive to gentamycin (100%), streptomycin (100%) chloramphenicol (94.4%), kanamycin (94.4%), penicillin G (94.4%), tetracycline (88.9%) and ampicillin (88.9%) (Table 2). It is important to note that all (100%) bacteria isolates were susceptible to gentamycin (Tables 1 and 2).

Table 1. Antibiotic sensitivity profiles of *Staphylococcus aureus*, coagulase-negative *Staphylococcus,* and *Escherichia coli* isolated from traditional milk of cattle from Kajiado Central Sub-County, Kenya

Antibiotic Used	*Staphylococcus aureus* (n=13)			CoNS (17)			*Escherichia coli* (5)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Ampicillin	2 (15.4.%)	1(7.7%)	10 (76.9%)	0(0%)	0(0%)	17(100%)	3 (60%)	0 (0%)	2(40%)
Chloramphenicol	2 (15.4%)	0 (0%)	11(84.6%)	0(0%)	0(0%)	17(100%)	0 (.0%)	0 (0%)	5(100%)
Kanamycin	8 (61.5%)	0 (0.%)	5 (38.5%)	16(94.1%)	1 (5.9%)	0(0%)	0 (0%)	0 (0%)	5(100%)
Penicillin G	2(15.4%)	0 (0%)	11 (84.6%)	0(0%)	0(0%)	17(100%)	0(0.%)	0 (0%)	5(100%)
Gentamycin	0 (0%)	0 (0%)	13 (100%)	0(0%)	0(0%)	17(100%)	0 (0%)	0 (0%)	5 (100%)
Streptomycin	9 (69.2%)	1 (7.7%)	2 (15.4%)	0(0%)	0(0%)	17(100%)	0(0%)	0 (0%)	5 (100%)
Oxytetracycline	9 (69.2%)	0 (0.%)	4 (30.8%)	0(0%)	5(29.4%)	12(70.6%)	4 (80%)	0(0%)	1 (20%)
Vancomycin	0 (0%)	0(0%)	13 (100%)	0(0%)	0(0%)	17(100%)	5 (100%)	0(0%)	0 (0%)

R: Resistant, I: Intermediate resistant, S: Sensitive, CoNS: Coagulase Negative *Staphylococcus*

Table 2. Antibiotic sensitivity profiles of *Klebsiella* spp. and *Salmonella typhi* isolated from traditional milk of cattle from Kajiado Central Sub-County, Kenya

Antibiotic Used	*Klebsiella* spp. (n=18)			*Salmonella typhi* (n=9)		
	R (%)	I (%)	R (%)	I (%)	R (%)	I (%)
Ampicillin	1(5.6%)	1(5.6%)	16(88.9%)	8 (88.9%)	0 (0%)	1 (11.1%)
Chloramphenicol	1(5.6%)	0(0%)	17(94.4%)	0(0%)	0 (0.%)	9(100%)
Kanamycin	0(%)	19(5.6%)	17(94.4%)	2 (22.2%)	0 0.%)	7 (77.7%)
Penicillin G	1(5.6%)	0(0%)	17(94.4%)	0 (0%)	0 (0%)	9 (100%)
Gentamycin	0(0%)	0(0%)	18(100%)	0(0%)	0 (0%)	9 (100%)
Streptomycin	0(0%)	0(0%)	18(100%)	0(0%)	0 (0%)	9 (100%)
Oxytetracycline	6(33.3%)	0(0%)	12(66.7%)	4 (44.4%)	0 (0%)	5(55.6%)
Vancomycin	18(100%)	0(0%)	0(0%)	8(88.9%)	0 (0%)	1(11.1%)
Tetracycline	1(5.6%)	1(5.6%)	16(88.9%)	4(44.4%)	0 (0%)	5 (55.6%)

R: Resistant, I: Intermediate resistant, S: Sensitive

4. Discussion

This study was aimed at assessing the antimicrobial susceptibility profiles of bacteria present in fermented milk produced and consumed by Maasai pastoralists in Kajiado Central Sub-County, Kenya. A previous publication has already discussed the prevalent bacteria in the fermented milk[20] in the study area.These bacteria included *S. aureus,* Coagulase negative *Staphylococcus, E. coli, S. typhi, Streptococcus* spp., *Lactobacillus* spp., *Pseudomonas* spp. and *Klebsiella* spp. In the current study, *Staphylococcus* spp., Coagulase-negative *Staphylococcus, E. coli, S. typhi,* and *Klebsiella* spp. were selected randomly for antimicrobial susceptibility tests.

In this study, *S. aureus* revealed the highest resistance to Oxytetracycline and Streptomycin. Locally, studies carried out on fresh milk also showed that *S. aureus* had high resistance to Streptomycin at 46.9%[9]. High antibiotic resistance levels in *S. aureus* isolated from milk were also documented against oxytetracycline in India (98%)[21] and Italy (84%)[22]. In the present study, *S. aureus* displayed resistance to Kanamycin at rates close to that reported in India (60%)[21] but higher resistance was reported in Italy (88.0%)[22]. According to the local veterinary officers (personal communications) in Kajiado County, oxytetracycline and streptomycin are the commonly used antibiotics for the treatment of cattle diseases in the study area. Most often, these drugs are administered without professional prescription, and drug withdrawal periods are often not observed. This may lead to the accumulation of these drugs in the milk and can lead to the emergence of drug resistance. On the positive side, the *S. aureus* isolates in the current study were all sensitive to gentamycin and vancomycin and moderately sensitive to chloramphenicol and penicillin. A similar study carried out in the study area on fresh milk showed that the isolated *S. aureus* was sensitive to gentamicin (81.3%) and vancomycin (84.4%)[9]. In Ethiopia, isolates of *S. aureus* from milk were highly sensitive to gentamycin and chloramphenicol[23].

Coagulase-negative staphylococci are currently posing a threat to global health due to increased cases of antibiotic resistance and being frequently associated with nosocomial infections[24,25]. The Coagulase Negative *Staphylococcus* species isolated from the current study were shown to be highly sensitive to ampicillin, chloramphenicol, penicillin G, gentamycin, and streptomycin. Sensitivity to tetracycline and oxytetracycline was moderate. These findings are in agreement with a study carried out in South Africa on unpasteurized cow milk locally consumed where all the isolates were generally susceptible to vancomycin, streptomycin, and ampicillin[26]. Another study carried out on goat milk sold in Juja Sub-County, Kenya recorded similar findings where CoNS had high sensitivity against gentamycin, chloramphenicol, and streptomycin[27]. However, these findings contradict those of similar studies carried out in Kajiado County, Kenya where CoNS resistance to vancomycin, gentamycin, and streptomycin varied from 3.7% to 32.7%[9].

In the current study, the *E. coli* isolates exhibited multi-drug resistance patterns against vancomycin, tetracycline, oxytetracycline, and ampicillin. These results are comparable to a study carried out in dairy farms on bovine mastitis in Bangladesh[28], where resistance to both ampicillin and oxytetracycline was high. In Ethiopia, *E. coli* isolates from milk were found to be resistant to Ampicillin 70%[29] and Tetracycline 40%[29]. Furthermore, a study carried out on *E. coli* isolated from raw milk in Northern Kenya indicated that 55% of them were resistant to Tetracycline[30]. However, in the current study, all *E. coli* isolates were susceptible to kanamycin, penicillin G, gentamycin, and streptomycin. The presence of *E. coli* in the finished products may be a result of poor techniques during milk handling and processing and is generally regarded as an indicator of fecal contamination[31,32].

In the present study, the isolated *Klebsiella* spp. exhibited high sensitivity to vancomycin, streptomycin, gentamycin, chloramphenicol, kanamycin, and penicillin G. These findings are comparable to a study carried out in Egypt which involved buffalo and cow mastitis milk[33]. However, the latter study recorded a high prevalence of *Klebsiella* spp. resistant to Ampicillin (100%), chloramphenicol (83%), streptomycin (82.6%), and penicillin G (78.3%). The findings of the current study is also close to a study carried out in Pakistan where a *Klebsiella* spp. had high resistance to vancomycin (95%)[34]. *Klebsiella* spp. such as K. *pneumoniae* and *K. oxytoca* are important causes of bovine mastitis, and there is zoonotic potential for transmission to humans where it can cause serious morbidities[35].

The findings from the present study indicated that *S.*

typhi isolates exhibited high sensitivity to chloramphenicol, penicillin G, gentamicin, and streptomycin. However, the bacteria demonstrated the highest resistance levels to ampicillin, vancomycin, oxytetracycline, tetracycline, and kanamycin. These findings are comparable to studies carried out in Ethiopia where *Salmonella* spp. resistance to ampicillin and tetracycline was 66.7- 94.4% and 52.8%, respectively[36,37]. In Iran, *Salmonella* spp. isolates from milk were resistant to chloramphenicol (21.42%), ampicillin, and tetracycline (42.6%)[38]. Sporadic outbreaks of antibiotic-resistant *S. typhi* are common, especially in resource-poor settings[39]. Since humans are the only source of *S. typhi* infection the milk could have been contaminated through poor milk hygiene and thus there is a need for sanitation improvements in this community.

5. Conclusion

The present study showed that several pathogenic bacteria present in fermented milk manifested antimicrobial resistance to various antibiotics. The *S. aureus,* Coagulase-Negative Staphylococci*, E. coli, Klebsiella* spp., and *S. typhi* showed the highest sensitivity to gentamycin, chloramphenicol and penicillin G. There was marked resistance of the isolates to oxytetracycline and tetracycline and this could be due to wide usage of the drugs in Kajiado County to treat animal diseases, such as mastitis. There is a need to educate the farmers concerning the proper use of antibiotics and the dangers that may arise as a result of the use and misuse of these antibiotics. These isolates can be transmitted to man since amongst most pastoralist communities, processing of milk products does not involve pasteurization before fermentation and is also stored in traditional condition for an indefinite time. Public health education could also go a long way in uplifting the general sanitary conditions in the farms, especially in regards to waste disposal and provision of clean water for domestic use and milking of animals. Further studies should also be undertaken to determine the zoonotic potential of the isolates and the implications of possible transfer to the local human population.

Declarations

Competing interests

The authors of this manuscript declare that they have no competing interests.

Authors' contributions

John Kagira, Naomi Maina, Maina Ngotho were involved in seeking for funds for the project. Jane Kiarie, Naomi Maina, John Kagira and John Maingi designed the study. Jane Kiarie, John Kagira, Maina Ngotho, Peter Achoki carried out the field studies and culturing of the samples. Jane Kiarie, John Kagira, Maina Ngotho, Naomi Maina, Peter Achoki and John Maingi undertook studies on testing the susceptibility of the isolates to antibiotics. Jane Kiarie and John Kagira made the first draft of the manuscript. Jane Kiarie, Peter Achoki, John Kagira undertook the data analysis. All authors reviewed the manuscript.

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Availability of data and materials

The data collected and analyzed during this study are available from the corresponding author upon request.

Ethical considerations

The authors considered farmers' ethical concerns and consent before conducting the study. This article was written originally without any copy from other published studies.

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References

1. World Health Organization (WHO). Antimicrobial resistance. 2023. Available at: https://www.who.int/health-topics/antimicrobial-resistance
2. World Bank. Drug-resistant infections: A threat to our economic future. 2017. Available at: https://www.worldbank.org/en/topic/health/publication/drug-resistant-infections-a-threat-to-our-economic-future
3. World Health Organization (WHO). WHO strategic priorities on antimicrobial resistance. Geneva: World Health Organization. 2021. Available at: https://www.who.int/publications/i/item/9789240041387
4. Taylor J, Marco H, Erez Y, Smith R, Bellasio J, Vardavas R, et al. Estimating the economic costs of antimicrobial resistance: Model and results. RAND Corporation, Santa Monica, Calif, and Cambridge, UK, 2014. Available at: https://www.rand.org/pubs/research_reports/RR911.html
5. Sharma C, Rokana N, Chandra M, Singh B, Gulhane R, Gill J, et al. Antimicrobial resistance: Its surveillance, impact, and alternative management strategies in dairy animals. Front Vet Sci. 2018; 4. 237. DOI: 10.3389/fvets.2017.00237
6. Sukmawinata E, Uemura R, Sato W, Htun MT, and Sueyoshi M. Multidrug-resistant ESBL/AmpC-producing *Klebsiella pneumoniae* isolated from healthy Thoroughbred racehorses in Japan. Animals, 2020; 10(3): 369. DOI: 10.3390%2Fani10030369
7. van den Brom, R., de Jong, A., van Engelen, E., Heuvelink, A., & Vellema, P. (2020). Zoonotic risks of pathogens from sheep and their milk borne transmission. *Small ruminant research*, *189*, 106123. Available at:https://doi.org/10.1016/j.smallrumres.2020.106123
8. James JE. Can public financing of the private sector defeat antimicrobial resistance?. JP Hlth. 2019; 41(2): 422-426. Available at:https://doi.org/10.1093/pubmed/fdy116

9. Ong'era E, Kagira J, Maina N, Kiboi D, Waititu K, Michira L, et al . Prevalence and potential risk factors for the acquisition of antibiotic-resistant *Staphylococcus* spp. bacteria among pastoralist farmers in Kajiado Central Subcounty, Kenya. Biomed *Res Internation*. 2023; 2023: 3573056. DOI: 10.1155/2023/3573056
10. Michira L, Kagira J, Maina N, Waititu K, Kiboi D, Ongera E, et al . Prevalence of subclinical mastitis, associated risk factors and antimicrobial susceptibility pattern of bacteria isolated from milk of dairy cattle in Kajiado Central sub-county, Kenya. *Vet Med Sci*. 2023; 9(6): 2885-2892. Available at:https://doi.org/10.1002/vms3.1291
11. Maske BL, de Melo Pereira GV, da Silva Vale A, Souza DSM, Lindner JDD, and Soccol CR. Viruses in fermented foods: Are they good or bad? Two sides of the same coin. *Food Microbiol. 2021*; 98: 103794. Available at : https://doi.org/10.1016/j.fm.2021.103794
12. Rana AM, Abd El latif, Mohammed EE, and Abdelkhalek AA. Prevalence and characterization of some pathogenic bacteria in fermented milk products and mish cheese in Dakahalia Governorate, Egypt. J Adv Vet Res. 2022; 12(4): 446-450. Available at: https://advetresearch.com/index.php/AVR/article/view/1051
13. Okon IJ, Adamu BB, Joseph RI, Ogbu LC, Emelogu JN, Akubue KN, et al. Isolation of *Salmonella* sp. and *E. coli* from fermented milk product (Nono) in Kuje Area Council (Fct, Abuja, Nigeria) and their antimicrobial resistant status. Intern J Pharmac Bio Med Sci. 2022; 2(10): 435-441. DOI: https://doi.org/10.47191/ijpbms/v2-i10-11
14. Schoustra S, van der Zon C, Groenenboom A, Moonga HB, Shindano J, Smid EJ et al. Microbiological safety of traditionally processed fermented foods based on raw milk, the case of Mabisi from Zambia. LWT. 2022; 171: 113997. DOI: 10.1016/j.lwt.2022.113997
15. Gunga P. Antibiotic resistance phenotypes and genotypes of *Staphylococcus aureus* isolated from milk submitted to the Central Veterinary Laboratories. Thesis, University of Nairobi. 2018. Available at: http://erepository.uonbi.ac.ke/handle/11295/105618
16. El-Sayed AS, Ibrahim H, and Farag MA. Detection of potential microbial contaminants and their toxins in fermented dairy products: A comprehensive review. *Food Anal Methods*, 2022; 15(7): 1880-1898.
17. Admasie A, Eshetu A, Tessema TS, Vipham J, Kovac J, and Zewdu A. Prevalence of Campylobacter species and associated risk factors for contamination of dairy products collected in a dry season from major milk sheds in Ethiopia. *Food Microbiol*. 2023; 109: 104-145. Available at : https://doi.org/10.1016/j.fm.2022.104145
18. Kenya National Bureau of Statistics (KNBS). 2019 Kenya population and housing census. Distribution of population by administrative units, Nairobi, Kenya. 2019. Available at: https://housingfinanceafrica.org/app/uploads/VOLUME-II-KPHC-2019.pdf
19. Cochran WG. Sampling techniques. 3rd ed. New York: John Wiley & Sons; 1977.
20. Kagira JM, Hussein A, Kiptanui A, Lkurasian L, Kiarie J, Cheruiyot K. Risk factors associated with prevalence of brucellosis and bacteria in fermented cow milk obtained from Kajiado Central Sub-County in Kenya. *Asian Journal of Research in Animal and Veterinary Sciences*, *6*(4), 441-448. Available at: http://asian.go4sending.com/id/eprint/1652
21. Quinn PJ, Carter ME, Markey B, and Carter GR. Clinical veterinary microbiology. London: Mosby. 1999; 27(1): 21-66.
22. Sagar A. Mueller Hinton Agar (MHA) – Composition, principle, uses and preparation. 2022. Available at: https://microbiologyinfo.com/mueller-hinton-agar-mha-composition-principle-uses-and-preparation/
23. Neelam, Jain VK, Singh M, Joshi VG, Chhabra R, Singh K, et al. Virulence and antimicrobial resistance gene profiles of *Staphylococcus aureus* associated with clinical mastitis in cattle. PLoS One. 2022; 17(5): e0264762. DOI: 10.1371/journal.pone.0264762
24. Virdis S, Scarano C, Cossu F, Spanu V, Spanu C, and De Santis EP. Antibiotic resistance in *Staphylococcus aureus* and Coagulase Negative Staphylococci isolated from goats with subclinical mastitis. Vet Med Int. 2010; 2010: 517060. DOI: 10.4061/2010/517060
25. Grima LYW, Leliso SA, Bulto AO, and Ashenafi D. Isolation, identification, and antimicrobial susceptibility profiles of *Staphylococcus aureus* from clinical mastitis in Sebeta Town Dairy farms. Vet Med Int. 2021; 2021: 1772658. DOI: 10.1155/2021/1772658
26. Asante J, Amoako DG, Abia AL, Somboro AM, Govinden U, Bester LA, et al . Review of clinically and epidemiologically relevant coagulase-negative staphylococci in Africa. Microbial Drug Res. 2020; 26(8): 951-970. Available at: https://doi.org/10.1089/mdr.2019.0381
27. Cunha, MD, Rugolo LM, and Lopes CA. Study of virulence factors in coagulase-negative staphylococci isolated from newborns. Mem Inst Oswaldo Cruz. 2006; 101: 661-668. Available at: https://doi.org/10.1590/S0074-02762006000600014
28. Abongile P, Uchechukwu UN, Anthony IO, and Green E. Distribution and antibiotic susceptibility profiles of *Staphylococcus* spp. isolated from unpasteurized cow milk locally consumed in Nkonkobe Local Municipality, South Africa, Intern. J Appl Res Vet Med. 2017; 15(2): 75-84. Available at: http://www.jarvm.com/articles/Vol15Iss2/Vol15%20Iss2%20Nwodo.pdf
29. Sombie JI, Kagira J, and Maina N. Prevalence and antibiogram of *Escherichia coli* and *Staphylococcus* spp. isolated from cattle milk products sold in Juja Sub-County, Kenya. J Trop Med.2022; DOI: https://doi.org/10.1155/2022/5251197
30. Bag MAS, Khan MSR, Sami MDH, Begum F, Islam MS, Rahman MM, et al. Virulence determinants and antimicrobial resistance of *E. coli* isolated from bovine clinical mastitis in some selected dairy farms of Bangladesh. Saudi J Biol Sci. 2021; 28(11): 6317-6323. DOI: 10.1016/j.sjbs.2021.06.099
31. Tadesse HA, Gidey NB, Workelule K, Hailu H, Gidey S, Bsrat A, et al. Antimicrobial resistance profile of *E. coli* isolated from raw cow milk and fresh fruit juice in Mekelle, Tigray, Ethiopia. Vet Med Int. 2018; 2018: 8903142. DOI: 10.1155/2018/8903142
32. Ngaywa C, Aboge GO, Obiero G, Omwenga N, Ngwili N, Wamwere G, et al. Antimicrobial-resistant *Escherichia coli* isolates detected in raw milk of livestock in pastoral areas of Northern Kenya. Food Control 2022; 102: 173-178. DOI: 10.1016/j.foodcont.2019.03.008
33. Ribeiro LF, Barbosa M, Pinto FR, Lavezzo LF, Rossi GA, Almeida H, et al. Diarrheagenic *Escherichia coli* in raw milk, water, and cattle feces in non-technified dairy farms. Ciência Anim Bras 2019, 20: 1-9. DOI: 10.1590/1089-6891v20e-47449
34. Disassa N, Sibhat B, Mengistu S, Muktar Y, and Belina D. Prevalence and antimicrobial susceptibility pattern of *E. coli* O157: H7 isolated from traditionally marketed raw cow milk in and around Asosa town, western Ethiopia. Vet Med Int. 2017; 2017: 7581531. DOI: 10.1155/2017/7581531
35. Osman KM, Hassan HM, OrabiA, and Abdelhafez AST. Phenotypic, antimicrobial susceptibility profile and virulence factors of *Klebsiella pneumoniae* isolated from buffalo and cow mastitic milk. Pathog Glob Health. 2014; 108(4): 191-199. Available at: https://doi.org/10.1179/2047773214Y.0000000141
36. Saddam S, Khan M, Jamal M, Rehman SU, Slama P, and Horky P. Multidrug-resistant *Klebsiella pneumoniae* reservoir and their capsular resistance genes in cow farms of district Peshawar, Pakistan. PLoS One. 2023; 18(2): e0282245. DOI: 10.1371/journal.pone.0282245
37. Song J, Xiang W, Wang Q, Yin J, Tian T, Yang Q, et al. Prevalence and risk factors of *Klebsiella* spp. in milk samples from dairy cows with mastitis-A global systematic review. Front Vet Sci. 2023; 10: 1143257. DOI: 10.3389/fvets.2023.1143257
38. Aliyo A, Seyoum A, and Teklemariam Z. Bacteriological quality and antimicrobial susceptibility patterns among raw milk producers and vendors in Gomole District, Borena Zone, Southern Ethiopia. Infect Drug Resist. 2022; 15: 2589-2602. DOI: 10.2147/IDR.S364578
39. Feyissa N, Alemu T, Birri DJ, Desalegn A, Sombo M, and Abera S. Isolation and determination of antibacterial sensitivity characteristics of *Staphylococcus aureus* from lactating cows in West Shewa Zone, Ethiopia. Vet Med Int. 2023; 2023: 3142231. DOI: 10.1155/2023/3142231
40. Dakorah MP. Prevalence of salmonella infections in patients attending St. Dominic hospital,(Akwatia)-Eastern Region (Doctoral dissertation). MSc Thesis, 2014. Kwame: Nkrumah University of Science and Technology. Available at https://ir.knust.edu.gh/handle/123456789/6209
41. Kariuki S, Revathi G, Muyodi J, Mwituria J, Munyalo A, Mirza S, et al. Characterization of multidrug-resistant typhoid outbreaks in Kenya. J Clin Microbiol. 2004; 42(4):1477-1482. DOI: 10.1128/jcm.42.4.1477-1482.2004