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ORIGINAL ARTICLE

Occurrence of Mastitis and Methicillin Resistant *Staphylococcus aureus* in Goats in Zaria, Kaduna State, Nigeria

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SUMMARY

The objective of this study was to conduct a cross sectional study to assess the occurrence of mastitis and Methicillin Resistant *Staphylococcus aureu*s (MRSA) in goats in Zaria, Kaduna State from May to July 2013. A total of 386 fresh goat milk samples from both right and left halves of 198 lactating goats from National Animal Production and Research Institute (NAPRI) and other locations within Zaria metropolis were examined for the occurrence of mastitis and MRSA. The observed prevalence of mastitis in goats, defined by positive (≥+1) California Mastitis Test (CMT) was 119 (60.1%). The prevalence of mastitis showed significant variation among goats of different breeds (p=0.048) but no significant variation between age groups (p=0.890), location and goats raised under the two different systems of management (p=0.438). Also, there was no significant difference as to which udder half was most sub clinically affected (left or right). From a total of 386 fresh goat milk samples examined, 60 strains of staphylococci were isolated and identified using the conventional biochemical tests and 32 of which were identified as *Staphylococcus aureus* using the Microgen™ STAPH-ID system. Furthermore, 12 of these 32 showed agglutinations with the PBP-2a Latex agglutination test reagent. The susceptibilities of the isolates to 14 antimicrobial agents were evaluated using the Kirby-Bauer disc diffusion method and interpreted as sensitive, intermediate or resistant following the Clinical and Laboratory Standards Institute (CLSI, 2017). Out of the 32 isolates of *S. aureus* ; 90.63% were resistant to cefoxitin, Penicillin G (93.75%), Ampicillin (100%), Amoxicillin-clavulanic acid (34.38%), Ceftriaxone (71.88%), Vancomycin (31.25%), Gentamicin (31.25%), Kanamycin (46.88%), Erythromycin (43.75%), Tetracycline (40.63%), Ciprofloxacin (3.13%), Nitrofurantoin (15.63%), Trimethoprim-sulfamethoxazole (25%) and Chloramphenicol (15.63%). All the isolates were resistant 2 or more antimicrobial agents, but none was simultaneously resistant to all. There were 28 antimicrobial resistant patterns in which resistance to Ampicillin, Penicillin G, Cefoxitin were the most frequent. Methicillin resistance was determined using Cefoxitin discs as a surrogate marker of *mec*A gene as recommended by Clinical Laboratory Standards International (CLSI). In conclusion, the findings of this study confirm the importance of *S. aureus* as a mastitis causing organism and the possible role of goats in the transmission of multiple drug resistant *S. aureus.*

Key words: mastitis, methicillin resistant *Staphylococcus aureus*, goats, Zaria.

INTRODUCTION

In Mastitis is the inflammation of mammary glands resulting in physical, chemical and microbiological changes characterized by an increase in somatic cells, especially leukocytes in the milk and by the pathological changes in the mammary tissue. Generally, mastitis occurs in two forms which include clinical (overt) and sub-clinical (hidden). In clinical mastitis all the five cardinal signs of udder inflammation (redness, heat, swelling, pain and loss of milk production) are present, while the sub-clinical form is bereft of any obvious manifestation of inflammation (Haftay *et al*., 2016). Mastitis is a highly prevalent problem in dairy animals and is also one of the most important threats affecting the world's dairy industry (Merz *et al*., 2016). This inflammatory condition of the mammary gland results in changes in the physical characteristics of the udder or milk (Dan Mallam and Pimnoy, 2019).

S. aureus has been reported as the causative agent of infections in many mammals as well as for wild and domestic birds and also in some reptiles. Some animals are asymptomatic while others suffer respiratory, gastrointestinal, or skin and soft tissue infections. *S. aureus* is a significant cause of mastitis in cows and small ruminants (Khaled *et al*., 2015).

Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogen that has attracted public health interest worldwide because it is a major cause of nosocomial infection and colonization, which often result in morbidity and mortality (Haran *et al*., 2015). Emerging disease reports have revealed that antibiotic-resistant pathogens are mostly responsible for the increased rate of hospital-acquired infections (Haran *et al*., 2015).

S. aureus has been considered as the major causative agent in mastitis (Khaled *et al*., 2015) especially in the Nigerian breeds of goats (Dan-Mallam and Pimenov, 2017). These goats are rarely milked by people and their milk is rarely tested for quality and therefore, it is difficult to detect sub clinically infected animals or early cases of mastitis with no swelling of the mammary glands (Dan-Mallam and Pimenov, 2017).

Methicillin resistant *Staphylococcus aureus* (MRSA), like methicillin-susceptible *S. aureus* can cause infections ranging from relatively mild skin infections to life threatening invasive bloodstream infections, pneumonia, central nervous system infections, and pericarditis. MRSA was considered only a human pathogen, until report of MRSA mastitis (udder infection) in a dairy cow surfaced in 1972 (Mohammed and Nigatu, 2015). It has become an increasingly urgent problem in veterinary medicine with MRSA infection reported in small animals and cattle (Rahimi *et al*., 2015; Mohammed and Nigatu, 2015), MRSA has recently been found to be emerging in livestock (Mohammed and Nigatu, 2015). The emergence of MRSA on dairy farms can be responsible for the colonization or infection of farm personnel. It is also a source of contamination for consumers via the food chain (Papadopoulos *et al*., 2018). Livestock- associated MRSA (LA-MRSA) has created public health concerns because it is a source of antimicrobial resistant bacteria, which can be transmitted to humans (Asiimwe *et al*., 2017). It is known that people working in close contact with animals, particularly on MRSA- positive farms are at a high risk of being colonized with LA-MRSA (Schmidt *et al*., 2017). Animals can act as reservoirs of MRSA, and the bacterium can be transmitted to humans in close contact with MRSA colonized animals. Persons in direct contact with MRSA-positive animals have an increased risk of becoming MRSA positive. This has been documented for individuals working in companion animal and equine clinics, and livestock production environments (Kaur and Chate, 2015).

It has attracted the attention of the medical research community, illustrating the urgent need

to develop better ways to diagnose and treat bacterial infections.

The aim of this research was to determine the occurrence of mastitis and Methicillin Resistant *Staphylococcus aureus* (MRSA) in goats in Zaria.

MATERIALS AND METHODS

Study Area

The study area spanned three L.G.As in Zaria and environs. Zaria is located in Kaduna state, within latitudes 11^{0} $7^{/}$ N and longitudes 07^{0} $41^{/}$ E. It is administratively divided into Zaria and Sabon gari Local Government Areas (Ministry of Economic Development, 1996).

Study Animals

Fresh milk samples were collected from 198 lactating goats based on convenience sampling method. Three hundred and eighty six (386) milk samples were collected from both right and left halves of 198 goats based on convenience sampling technique. Samples were collected from Kano brown, Sokoto red, West African dwarf and Sahelian breed of goats.

Milk Sample Collection

At least 3ml of milk sample was aseptically collected in a sterile sample bottle following the standard procedures described by National Mastitis Council (NMC, 2014). Each teat was disinfected with a cotton wool soaked in 70% ethyl alcohol and the fore milk was discarded before the collection of the milk samples. The samples were properly labelled and transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria in Coleman box with ice packs. Samples were collected between May and July 2013.

California Mastitis Test

The California mastitis test was conducted to detect the presence of subclinical mastitis and it was carried out according to procedures given by Quinn *et al*. (2002). About 2ml of milk sample was mixed in 2ml of the CMT reagent (Alkyl aryl sulphuric acid) (Kruuse Denmark) in each well of the test plate (CMT paddle). A gentle circular motion was made with the plate for 10 seconds to mix the reagent and milk; the formation of milk clots upon addition of the reagent was recorded based on changes in the milk (colour change, viscosity and gel formation). Positive samples showed change in viscosity or gel formation within a few seconds. Results were read immediately as per manufacturer's recommendation and were scored as 0 (negative), ± (trace), 1+ (weak positive), 2+ (distinct positive), 3+ (strong positive) depending on the degree of viscosity and of gel formed as described (Kandeel *et al.,* 2018). A goat was considered positive if at least one half of the udder was positive by CMT with or without the isolation of microorganisms.

Isolation and Identification of *S. aureus*

One ml of goat milk sample was aseptically added to 9ml of Modified Tryptone Soy Broth (MTSB) supplemented with 6.5% NaCl and incubated at 37^{o}C for 24 hours. After the incubation, a loopful of growth inoculum from MTSB was streaked onto the surface of Baird Parker Agar (Oxoid®) plates and incubated at 37^{o}C for 24 hours. Presumptive colonies of *Staphyloccocus aureus* species appearing as black shiny colonies on BPA were picked, inoculated on nutrient agar slants and grown at 37^{o}C for 24 hours. The isolates were stored in the refrigerator at 4^{o}C for further characterization.

Conventional Biochemical Tests

The following biochemical tests were carried out for the presumptive identification of *Staphylococcus aureus*; Gram staining, catalase, coagulase, sugar fermentation (glucose, lactose, maltose, sucrose, xylose, arabinose and raffinose), haemolysis on 5% sheep blood agar, pigmentation with mannitol salt agar and DNase activity.

Microgen Staphylococcus-ID testing of Staphylococcus

Commercially available biochemical test strip (Microgen^{TM} STAPH-ID) system was used according to the manufacturer's instructions to confirm isolates as *S. aureus.*

Antimicrobial Susceptibility Test

The susceptibilities of *S. aureus* isolates to 14 antimicrobial agents were determined by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (Bauer *et al*., 1966), and the results were interpreted according to the recommendations of Clinical and Laboratory Standards Institute (CLSI, 2017). The following antimicrobial disks (Oxoid, Basingstoke, UK) with their corresponding concentrations were used: Penicillin G (10units), Ampicillin (10ug), Cefoxitin (30ug), Gentamicin (10ug), kanamycin (30ug), Tetracycline (30ug), Vancomycin (30ug), Chloramphenicol (30ug), Ciprofloxacin (5ug), Erythromycin (15ug), Trimethoprim-sulphamethoxazole (25ug), Nitrofurantoin (300ug), Ceftriaxone (30ug), Amoxicillin–clavulanic acid (30ug). A sterile wire loop was used to pick 3-5 well isolated colonies of the test organism and they were emulsified in 3ml of normal saline. The turbidity of the suspension was matched to the turbidity of 0.5 McFarland standard. A sterile swab was used to inoculate the test organism on Muller Hinton agar plates. The swab was evenly streaked over the surface of the plate and left un-covered for about 3-5 minutes for the surface of the agar to dry. A multidisc dispenser (Oxoid) was used to distribute 7 discs of the antimicrobial agents per inoculated plate. The plates were inverted and incubated aerobically at 35^{o}C for 18-24 hours.

RESULTS

A cross sectional study on subclinical mastitis (SCM) was carried out on 198 lactating goats in Zaria, Kaduna State. Milk samples were collected from 386 udder halves (194 right udder halves and 192 left udder halves) while 10 udder halves had blind ends. Of these, samples from
101 right udder halves and 100 left udder halves had CMT values ≥ +1. The overall prevalence of mastitis by CMT was 60.1%. Goats sampled from Sabon-gari L.G.A had the highest prevalence 64.40% of subclinical mastitis (SCM) while the goats sampled from NAPRI and Zaria L.G.A had prevalence rates of 57.84% and 59.46% respectively (TABLE I). There was no significant difference in the prevalence of mastitis based on location (p=0.569).
The prevalence of subclinical mastitis by CMT with respect to the breed of goats sampled is shown on TABLE II. Kano brown had the highest prevalence 79.4%, Sahelian 54.9%, Sokoto red 55.77% and West African Dwarf (66.66%). There was a significant association between the breeds of goats and the occurrence of mastitis (p=0.048).

TABLE 1: Occurrence of Mastitis in Goats in Zaria, Kaduna state, Nigeria

Location	Total no. Sampled	No. Positive (%)	No. Negative (%)
NAPRI	102	59 (57.84)	43 (42.16)
Zaria L.G.A	37	22 (59.46)	15 (40.54)
Sabon gari L.G.A	59	38 (64.40)	21(35.59)
Total	198	119 (60.1)	79 (39. 9)

Chi Square =1.126, p=0.569

Based on age distribution of the goats, does aged 7 years and above had the highest prevalence of subclinical mastitis, 66.67%, while the goats aged between 1-3 years and 4-6 years had prevalence rates of 59.8% and 59.8% respectively (TABLE III). The differences between the three age groups were not significant (p=0.89).

TABLE II: Occurrence of SCM in four Breeds of Goats in Zaria, Kaduna state, Nigeria

Location	Total no. Sampled	No. Positive (%)	No. Negative (%)

Sokoto Red	102	58 (55.77)	46 (44.2)
Sahel	51	28 (54.90)	23 (45.1)
Kano Brown	34	27 (79.4)	7 (20.59)
West African Dwarf	198	6 (66.66)	3 (33.33)

Chi Square =7.884, p=0.048

TABLE III: Occurrence of SCM in the different Age groups of Goats

Location	Total no. Sampled	No. Positive (%)	No. Negative (%)
1-3	82	49 (59.8)	33 (40.24)
4-6	107	64 (59.8)	43 (40.19)
7 and above	9	6 (66.67)	3 (33.33)
Total	198	119 (60.1)	79 (39.9)

Chi Square =0.232, p=0.89

The prevalence of subclinical mastitis with respect to the management system under which the goats were kept is shown on TABLE IV. The results showed that goats kept under intensive system of management had a lower prevalence of subclinical mastitis (57.8%) compared to the goats kept under the extensive system of management (62.5%). The difference between the two system of management was not significant (p=0.438).

The results of this study showed the overall prevalence of *S. aureus* isolated from goats in Zaria to be 8.3% (TABLE V). From a total of 386 goat milk samples collected, 60 (15.5%) milk samples contained *Staphylococcus* species while 32 (8.3%) were confirmed as *S. aureus* by conventional biochemical testing and microgen Staph-ID system. The other *Staphylococcus* species identified in this study were *S. chromogenes*, 4 (1.04%), 8 (2.1%) *S. hominis*, 5 (1.3%) *S. caprae* and 11 (2.85%) *S. haemolyticus*.

TABLE IV: California Mastitis Test (CMT) results for Subclinical Mastitis (SCM) in Goats in Zaria based on system of management

Location	Total no. Sampled	No. Positive (%)	No. Negative (%)
Intensive	102	59 (57.8)	43 (42.15)
Semi-intensive	96	60 (62.5)	36 (37.5)
Total	198	119 (60.1)	79 (39.9)

Chi Square =0.601, p=0.438

TABLE V: Association between CMT Scores and Occurrence of *S. aureus*

CMT Score	+ve	-ve	SR (%)	OR	95% CI on OR
0	9	112	7.4	1	
Trace	12	51	20.3	3.17	1.27, 7.90 *
1+	6	94	6	0.79	0.27, 2.31
2+	3	51	5.5	0.73	0.19, 2.82
3+	1	46	2.1	0.27	0.33, 2.20
Total	32	354			

* Significant at P< 0.05
SR= specific rate , OR=3.17, 95% C.I on OR=1.27, 7.90

The association between CMT scores and the occurrence of *S. aureus* in goat milk is shown in TABLE VI. Out of a total of 386 milk samples tested, 32 *S. aureus* were isolated. Nine *S. aureus* were isolated from milk samples with CMT negative score while 13, 6, 3 and 1 *S. aureus* isolates were observed from trace, weak, distinct and strong positive CMT scores, respectively. From the odds ratio calculated, *S. aureus* was more likely to be isolated from milk samples that showed trace CMT score than from those with

negative, weak, distinct and strong positive CMT scores.

Of a total of 386 milk samples tested by CMT, 201 samples were CMT-positive while 185 milk samples were CMT negative (CMT score 0 or trace (±). Of the 185 milk samples taken as CMT-negative, 22 (11.9%) yielded *Staphylococcus aureus*, while only 10 (5%) out of the CMT-positive samples yielded *S. aureus*.

The association between CMT results and the frequency of isolation of *S. aureus* from the udder halves of goats is shown on TABLE VII. The result shows that a higher prevalence of *S. aureus,* (62.5%) was isolated from the left udder halves in comparison to the right udder half (37.5%). The odds ratio calculation showed that *S. aureus* were more likely to be isolated from milk samples with CMT trace scores than the negative, weak positive, distinct and strong positive CMT scores.

The results of the antimicrobial testing showed high percentage of resistance by the isolates to ampicillin (100%), penicillin G (93.75%), cefoxitin (90.63%), ceftriaxone (71.88%). The isolates were moderately resistant to kanamycin (46.88%), tetracycline (40.63%), erythromycin (43.75%), gentamicin (31.25%), amoxicillin/clavulanic acid (34.38%), vancomycin (31.25%), trimethoprim-sulfamethozole (25%) and relatively low frequency of resistance was observed to chloramphenicol (15.63%), nitrofurantoin (15.63%) and ciprofloxacin (3.13%) (Fig.1).

TABLE VI: Association between CMT Scores and Occurrence of *S. aureus*

S. aureus CMT Score	+ve	-ve	SR (%)	OR	95% CI on OR
0	9	112	7.4	1	
Trace	13	51	20.3	3.17	1.27, 7.90 *
1+	6	94	6	0.79	0.27, 2.31
2+	3	51	5.5	0.73	0.19, 2.82
3+	1	46	2.1	0.27	0.33, 2.20
Total	32	354			

* Significant at P< 0.05

SR= specific rate

OR=3.17, 95% C.I on OR=1.27, 7.90

All the 32 *S. aureus* strains tested exhibited multiple antibiotic resistance patterns to the 14 antibiotics tested. Thirty antimicrobial resistance patterns were observed against the tested antimicrobials. The antimicrobial agents most commonly encountered among the patterns were ampicillin, penicillin G, cefoxitin and ceftriaxone and the patterns AMP-CRO-P-FOX-CN and AMP-CRO-K-P-FOX-CN appeared twice, while all the others once (TABLE VIII).

TABLE VII: Association between CMT Scores and Udder-wise Isolation of *S. aureus*

CMT Score	S. aureus L +ve	L -ve	R +ve	R _ve	OR	95% CI on OR
0	2	61	7	51	0.239	0.048, 1.20
Trace	10	19	3	32	5.614	1.371, 23.00*
1+	5	48	1	46	4.792	0.539, 42.59
≥2+	3	44	1	53	3.614	0.363, 35.98
Total	20	172	12	182		

*Significant at < 0.05

OR=5.61, 95% C.I on OR=1.37, 23.00

TABLE VIII: Antimicrobial Resistance Patterns of 32 *Staphylococcus aureus* Isolates

S/N	Pattern	Frequency
1	AMP, CRO	1
2	AMP, CRO, P	1
3	AMP,FOX,P	1
4	AMP, FOX, E, CN	1
5	AMP, SXT, FOX, P	1
6	AMP, CRO, K, P	1
7	AMP, FOX, CRO, P	1
8	AMP, FOX, AMC, K, P	1
9	AMP, FOX, E, CRO, P	1
10	AMP,FOX, CRO, K, P	1
11	AMP, FOX, CN, TE, CRO	1

12	AMP, FOX, CN, CRO, K, P	2
13	AMP, FOX, CN, CRO, P	2
14	AMP,FOX, E,TE,C, CRO, P	1
15	AMP, FOX, E, VA, CRO, P	1
16	AMP, FOX, E, CRO, K, P	1
17	AMP, FOX, TE, CRO, K, P	1
18	AMP, SXT, FOX, AMC, F, P	1
19	AMP, FOX, TE, VA, CRO, K, P	1
20	AMP, FOX, E, AMC, TE, VA, K, P	1
21	AMP, FOX, E, TE, VA, CRO, K, P	1
22	AMP, FOX, E, CN, VA, CRO, K, P	1
23	AMP, SXT, FOX, AMC, F, CRO, P	1
24	AMP, SXT, FOX, E, AMC, TE, C, K, P	1
25	AMP, FOX, E, AMC, TE,VA , CRO, P	1
26	AMP, CIP, FOX, AMC, TE, C, CRO, K, P	1
27	AMP, SXT, FOX, E, CN, AMC, TE,VA, C, F,	1
28	AMP, SXT, FOX, E, AMC,TE, VA, F, CRO, P	1
29	AMP, SXT, FOX, E, CN, AMC, TE, VA, C, K, P,	1
30	AMP, SXT, FOX, E, CN, AMC, TE, VA, F, CRO, K, P	1

The results of the multiple antibiotics resistance index showed that 96.9% of the *S. aureus* isolates tested were multidrug resistant. A total of 31 isolates were resistant to three or more antimicrobial agents tested while only one isolate showed resistance to two antimicrobial agents. None of the test isolates was simultaneously resistant to all the antimicrobial agents. The MAR index was determined using the formula; MAR=a/b, where 'a' was the total number of antibiotics to which the test isolate was resistant to and 'b' is the total number of antibiotics to which the test isolate has been evaluated for sensitivity. The MAR index for the *S. aureus* isolates tested ranged from 0.14 to 0.86 where 40.6% of the isolates were resistant to at least 7 antimicrobial agents (TABLE IX).

TABLE IX: MAR Index Analysis of the 32 *S. aureus* isolates obtained from goat milk in Zaria

S/N	Isolate	No.of antibiotics to which the isolate was resistant (a)	MAR Index (a/b)*
1	L134	12	0.86
2	L127	11	0.79
3	L97	11	0.79
4	L156	10	0.71
5	R147	9	0.64
6	L151	9	0.64
7	R161	8	0.57
8	L160	8	0.57
9	L137	8	0.57
10	L150	8	0.57
11	L175	7	0.50
12	R159	7	0.50
13	L157	7	0.50
14	R136	6	0.43
15	L148	6	0.43
16	R110	6	0.43
17	L161	6	0.43
18	L111	6	0.43
19	L13	6	0.43
20	L105	6	0.43
21	L136	5	0.36
22	L19	5	0.36
23	L50	5	0.36
24	R105	5	0.36
25	R160	5	0.36
26	R114	4	0.29
27	R13	4	0.29
28	R184	4	0.29
29	L94	4	0.29
30	R89	3	0.21
31	R94	3	0.21
32	L49	2	0.14

B = Total no. of antibiotics used; 14

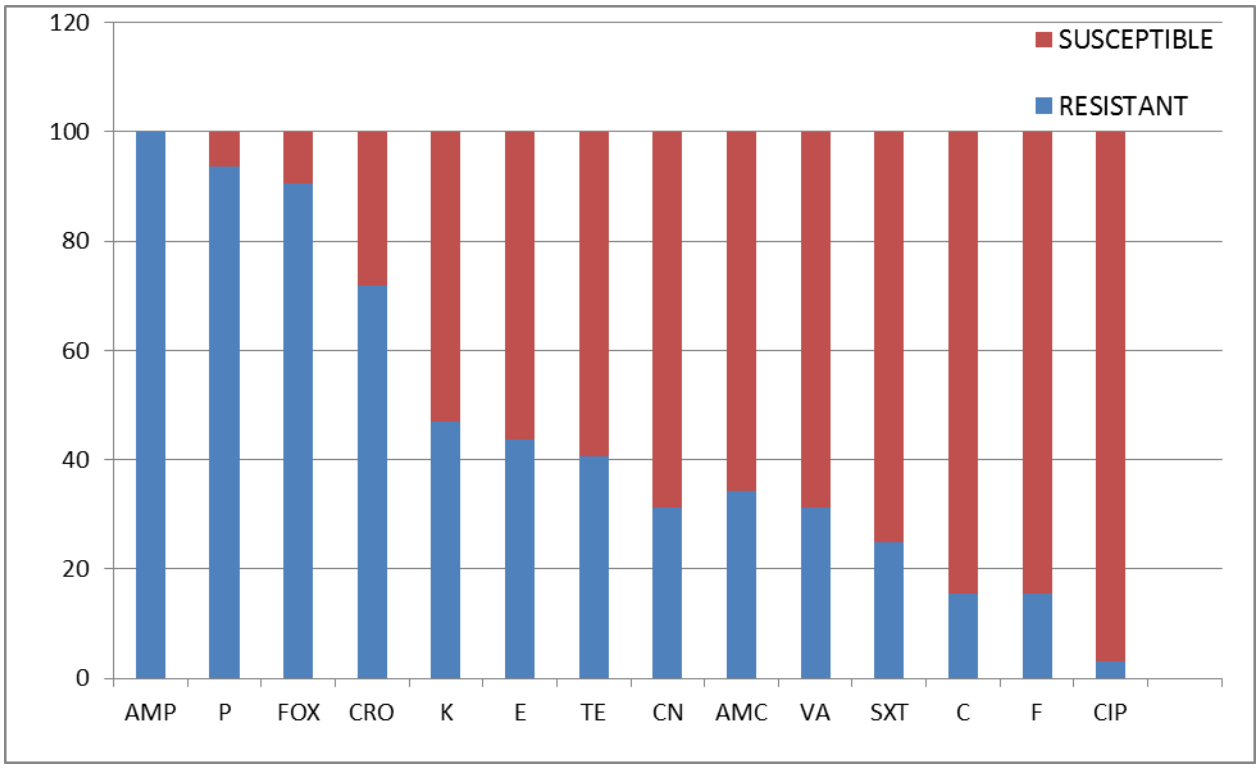

Figure 1: Percentage Distribution of resistance among *S. aureus* tested against 14 antimicrobial

KEY

TE	-	Tetracycline
C	-	Chloramphenicol
F	-	Nitrofuran
CRO	-	Ceftriaxone
K	-	Kanamycin
P	-	Penicillin G
AMP	-	Ampicillin
CIP	-	Ciprofloxacin
SXT	-	Trimetoprim-sulphamethoxazole
AMC	-	Amoxicillin/clavulanic acid
VA	-	Vancomycin
CN	-	Gentamicin
E	-	Erythromycin

DISCUSSION

Mastitis is one of the most important and frequent disease of goats (El-Shymaa *et al*., 2018). California Mastitis Test (CMT) was conducted on 386 milk samples collected from 198 lactating goats in Zaria to detect the presence of subclinical mastitis. A total of 201 (52.1%) milk samples from both the right and left udder halves were CMT positive (CMT ≥+1) while 185 (47.9%) gave a negative CMT result (CMT=0, trace; ±). In this study, the CMT scores of 0 and trace (±) were considered as negative and 1+, 2+ and 3+ as positive. Out of the 198 lactating goats sampled, 119 (60.1%) goats had subclinical mastitis in that a positive CMT result was detected in at least one of the udder halves, while 79 (39. 9%) showed no evidence of subclinical mastitis from the CMT test.

In dairy goats, the incidence of clinical mastitis should not exceed 5%, while sub-clinical mastitis (SCM) is common and is about 6 times more than clinical cases (Nabil*et al*., 2018). In this study, the prevalence of SCM by CMT was 60.1%. The findings of this study was in close agreement with results (61%) reported in Tanzania (Mbindyo *et al*., 2014) and the result (61%) reported by El-Shymaa *et al*., (2018) in Egypt, and is higher than the 32.4% obtained by Dan Mallam & Pimenov (2019) from goats in Bauchi, Plateau and Edo States, Nigeria and the result (43.33%) reported by Ferdous *et al*., (2018) in Bangladesh. As mastitis is a complex disease involving the

interaction of various factors such as differences in management and husbandry practices, geographical distribution, health status of the flock, causative agent, environmental conditions (weather), nutritional status and finally the size of the study samples may be the reason for the variation in the prevalence rates between the present study and the studies mentioned (El-Shymaa *et al*., 2018; Ferdous *et al*., 2018). The high prevalence of *S. aureus* in this study is in accordance with other works which found a higher prevalence of this organism from goat milk samples. These findings and other research works justify that *S. aureus* is the most important and predominant mastitis causing pathogen globally, including Nigeria. The high prevalence of *S. aureus* in goats is suggestive of re- infection of mammary glands and teat lesions which transfer during milking operations, and in Nigeria, mostly, hand milking is practised. Intra-mammary infections caused by *S. aureus* are very important from the public health point of view due to its ability to produce thermo stable enterotoxins and leukotoxins (Khaled *et al*., 2015).

This result is also comparable with the findings of Pirzada *et al*. (2016) who obtained a prevalence of 52.63% of sub clinical mastitis in Pakistan, but higher than the result recorded in India (19.89%) by Mishra *et al*. (2018). The high prevalence of SCM observed in this study could be due to the period when the goats were sampled (May- July). In general, the variability in the prevalence of caprine mastitis between reports could be attributed to the difference in management systems, breed of goats or technical knowledge of the investigator (Mishra *et al*., 2018).

In this study, goats that were sampled from Sabon-gari L.G.A had a higher prevalence of subclinical mastitis (64.4%) compared with goats sampled from Zaria L.G.A and NAPRI with prevalence of 59.46% and 57.84% respectively. This may be due to the lower hygienic standards of the environment where the goats were kept and other management practices, since all the goats sampled from Sabon-gari L.G.A. were kept under the semi-intensive management system. Goats sampled from NAPRI were kept under intensive system of management where the hygienic standard of the environment is higher compared with the other locations from which the goats were sampled.

Goats that were aged 7 years and above had higher prevalence (66.67%) of subclinical mastitis compared with goats aged between 1-3 years and 4-6 years with prevalence rates of 59.8% and 59.8% respectively. The differences between the three age groups was not statistically significant (p=0.89). The increase in prevalence of mastitis with age may be due to the fact that older goats are long term nannies that have stayed longer in the herd and hence are at higher risk of coming into contact with mastitis causing organisms than the younger does. Old age and multiple parturitions have been reported to produce stress on animals and as a result, such animals become susceptible to infectious agents due to low immunity level (Mahlangu *et al*., 2018). The result of this study differs from the findings of the study of Mahlangu *et al*. (2018) who reported that goats of age 1year had a prevalence of SCM of 67% as compared to older goats of 2 and 3 years which had a prevalence of 46% and 50% respectively.

A higher prevalence of subclinical mastitis was found in goats raised under the semi-intensive system of management (62.5%) compared with goats kept under the intensive management system (57.8%). The reason for this difference in prevalence may be due to the fact that goats kept under the semi-intensive management system are allowed to graze outside their pens during day time and their udder could be exposed to trauma from hard objects like broken bottles and rusty metals in their scavenging environment, which makes them susceptible to mastitis causing organisms. Also, these animals have greater chances of mixing up with other animals harbouring mastitis-causing organisms and could get infected as a result of this exposure. This result is comparable with the findings of Makau *et al*. (2017) who recorded a high prevalence of mastitis

in West African dwarf goats kept under extensive system in Ogbomosho, Nigeria.

Of a total of 386 milk samples tested by CMT, 201 samples were CMT-positive while 185 milk samples were CMT negative (CMT score 0 or trace ($\pm$). Of the 185 milk samples taken as CMT-negative, 22 (11.9%) yielded *Staphylococcus aureus*, while only 10 out of the CMT-positive samples yielded *S. aureus* (TABLE VI). The CMT-positive milk samples which yielded no bacterial growth on culture could be due to less pathogenic bacteria that do not induce detectable levels of somatic cell count. It could also be partly explained by the fact that the udder could be injured and is recovering from infection or the infection was not caused by bacterial pathogens (Hristov *et al*., 2016) or bacterial pathogens other than *S. aureus* would have been responsible for the infection since a selective medium was used for the cultural isolation. It could also be due to an organism such as *Mycoplasma* species which requires a special media and cannot be identified in the routine bacterial isolation techniques (Hristov *et al*., 2016). Also, it has been reported that a CMT score of 1+ or higher is a good indicator of mastitis in goats which corresponds to a somatic cell count of greater than $0.8x10^6$cells/mL (Kandeel *et al*., 2018). CMT negative and trace results could be culture positive (false negative) and a number of samples with CMT scores of $\geq +1$could also be culture negative (false positive; 20-40%) (Kandeel *et al*., 2018). This result is in agreement with the reports of National Mastitis Council which states that 25-40% of milk samples of animals with clinical mastitis do not have a bacterial isolate. Also, an infected quarter with a bacterial concentration below 100cfu/ml which would cause the bacteria to be shed intermittently; white blood cells in the milk may also have engulfed and sequestered bacteria preventing isolation or some other mastitis causing pathogens such as viruses and fungi may have accounted for the high number of culture negative samples (NMC, 2014). The result of this study is lower than the result of Wang *et al*. (2018), who reported that 46.2% of the milk samples were positive for *Staphylococcus aureus*. The results of this study showed the overall prevalence of *S. aureus* isolated from goats in Zaria to be 8.3%. The other *Staphylococcus* species identified in this study were *S. chromogenes*, 4(1.04%), 8 (2.1%) *S. hominis*, 5 (1.3%) *S. caprae* and 11 (2.85%) *S. haemolyticus*. Previous studies on subclinical mastitis (SCM) in dairy goats revealed that Coagulase Negative Staphylococci (CNS) make up 44.7% to 95.9% of the isolated pathogens from milk samples, and *S. aureus*, which is usually considered to have the greater pathogenicity, accounts from 4.1% to 18.0% of SCM agents (Dore *et al*., 2016; Makau *et al*., 2017). This could be linked to the wide distribution of *S. aureus* inside the mammary glands and the skin of teats and udders (Mahlangu *et al*., 2018) and the fact that the organism has adapted to survive in the udder to establish chronic and subclinical infections (Mahlangu *et al*., 2018). The prevalence of *S. aureus* in this study is in agreement with the 10.65% prevalence of *S. aureus* reported by Mahlangu *et al*. (2018) from milk of goats with subclinical mastitis in Thika East subcounty, Kenya. The high prevalence of *S. aureus* intramammary infection can be of veterinary and public health concern because it is an important zoonotic bacterial pathogen which can also be transmitted to humans through raw milk of goats or sheep and can cause food borne intoxication associated with enterotoxin production.

In the present study, the prevalence of SCM was higher in milk samples collected from the right udder halves compared to the left udder halves, which was found not to be statistically significant ($P<0.05$). This is in agreement with the reports of Pirzada *et al*. (2016) in Pakistan who reported 52.63% prevalence for the right udder halves and 47.36% for the left udder halves. This was thought to be due to the greater milk production capacity of the right udder halves (Mangi *et al*. 2015).

Staphylococcus aureus has been known to show multiple antimicrobial resistance patterns. The

widespread use of antibiotics has undoubtedly accelerated the evolution of *S. aureus*, which as a result of the acquisition of multiple resistance genes has become able to survive almost all antibiotic families (Liu, *et al*. 2017).

In this study, resistance of *Staphylococcus aureus* isolates to ampicillin was higher compared to cefoxitin, penicillin G and ceftriaxone. The susceptibility of the isolates to ampicillin was within the range reported by Widianingrum *et al*. in 2016. The resistance observed for ampicillin (100%) is also in agreement with the reports by Mahlangu *et al*. (2018) who observed a resistivity of 100% to ampilcillin in a study carried out in Zaria. The resistance (40.63%) of the isolates to tetracycline in this study may reflect the excessive use of the drug in treatment in the study area.

The susceptibility by *S. aureus* isolates to chloramphenicol, gentamicin and kanamycin observed in this study is comparable with the findings of Klimesova *et al*. (2017) who reported resistance to chloramphenicol to be 15.63 %. The high proportion of susceptibility by the isolates to nitrofurantoin (84.38%), ciprofloxacin (78.13%) and chloramphenicol (78.13%) is suggestive of the potential efficacy of these drugs in treating MRSA infections and may reflect the fact that they may be less commonly abused in the environment. Also, the resistance to sulphamethozole/trimethoprim (25%) was slightly lower than that reported by Merz *et al*. (2016). The high resistance to Penicillin G (93.75%) reported in this study is similar to that reported by Mahlangu *et al*. (2018), who reported that all the isolates were resistant to penicillin G, also similar resistance was reported in India (Priya, 2016). In South Africa, Akondilire *et al*. (2015) reported a 100% resistance of the *Staphylococcus aureus* isolates from milk to penicillin G. The high resistance is probably due to the long time and extensive use of penicillin in the treatment of mastitis (Priya, 2016).

The evaluation of the antimicrobial susceptibility of *Staphylococcus* species isolated from goats with subclinical mastitis is of interest for clinical purposes in order to decide which antibiotics should be administered, as well as, for monitoring the spread of multiple resistant strains on farms (Widianingrum *et al*., 2016).

The multiple antibiotic resistance index (MAR) showed that 90.6% of isolates had MAR index greater than 0.25. This suggests that the isolates may have originated from a high risk source of contamination where antibiotics are often used and probably abused. This may be due to the fact that most goat owners prefer administering drugs to their animals themselves without employing the services of veterinarians.

In conclusion, the prevalence of subclinical caprine mastitis by California Mastitis Test (CMT) was 60.1% and the prevalence of *S. aureus* in goat milk in Zaria was found to be 8.3%. The prevalence of MRSA detected phenotypically was found to be 7.5%. The percentage resistance of the isolates to various antimicrobial agents ranged from 3.13% to 100%. Routine milking of lactating does and testing for mastitis would lead to early detection and treatment of subclinical cases of mastitis while culling of advanced and chronic cases would reduce the prevalence and spread of mastitis.

CONFLICT OF INTEREST

We declare no conflict of interest.

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