



OCCURRENCE OF BOVINE MASTITIS AND ISOLATION OF *Staphylococcus* SPECIES FROM FRESH COW MILK IN SETTLED FULANI HERDS IN KADUNA STATE, NIGERIA

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ABSTRACT

A study was conducted on 360 dairy cows (1440 quarters) to determine the prevalence of mastitis by California Mastitis Test (CMT) and Staphylococci in settled Fulani herds in Kaduna State, Nigeria. The prevalence of mastitis at cow level was 26.9% (97/360) and out of which 23.9% (86/360) and 3.1% (11/360) were subclinical mastitis and clinical mastitis respectively. The prevalence at quarter level was 21.7% (312/1440), and out of which 6.5% (28/1440) was clinical mastitis and 19.7% (284/1440) was subclinical mastitis respectively. Statistical analysis showed that there was significant difference in the occurrence of mastitis among the age group ($P < 0.05$). The prevalence at quarter level was 25.2% (157/624) in adult cows, 20.2% (97/480) in young adults and 17.3% (58/336) in old cows. Similar pattern of occurrence was recorded for both clinical and subclinical mastitis as the adults had 21.8% and 3.4 %, young adults had 19.8% and 0.4% and old cows had 15.8% and 1.5% respectively. The CMT scores showed that 55 (64.0%) of the milk samples had a score of 1+, 26 (30.2%) had a score of 2+, 3 (3.5) had a score of 3+ while only 2 (2.3%) had a score of "Trace". Sixty seven (69.1%) of the samples that were positive for CMT were found to be culture positive and out of which 56.7% (55/97) were *S. aureus* and 12.3% (12/97) were coagulase-negative staphylococci (CONS). The high prevalence of both clinical and subclinical mastitis coupled with the isolation of *S. aureus* and CONS are of great public health concern and requires strict control and preventive measures. The study recommends standards hygienic measures during milking and culling of infected cows to stop the spread of pathogens to uninfected cows.

Key words: mastitis, prevalence, Cow milk, Staphylococcus spp, settled Fulani herds, Kaduna State

INTRODUCTION

Dairy production systems provide milk that is rich in carbohydrates, fats, vitamins and minerals for the growing population (Wilson *et al.*, 2004; Mekibib *et al.*, 2010). However, milk production does not always meet the population's demands due to several factors, common among which is mastitis (Islam *et al.*, 2010; Cervinkova *et al.*, 2013; Kurjogi and Kaliwal, 2014). Mastitis is a complex and costly disease of dairy animals resulting from interplay between the dairy animal and the environment including the milking machines and milkers (Azmi *et al.*, 2008). Mastitis is inflammation of mammary gland affecting all the species of domestic animals and characterized by the changes in physical appearance of milk, swelling, redness, and rises in temperature of udder (clinical mastitis) and in many case no visible changes in milk or udder (sub clinical mastitis) are apparent (Wilson *et al.*, 2004; Samad, 2008; Reza *et al.*, 2011; Kurjogi *et al.*, 2014). Bovine mastitis has great economic impact on dairy industry and is associated with multi-etiological

agents, with one third of all dairy cows estimated to be infected with some form of mastitis in one or more quarters (Samad, 2008; Islam *et al.*, 2010). In almost all dairy animals, microorganisms are mainly incriminated in mastitis, but some predisposing factors such as poor management and hygiene, teat injuries and faulty milking machines, pendulous udder with long teats, larger size of teat orifice in high yielding cows, etc. play an important role in the development of mastitis (Islam *et al.*, 2010). Microorganisms causing mastitis are either contagious or environmental, with an infected udder serving as the primary reservoir of contagious pathogens whereas a contaminated environment as the primary reservoir of organisms causing environmental mastitis (Cervinkova *et al.*, 2013). *Streptococcus agalactiae*, *Staphylococcus aureus* subsp. *aureus* and *Mycoplasma* spp. are the most typical contagious organisms causing mastitis (Suleiman *et al.*, 2013; Cervinkova *et al.*, 2013),

while the environmental organisms are streptococci, *Enterobacteriaceae* and coagulase-negative staphylococci (CONS) (Bradley 2002; Barkema *et al.*, 2009). Other organisms such as *Pseudomonas* spp., *Pasteurellaceae*, some pyogenic and anaerobic bacteria, yeasts and algae are also implicated in some cases of mastitis

Economic losses involving mastitis are mainly decrease in milk production, increased culling rate, cost involved with veterinary care and to a larger extent poor milk quality (Seegers *et al.*, 2003; Rahman *et al.*, 2009; Hashemi *et al.*, 2011). Despite the economic importance associated with mastitis, it is not thoroughly investigated in Nigeria (Ameh *et al.*, 1999; Junaidu *et al.*, 2011).

Staphylococcus aureus is a major bacterium implicated with contagious bovine mastitis (Suleiman *et al.*, 2013), that is difficult to prevent and control in the affected farms. Therefore, identification of *S. aureus* from mastitic milk is necessary for the control and prevention of this disease. This study is aimed at evaluating the prevalence of mastitis in cows by California Mastitis Test (CMT) and also isolating *Staphylococci* as the causative agent in Kaduna State, Nigeria.

MATERIALS AND METHODS

Sample collection

The study was conducted in Kaduna State, Nigeria. Cohort study was formed by identifying and selecting five (5) herds from each of the six (6) selected Local Government Areas namely: Kaduna South, Igabi, Lere, Sabon-Gari, Giwa and Zaria. Twelve (12) milk samples were collected from each of five (5) cohort herds making a total of sixty (60) from each Local Government Area. Thus a total of 400 milk samples were collected during the study period. All the samples collected were placed on ice and transported to the Bacterial Zoonoses laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for standard laboratory procedures.

Clinical and subclinical mastitis test by California Mastitis Test (CMT)

The age of the cows were determined by dentition and confirmed by owners as Young adults (3-6 yrs), adults (7-10 yrs) and old (> 10 yrs) (Mekibib *et al.*, 2010). Clinical mastitis was assessed by observing the udder visually and by palpation for visible trauma, tick infestation, pain, warmth and swelling of the super mammary gland (Mekibib *et al.*, 2010). Abnormality in milk characteristics like blood tinged milk, watery secretions, clots and pus were checked (Dingwell *et al.* (2003).

Cows that did not have clinical mastitis were subjected to further examination for subclinical mastitis by using California Mastitis Test (CMT) as described by Dingwell *et al.* (2003).

Foremilk from each quarter was milked into cups of four-cup plastic paddle. The paddle was tilted to equalize milk quantities in the cups at 2.5 ml each. Equal volume of the CMT reagent (Kruuse, Denmark) was added to each cup. The paddle was rotated to mix thoroughly. Changes in colour and gel formation were observed within 10-15 seconds after mixing and

then scored depending upon the amount of gel formation as follows:

No reaction= Negative

Appearance of streaks visible during rotation of the plate= Trace

Distinct thickening during rotation, but no gel= 1+

Slight formation of gel which follows the rotating plate very slowly= 2+

Solid formation of gel that adheres to the base of the plate= 3+

Isolation of *Staphylococcus aureus*

One (1) ml of milk was enriched in 10 ml Tryptone Soya Broth (TSB, Oxoid) containing 6.5% sodium chloride. Enrichment cultures and milk samples was then inoculated on to the surface of the Baird Parker medium supplemented with egg yolk and potassium tellurite, and incubated aerobically for 24 hours at 37° C (Tamagnini *et al.*, 2006; O' Brien *et al.*, 2009). The *S. aureus* isolates were identified using colony morphology, Gram's staining, catalase, coagulase, motility, DNase and haemolysis tests (Normano, 2005; Tagmanini *et al.*, 2006) and further confirmed using Microbact™ *Staphylococcal* identification system 12S (Oxoid, Basingstoke, UK).

Statistical analysis

The prevalence of subclinical and clinical mastitis was calculated using the following formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of animals positive}}{\text{Number of animals tested}} \times 100\%$$

The data was statistically analyzed using SPSS package (Version 16.0). Chi-square and Fisher's Exact Tests at 5% level of confidence were used to determine the level of significant. A P value of $P < 0.05$ was considered as significant.

RESULTS

The results obtained from the study are presented in Tables 1-4. The prevalence of mastitis at cow level was 26.9% (97/360) and out of which 3.1% (11/360) and 23.9% (86/360) were clinical and subclinical mastitis (Table 1). The prevalence of mastitis at quarter level was 21.7% (312/1440), and out of which 6.5% (28/1440) was clinical mastitis and 19.7% (284/1440) was subclinical mastitis respectively (Table 4). Statistical analysis showed that there was significant difference in the occurrence of mastitis among the age group ($P < 0.05$). The prevalence at quarter level was 25.2% (157/624) in adult cows, 20.2% (97/480) in young adults and 17.3% (58/360) in old cows (Table 4). Similar pattern of occurrence was recorded for both clinical and subclinical mastitis as the adults had rates of 21.8% and 3.4 %, young adults had 19.8% and 0.4% and old cows had 15.8% and 1.5% respectively.

Samples from Igabi, Lere and Kaduna South had the highest rates of mastitis (35.0%, 31.7% and 30.0%) than those from Zaria (26.7%), Giwa (25.0%) and Sabon-Gari (13.3%), but all occurred at a significant rate ($P < 0.05$). Similar pattern of occurrence was observed with both clinical and subclinical mastitis as samples from Igabi had the highest prevalence of 30.0%,

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followed by Kaduna South and Zaria with 26.7% each, while the least was Sabon-Gari with 13.3%. In contrast, clinical mastitis was highest in Lere (6.7%) and lowest in Giwa (3.3%) and Kaduna South (3.3) respectively. No case of clinical mastitis was recorded in Sabon-Gari and Zaria.

The CMT results showed that 55 (15.3%) of the milk samples had a score of 1+, 26 (7.2%) had a score of 2+, 3 (0.8) had a score of 3+ while only 2 (0.6%) had a score of "Trace" (Table 2).

Sixty seven samples (69.1%) of the mastitis positive samples were found to be culture positive and out of which 44.2% (43/97) were *S. aureus* and 24.7% (24/97) were coagulase negative staphylococci (Table 3). The prevalence of both *S. aureus* and coagulase negative staphylococci (CONS) was significantly higher ($P < 0.05$) in subclinical mastitis (35.1% and 21.6%) than in clinical mastitis (9.3% and 3.1%) respectively (Table 3).

Table 1: PREVALENCE OF CLINICAL AND SUBCLINICAL MASTITIS IN KADUNA STATE, NIGERIA

Location	No. of samples examined	No. (%) of Clinical mastitis	No. (%) of Subclinical mastitis	Total mastitis
Giwa	60	2 (3.3)	13 (21.7)	15 (25.0)
Igabi	60	3 (5.0)	18 (30.0)	21 (35.0)
Kaduna South	60	2 (3.3)	16 (26.7.0)	18 (30.0)
Lere	60	4 (6.7)	15 (25.0)	19 (31.7)
Sabon-Gari	60	0 (0.0)	8 (13.3)	8 (13.3)
Zaria	60	0 (0.0)	16 (26.7)	16 (26.7)
Total	360	11 (3.1)	86 (23.9)	97 (26.9)

$\chi^2 = 8.864$ $df = 5$ $P = 0.100$

Table 2: CALIFORNIA MASTITIS TEST (CMT) SCORES OF BOVINE MILK ACCORDING TO THE GEL FORMATION IN KADUNA STATE, NIGERIA (N=86)

Location	No. of samples examined	No. (%) CMT score				
		Negative	Trace	1+	2+	3+
Giwa	60	47	-	4	8	1
Igabi	60	42	-	10	7	1
Kaduna South	60	44	2	12	2	-
Lere	60	45	-	12	3	-
Sabon-Gari	60	52	-	6	2	-
Zaria	60	44	-	11	4	1
Total	360	274	2 (0.6)	55 (15.3)	26 (7.2)	3 (0.8)

Table 3: PREVALENCE OF STAPHYLOCOCCI IN THE MASTITIC MILK IN KADUNA STATE, NIGERIA

Mastitis	No. (%) positive	No. (%) <i>S. aureus</i>	No. (%) CONS	Total
Clinical	11 (2.8)	9 (20.0)	3 (3.1)	12 (17.9)
Subclinical	86 (21.5)	34 (58.2)	21 (21.6)	55 (82.1)
Total	97 (24.3)	43 (44.4)	24 (24.7)	67 (69.1)

Key: CONS= Coagulase negative staphylococci

$\chi^2 = 12.211$

$df = 5$

$P = 0.01$

Table 4: OCCURRENCE OF MASTITIS ACCORDING TO THE QUARTERS AND AGE OF THE COWS

Age	No. of cows/ quarters	No. (%) clinical mastitis	No. (%) subclinical mastitis	Total
Young adults	120/480	2 (0.4) ^a	95 (19.8) ^a	97 (20.2)
Adults	156/624	21 (3.4) ^b	136 (21.8) ^b	157 (25.2)
Old	84/336	5 (1.5) ^c	53 (15.8) ^c	58 (17.3)
Total	360/1440	28 (1.9)^d	284 (19.7)^d	312 (21.7)

Values in the same row with different superscript differ significantly ($P < 0.05$)

DISCUSSION

The present study was carried out on settled Fulani herds in Kaduna State to determine status of mastitis in cow/quarters by California Mastitis test (CMT) and microbiological analysis of both suspected and apparently healthy milk samples for *Staphylococcus* spp. The prevalence of clinical mastitis at cow level was 26.9% (n=97) (Table 1), the prevalence of

subclinical mastitis as detected by CMT was 23.9% while that of the clinical mastitis is 3.1% (Table 1). The prevalence in the present study is lower than the 52% reported by Junaidu *et al.* (2011) in Sokoto, Nigeria, 85.33% by Shittu *et al.* (2012) in the Savannah region of Nigeria, 30.9% by Suleiman *et al.* (2013) in Plateau State, Nigeria and 71.0% by Mekibib *et al.* (2010) in Ethiopia.

However, the result is higher than 2.1% reported by Ameh *et al.* (1999) in Maiduguri, Borno State, Nigeria and those reported elsewhere (19.9%, 2.12% and 21.6%) respectively in Bangladesh, Sirajganj and Iran) (Rahman *et al.*, 2009; Islam *et al.*, 2010; Hashemi *et al.*, 2011) The discrepancies in the figures recorded above could be due to differences in the management systems, stage of lactation, parity and breed of the dairy animals (Hashemi *et al.*, 2011).

The overall quarter prevalence of mastitis in the present study was 21.7% (312/1440) with 6.5% (28/1440) as clinical mastitis and 19.7% (284/1440) as subclinical mastitis respectively. This is comparable with the reports of Ameh *et al.* (1999), but differ with the 44.9% and 43.25% overall quarter prevalence reported by Mekibib *et al.* (2010) and Shittu *et al.* (2012) in HOLETA town, Central Ethiopia and Savannah region of Nigeria respectively.

The traditional method and protocol of milking cows was the method practiced in all the herds sampled, thus milking hygiene was generally poor in the majority of the farms sampled. Mastitis and infection preventive measures like the use of udder antiseptics, hand disinfectants, post-milking teat dipping, dry cow therapy and intensive surveillance were also not practiced in these herds. This may be the possible reasons for the high prevalence of mastitis in these areas. Another reason could be the traditional practice of keeping calves away from the dam for long periods and only allowed to suckle for a short period coupled with low milk supply that resulted in the calves suckling vigorously leading to teat injuries, which subsequently predisposed to infection (Junaidu *et al.*, 2011).

In this study, the prevalence of subclinical mastitis (21.5%) was higher than clinical mastitis (2.8%) as observed by many authors (Ameh *et al.*, 1999; Rahman *et al.*, 2009; Mekibib *et al.*, 2010; Islam *et al.*, 2010; Hashemi *et al.*, 2011; Suleiman *et al.*, 2013). The possible explanation is that subclinical mastitis is characterized by gross visible pathologies which are familiar to the farmers and so are treated as soon as noticed. It was also shown that subclinical mastitis is normally found to be higher than clinical mastitis due to host's defense mechanism that reduces the severity of the disease (Mekibib *et al.*, 2010). The CMT results showed that 55 (64.0%) of the milk samples had a score of 1+, 26 (30.2%) had a score of 2+, 3 (3.5) had a score of 3+, while only 2 (2.3%) had a score of "Trace" (Table 2). This agrees with the acceptable fact that the CMT score of 1+ which corresponds to somatic cell count SCC of 500,000 is the threshold index of intra mammary infection (IMI).

Statistical analysis showed that there is significant difference in the occurrence of mastitis among the age groups ($P < 0.05$). The prevalence at quarter level was 25.2% (157/624) in adult cows, 20.2% (97/480) in young adults and 17.3% (58/3360) in old cows (Table 4). Similar pattern of occurrence was recorded for both clinical and subclinical mastitis as the adults had 21.8% and 3.4%, young adults had 19.8% and 0.4% and old cows had 15.8% and 1.5% respectively. This trend is in line with the reports by Ameh *et al.* (1999) who recorded high occurrence of mastitis in

adults cows ($n=50$) than in young cows ($n=25$) and old ($n=8$). Mekibib *et al.* (2010) also showed that the likelihood of mastitis in adult cows was more than in young adults. They reported the prevalence to be 56.0% in adults, 33.5% in young adults and 7.3% in old cows. It was observed that there was increase in cases of mastitis (clinical and subclinical) with advancing age. This is evident in the present study as the prevalence of mastitis increased from young adults to adult cows and decreased when the cows were old.

Even though, other pathogens were not examined, laboratory results showed that *Staphylococcus aureus* and coagulase negative staphylococci (CONS) were implicated as causes of mastitis in this study. Sixty seven (69.1%) of the CMT positive samples were found to be culture positive and out of which 56.7% (55/97) were *S. aureus* and 12.3% (12/97) were coagulase negative staphylococci (Table 3). Ameh *et al.* (1999) showed that coagulase negative staphylococci and *S. aureus* occurred predominantly in both clinical and sub-clinical bovine mastitis. In this study, 56.7% and 12.3% of *S. aureus* and CONS were respectively isolated from the 97 mastitic milk. This indicates that 69.0% of the mastitis was caused by staphylococci. This may also mean that staphylococci are not the only bacterial agents responsible for both clinical and subclinical mastitis in this study, hence other pathogens and agents were likely responsible for the remaining 31.0%. This result is of public health concern due to the involvement of *S. aureus* in food poisoning outbreaks. The study is also in agreement with Mekibib *et al.* (2010) who revealed that *S. aureus* and CONS were the predominant bacteria isolated in mastitic milk in Ethiopia with a 47.1% and 30.1% prevalence rates respectively. A report by Suleiman *et al.* (2013) showed the prevalence of *S. aureus* in bovine mastitic milk to be 98.1% in Plateau State, Nigeria and suggested that most of the *S. aureus* were of human origin since *S. aureus* of animal origin coagulate bovine plasma, produce beta-hemolysis and are less often enterotoxigenic than the human isolates.

Conclusion and recommendations

The high prevalence of both clinical and subclinical mastitis coupled with the isolation of *S. aureus* and CONS are of great public health concern and requires strict control and preventive measures. Also, cows with mastitis as evident in this study would lead to decrease in the yield of high quality milk, thereby affecting nutritional demands of the populace. This has also an economic consequence for the farmer as the milk would not be readily marketable. Therefore, farmers should adhere to good hygienic procedures during milking and proper management practices in order to reduced or eliminate infection of dairy cows. Culling of chronically infected cows is also recommended to avoid spread of pathogens to uninfected ones. All milk should be pasteurized before consumption or processing into other milk products to avoid transmission of pathogenic bacteria to humans through milk or milk products.

Conflict of Interests

We declare that there are no conflicting interests as regard to this research work.

Authors' contributions

Umaru, G. A. designed and conducted the research work; Kwaga, J. K. P., Bello, M. and Raji, M. A. supervised the research work, participated in drafting the manuscript and final acceptance, while Maitala, Y. S. provided the technical assistance. The manuscript

was read and accepted by all authors prior to submission for publication.

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