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Enterotoxigenicity of staphylococci isolated from raw milk obtained from settled and nomadic herds around Zaria, Nigeria

UMOH (V.I.), ADESIYUN (A.A.), COMWALK (N.E.). Infection intestinale par staphylocoques isolés du lait cru de troupeaux sédentaires et nomades de la région de Zaria, Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (1) : 43-47.

Des isolats de staphylocoques provenant de 135 échantillons de lait cru de 42 troupeaux sédentaires et de 93 troupeaux nomades ont été caractérisés et testés pour leur production d'entérotoxine. Sur 42 prélèvements provenant des troupeaux sédentaires, 13 (31 p. 100) étaient positifs avec le CMT mais tous contenaient des staphylocoques. Seuls 3, soit 3,2 p. 100 des 93 échantillons de lait cru des troupeaux nomades, étaient positifs avec le CMT mais 58 (62,4 p. 100) contenaient des staphylocoques. Sur les 13 isolats du lait des troupeaux sédentaires, positifs avec le CMT, 1 a produit des entérotoxines A, tandis que parmi les 29 autres, 4 ont élaboré des entérotoxines A et 3 des entérotoxines D. Aucun des isolats obtenus à partir du lait des troupeaux nomades n'était entérotoxigénique. *Mots clés* : Vache - Staphylocoque - Entérotoxine - Prélèvement - Lait - Analyse microbiologique - Nigeria.

INTRODUCTION

The key factor in the manufacture of good quality dairy food is starting with top quality raw milk materials. There are many screening tests used to check the quality of raw milk for processing ; some of these tests include California Mastitis Test and Catalase test and when the results exceed recommended values, confirmation is obtained by direct microscopic somatic cell count (19). Generally, the quality of raw milk does not depend on the results of the above tests alone, but also on such factors as flavour, level of solids, freezing point, absence of antibiotics and other inhibitory residues, sediment content, microbial populations and types of organisms (21).

One of the pathogenic microorganisms frequently isolated from milk and always implicated as one of the current aetiologic agents of both clinical and subclinical mastitis is *Staphylococcus aureus* (4, 6, 7, 26). *S. aureus* causes inflammations of udder in cows (7) and mammary gland infections in humans (13).

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Reçu le 31.08.89, accepté le 12.09.89.

Dairy foods are frequently contamined with staphylococci and mastitic milk may be an important source of these strains, some of which have been shown to be enterotoxigenic (12, 14, 29). Staphylococci grow slowly in raw milk, due to competing floras, but when milk is heat-treated or in the presence of low concentrations of bacterial cells, they can multiply at suitable temperatures to a high level and produce detectable amounts of enterotoxins when staphylococcus count was 10^6 cells or more per ml of milk. In an experimentally induced mastitis, enterotoxin C was detected in the infected udder and in milk samples at very low *S. aureus* population levels (10^2 to 10^3 cells/ml (18).

Toxigenic strains of *S. aureus* isolated from milk samples often produce either enterotoxin C or D or both (17) whereas strains isolated in case of food poisoning have usually been producers of enterotoxin A (23).

This study was conducted to determine the occurrence of subclinical mastitis in cattle kept in settled governmental and Fulani nomadic herds. The enterotoxigenicity of the staphylococcal isolates from raw milk samples from the two management systems was also determined.

MATERIALS AND METHODS

Sample collection

All samples were collected early in the morning from individual cows to fill a 100 ml sterile medical bottle. The samples were carried in an ice-packed container to the laboratory for analysis. Milk samples were obtained from all the milking cows in two governmental farms and ten Fulani herds in Zaria area.

California Mastitis Test (CMT)

California Mastitis Test was carried out on 2 ml of each milk sample mixed with the rest of the reagent as described by ULLMANN *et al.* (28). All scores of 0, trace and 1 were regarded as negative CMT results and scores of 2 and 3 as positive.

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Isolation of staphylococci

One tenth of the raw milk and a 1:10 dilution in saline were surface-plated in duplicate on Baird-Parker agar (BPA), (Oxoid), incubated at 37 °C for 48 h and the colonies counted. Typical colonies of staphylococci were picked and tested for catalase production and Gram reaction. All catalase and Gram-positive organisms were cultured on heart infusion agar (DIFCO) slants and stored at 4 °C until needed.

Identification of staphylococci

Anaerobic fermentation of carbohydrate :

Anaerobic Fermentation of glucose and mannitol by isolates was determined as recommended by Subcommitee on Taxonomy of staphylococci and micrococci (25). Acid production for 5 days at 37 °C was considered as positive.

Coagulase detection

The tube coagulase test was performed as described by BAER *et al.* (2), using freshly prepared human, rabbit and bovine plasmas, on an overnight culture in brain heart infusion broth, and incubated at 37 °C. The test was read after 4, 6 and 24 h. The test interpretation of SPERBER and TATINI (24) was used. *Staphylococcus aureus* strain (F265) and *S. epidermidis* strain (ATCC 14990), kindly provided by Professor S. R. TATINI of the University of Minnesota, USA, were used as positive and negative controls, respectively for each test run.

Hemolysin production

The ability of the staphylococcal isolates to produce hemolysin was determined on 3 % of sheep blood agar plates. Interpretation of the hemolytic patterns was as described by ELEK and LEVY (9).

Thermonuclease test

Thermonuclease production was determined by the method of LACHICA *et al.* (15). *Staphylococcus aureus* (F265) and sterile brain heart infusion broth (DIFCO) were used as positive and negative control, respectively.

Enterotoxin production

To demonstrate enterotoxin production, isolates were grown using the cellophane-over-agar method of ROBBINS *et al.* (20), using brain heart infusion agar (DIFCO) plates. Staphylococcal Enterotoxin (SE) was detected by microslide gel double diffusion test of CASMAN and BENNET (5).

RESULTS

Of the 42 samples from settled herds 13 (31,0 %) were CMT-positive and all the samples were contaminated with staphylococci. Among the nomadic herds, only 3 (3.2 %) of the 93 samples, were CMT-positive and only 58 (62.4 %) contained staphylococci (Table I). There was a significant difference ($P < 0.01$) in the number of CMT-positive and negative samples from the two farm systems.

TABLE I California Mastitis Test (CMT) results on raw milk obtained from settled and nomadic herds.

CMT reaction	Source of samples		
	Settled herds	Nomadic herds	Total
Negative Positive	29 (69.0)* 13 (31.0)	90 (96.8)* 3 (3.2)	119 (88.1)* 16 (11.9)
Total	42 (100.0)	93 (100.0)	135 (100.0)

* *Number of isolates examined and percentage (percent of total number of samples collected from each management system).*

Statistical analysis using Student's t-test revealed no significant difference in staphylococcal counts for the two management systems and the CMT test either positive or negative (Table II).

TABLE II Staphylococcal count for raw milk samples obtained from cows in two different management systems.

CMT reaction	Source of milk			
	Settled herds		Nomadic herds	
	Number tested*	Mean staphylococcal count/ml	Number tested**	Mean staphylococcal count/ml
Negative Positive	29 13	$7.7 \times 10^3 \pm 5.2 \times 10^3$ $1.3 \times 10^4 \pm 1.8 \times 10^4$	55 3	$6.3 \times 10^3 \pm 7.1 \times 10^3$ $2.2 \times 10^3 \pm 2.7 \times 10^3$
Total	42	$1.0 \times 10^4 \pm 1.2 \times 10^4$	58	$4.2 \times 10^3 \pm 4.9 \times 10^3$

CMT : California Mastitis Test.
* *All samples were contaminated with staphylococci.*
** *Thirty-five (37.6 %) samples collected were negative for staphylococci.*

Statistical analysis revealed that a significant ($P < 0.01$) number of 41 isolates (97.6 %) from the settled herds, were coagulase-positive using bovine plasma, 28 (66.7 %), were thermonuclease-positive and 18 (42.9 %) produced beta hemolysin, whereas from nomadic herds 29 (69.0 %) were coagulase-positive using bovine plasma, 11 (26.2 %) thermonuclease-

positive and 9 (21.4 %) produced beta hemolysin (Table III). Compared to other plasmas, bovine plasma was superior in detecting coagulase production by the isolates from both sources. Milk from settled herds had more staphylococcal isolates producing beta-hemolysin 18 (42.9 %) than alpha and gamma hemolysins with 12 (28.6 %) isolates each, while more isolates from nomadic herds produced gamma-hemolysin 28 (66.7 %) than beta-hemolysin 9 (21.4 %) and alpha 5 (11.9 %) (Table III). The number of isolates from settled herds producing alpha and beta-hemolysins were significantly higher ($P < 0.05$) than those from nomadic herds.

TABLE III Characteristics of staphylococcal isolates from raw milk collected from cows in two management systems.

Biochemical test	Sources of staphylococcal isolates and number positive	
	Settled herds	Nomadic herds
	Number (percent.)	Number (percent.)
Mannitol	40 (95.3)	40 (95.2)
Glucose fermentation	42 (100.0)	42 (100.0)
Hemolysis alpha	12 (28.6)	5 (11.9)
Beta	18 (42.9)	9 (21.4)
Gamma	12 (28.6)	28 (66.7)
Coagulase detection in :		
rabbit plasma	8 (19.0)	16 (38.1)
human plasma	25 (59.5)	25 (59.5)
bovine plasma	41 (97.6)	29 (69.0)
Thermonuclease production	28 (66.7)	11 (26.2)
Enterotoxin production*	8 (19.0)	0 (0.0)

Numbers indicated as those that were positive for the biochemical tests. A total of 42 isolates were each tested from each of the management system.
** 5 produced enterotoxin A, 3 produced D, none produced B, C, E and multiple enterotoxin.*

Enterotoxin production was only detected among *S. aureus* strains isolated from settled governmental herds. Of the 13 isolates from CMT-positive milk samples 1 (2.4 %) elaborated staphylococcal enterotoxin A (SEA) with the remaining being non-enterotoxigenic strains. However, 4 (9.5 %) and 3 (7.1 %) of the 29 strains of *S. aureus* isolated from CMT-negative milk samples produced SEA and SED, respectively, but negative for SEB, SEC and SEE. No multiple enterotoxin production was observed.

DISCUSSION

A significant number ($P < 0.05$) of raw milk samples from the settled herds was CMT-positive and all the samples contained staphylococci. The difference between the two management systems could be attributed to the method of milking. In the settled herds the machine used for milking might exert direct trauma on the test udder tissue leaving it susceptible to bacterial invasion and also to clinical mastitis (16). The finding in milk samples from settled herds according to which 31 and 69 % were CMT-positive and CMT-negative, respectively, agrees with earlier reports by other authors like SANCHEZ *et al* (22), who studied the CMT reaction of 146 milk samples from settled herds and reported that 46.5 % were negative and 13.6 % slightly positive. The slight difference in CMT reaction between the two settled farms was probably due to the number of samples collected.

The staphylococcal count of CMT-positive milk was slightly higher than that from CMT-negative milk but the difference was not statistically significant. This suggests that the staphylococci isolated were probably not the main aetiologic agent of the subclinical mastitis detected by this screening test. It might occur as part of a mixed infection. Other authors noted that for the initial infection, higher percentage was due to streptococci, followed by staphylococci and other organisms (10, 11).

In this study it was observed that a highly significant number of isolates from the settled herds coagulated bovine plasma, produced thermonuclease and beta-hemolysin while a few were enterotoxigenic. These strains of *S. aureus* therefore, were probably of animal origin. In this environment, ADESIYUN and SHEBU (1) found that bovine plasma was superior to human and rabbit plasma in detecting coagulase production by *S. aureus* strains of animal origin while human and rabbit plasma were better for strains from foods. Also, DEVRIESE *et al.* (8) reported that bovine *S. aureus* strains coagulate bovine plasma, produce beta-hemolysin and are less often enterotoxigenic and do not produce staphylokinase.

The fact that some of the examined milk samples contained enterotoxigenic strains of staphylococci is of public health significance, because milk from these sources is normally heated slightly and taken as fresh milk or fermented as « nono ». NISKANEN *et al.* (18) experimentally inoculated enterotoxin C producing *S. aureus* strains into the udder of cows and detected enterotoxin in the infected udder and in the mastitic milk. Enterotoxin are also known to be stable at low pH (4.0 to 4.5) even when the staphylococcal count is low (27). VARADARAJ and NAMBUDROPAD (30) stated that when they used *S. aureus* contaminated milk to prepare khoa (a heat concentrated Indian milk product), it retained enterotoxins and thermostable DNAse. Also staphylococcal enterotoxins A and D added to infant diet have been found to remain active under some canning conditions (3).

CONCLUSION

This study demonstrates that most cows from the nomadic herds are free from subclinical mastitis using California mastitis test, while a small number of cows from settled herds have subclinical mastitis. The fact that some of the strains of isolated staphylococci are enterotoxigenic is significant, indicating the possibility of enterotoxin containing milk reaching the consumers. Therefore, improved sanitation and milking methods could result in better quality of raw milk from settled herds.

UMOH (V.J.), ADESIYUN (A.A.), COMWALK (N.E.). Enterotoxigenicity of staphylococci isolated from raw milk obtained from settled and nomadic herds around Zaria, Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (1) : 43-47.

Staphylococcal isolates from 135 raw milk samples obtained from settled herds (42) and nomadic herds (93), were characterized and assayed for enterotoxin production. Of the 42 samples from settled herds, 13 (31 %) were California Mastitis Test (CMT)-positive, but all the samples contained staphylococci. Only 3 (3.2 %) of the 93 raw milk samples from nomadic herds were CMT-positive but 58 (62.4 %) contained staphylococci. Of the 13 isolates from CMT-positive milk obtained from settled herds, one produced enterotoxin A, while amongst the 29 from CMT-positive milk, 4 elaborated enterotoxin A and 3 produced type D. None of the isolates from milk obtained from nomadic herds was enterotoxigenic. *Key words* : Cow - Staphylococcus - Raw milk sample - Enterotoxin - California Mastitis Test - Nigeria

UMOH (V.I.), ADESIYUN (A.A.), COMWALK (N.E.). Enterotoxigenicidad de estafilococos aislados de leche cruda, obtenida de hatos nómadas y sedentarios alrededor de Zaria, Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (1) : 43-47.

Aislamientos de estafilococos se caracterizaron y examinaron para la producción de enterotoxinas, fueron obtenidos a partir de 135 muestras de leche cruda, provenientes de hatos sedentarios (42) y nómadas (93). De las 42 muestras obtenidas de hatos sedentarios, 13 (31 p. 100) fueron California Mastitis Test (CMT) positivas, sin embargo, todas las muestras contenían estafilococos. Sólo 3 (3.2 p. 100) de las 93 muestras provenientes de hatos nómadas fueron CMT positivas, pero 58 (62.4 p. 100) contenían estafilococos. De los 13 aislamientos de leche CMT positiva, obtenida de los hatos sedentarios, una produjo enterotoxina A, mientras que de los 29 restantes 4 elaboraron enterotoxina A y 3 produjeron el typo D. Ninguno de los organismos presentes en los aislamientos provenientes de leche obtenida de hatos nómadas fue enterotoxigénico. *Palabras clave* : Estafilococos - Enterotoxina - Leche cruda - Test - Nigeria.

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