Original Research

Isolation and Phenotypic Characterization of *Staphylococcus aureus* from Mastitic Milk among Cattle Population in Sokoto, Nigeria

K. H. Ahmad[1*], J. S. Dalis[1], M. B. Abubakar[2] and B. R. Alkali[2]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, NIGERIA
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, NIGERIA

***Corresponding author:** kabirbka@gmail.com

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Abstract

The purpose of this study was to isolate and characterize Staphylococcus aureus from cattle milk in Sokoto metropolis. A total of three hundred mastitic milk samples were collected from dairy farms, abattoir and livestock market (Kara). Phenotypic identification of the S. aureus isolates by culture, microscopy and biochemical reactions were performed. Two hundred and nineteen (73%) of the samples were positive for species of mastitis causing bacteria. The isolates were S. aureus (47.9%), Streptococcus spp. (24.7%), Corynebacterium spp. (14.6%), Escherichia coli (5.9%), Bacillus spp (5.0%) and Klebsiella spp. (1.8%).The result obtained shows that S. aureus was the principal pathogen responsible for bovine mastitis in the study area with 35.0% prevalence. The need for strict hygienic and improved milking techniques as preventive measures against the disease was emphasized.

Key words: Cattle, Mastitis, Nigeria, *S. aureus*, Sokoto

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Introduction

Nigeria has abundant livestock resources, majority of which are concentrated in the northern parts of the country (Ajayi *et al.*, 1987; MOCIT, 2002; PACE, 2003). Sokoto state is the second largest producer of livestock in Nigeria and rearing of livestock and consumption of milk and milk products is habitual to the people in the area (Mamman, 2005; Junaidu and Garba, 2006). Mastitis is the inflammation of the parenchyma of the mammary gland. It is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissue (Amosun *et al.*, 2010; Junaidu *et al.*, 2011; Mohanty *et al.*, 2013). Udder characteristics, breeds, teat injuries, poor hygiene, poor management, milker's

hand, faulty milking machines, accumulation of milk and the presence of bacteria in or around the udder, are all factors which predisposed cows to mastitis (Amosun *et al.*, 2010; Junaidu *et al.*, 2011; Mohanty *et al.*, 2013; Anayo *et al.*, 2013). Mastitis is continuously the most frequent and most expensive disease of dairy cows (Bradley and Green, 2001; Momtaz *et al.*, 2010; Samah and Hanaa, 2011; Castro *et al.*, 2013). Mastitis leads to economic losses in terms of reduced milk yield or quality and early culling of severely infected animals. Also, mastitis results to expensive antibiotic treatment, veterinary services and losses of the young animals (Leitner *et al.*, 2001; Momtaz *et al.*, 2010; Anayo *et al.*, 2013). Mastitis has been a major problem in the area and in spite of its importance there is little study done on mastitis of cattle in Sokoto state (Junaidu *et al.*, 2011).

Materials and Methods

Study Area

Sokoto state is located in the north-western part of Nigeria, and is between the geographic coordinates of longitude 4° 8′E and 6° 54′E and between latitude 12°N and 13° 58′N. The state shares boundaries with the Republic of Niger to the north, Kebbi state to the west and Zamfara state to the east as reported by Junaidu *et al.* (2011).

Study Design

Commercial dairy farms, livestock market (Kara) and abattoir were used as the sources of the animals and purposive sampling method was carried out, where an animal typically showing the clinical sign of mastitis were sampled.

Sample Collection and Distribution

A total of three hundred (300) milk samples were collected from cattle typically showing the clinical signs of mastitis. From the total number of samples collected, 136 (45.3%) samples were from dairy farms, while 108 (36.0%) samples were from abattoir and the remaining 56 (18.7%) samples from livestock market (Kara). The distribution of sample was provided in (Table1).

Sampling Procedure

Milk samples were collected aseptically from individual quarters of the cows. Prior to milking, the teat and udder were washed with water, dried with individual disposable towels and disinfected with Savlon (antiseptics), the first few squirts of milk were discarded and 5mls of milk samples were taken into a sterile bijou bottle, properly labelled and transported in an ice-packed container to the Veterinary Microbiology Laboratory of Usmanu Danfodiyo University Sokoto, for bacteriological analysis.

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Bacterial Isolation and Identification

All samples were first inoculated onto freshly prepared nutrient agar and incubated at 37ºC for 24 hours. Bacterial colonies were identified based on colonial morphology, cultural characteristics, Gram's staining and biochemical reactions. *Staphylococcus aureus* produces golden colonies on nutrient agar, it is non-motile, coagulase positive, catalase positive, ferments mannitol and produces double pattern of haemolysis on sheep blood agar (Quinn *et al.*, 2004). The isolates that were suspected to be *Staphylococcus species* were again subcultured onto freshly prepared mannitol salt agar (MSA) and incubated at 37ºC for 24 hours. Golden yellow colonies were presumptively identified as *S. aureus.* Furthermore, a single golden yellow colony from the culture plate was subcultured on freshly prepared nutrient agar slants at 37ºC for 24 hours, after which the slants were stored in the refrigerator for further analysis. The haemolytic activities of the isolates were determined using the blood agar plate as described previously by Rebecca (2013).

Phenotypic Identification of *Staphylococcus aureus* Isolates from Mastitic Milk of Cattles in Sokoto Metropolis

Following culture, bacterial isolation, grams staining, and microscopy, all suspected staphylococcus isolates were further identified by standard biochemical test as described by Quinn *et al.* (2004). Isolate with the following features; gram positive (G +ve), cocci in appearance, catalase positive, coagulase positive and ferment mannitol sugar were identified as *Staphylococcus aureus*.

Results

Distribution and Prevalence of Different Bacterial Isolate

Out of the 300 samples collected, 136 (45.33%) were from dairy farms, 108 (36.0%) from abattoir and 56 (18.67%) from livestock market (Kara) Table1.

Table 1: Distribution of samples collected from bovine mastitic milk in Sokoto metropolis

S. No.	Sample	Number Sample	Percentage of Sample (%)
1	Farms	136	45.33
2	Abattoir	108	36
3	Livestock market	56	18.67
	Total	**300**	**100**

Overall isolation rate was 219 (73.0%) and *Staphylococcus aureus* was the most frequently isolated bacterial species with isolation rate of 105 (47.95%), other bacterial species were; *Streptococci spp.* 54 (24.66%), *Corynebacteria spp.* 32 (14.61%), *Bacillus spp.* 11 (5.02%), *E. coli* 13 (5.94%) *and Klebsiella spp.* with 4 (1.83%) isolation rate (Table 2).

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Table 2: Isolation rates of bacterial species from cattle mastitis milk in Sokoto metropolis

S. No.	Organism	No. of Samples	% of Isolate
1	*Staphylococcus aureus*	105	47.95
2	*Streptococcus* spp	54	24.66
3	*Corynebacterium* spp	32	14.61
4	*Bacillus* spp	11	5.02
5	*Escherichia coli*	13	5.94
6	*Klebsiella* spp	4	1.83
Total		219	100

n=300; n = Total number of samples collected

Discussion

Staphylococcus aureus was the most common prevalent bacteria (35.0%), this was followed by *Streptococcus spp* (18%), *Corynebacterium spp* (10.7), *Escherichia coli* (4.3%), *Baccilus spp* (3.7) and *Klebsiella spp* (1.3%) respectively. Prevalence of 35.0% *S. aureus* isolate was slightly higher to what was reported by (Junaidu *et al.,* 2011; Suleiman *et al.,* 2012) who observed a prevalence of 22.8% and 30.9% respectively, similar to Ameh *et al.* (1999) who reported a prevalence of 34.6% and significantly lower than what was reported by Sori *et al.* (2011) who observed a prevalence of 52.4%. The prevalence could be as a result of tick infestation as high tick infestation and vigorous suckling by calves are known to cause direct inflammatory reaction to the mammary gland, necrosis and abscess formation, which may lead to udder damage and or exposure to serious secondary infection as reported by FAO, (1990). It could also be due to traditional dairy husbandry practices whereby calves are kept away from their dam over a long period of time and are only allowed to suckled for a short period as well as inadequate milk supply which leads to calves suckling vigorously, inducing teat injuries and subsequent infection of the mammary gland as reported by Junaidu *et al.* (2011). The prevalence recorded could as well be attributed to the poor milk hygiene practices such as lack of usage of disinfectant on udder, use of hand glove and lack of instituting dry cow therapy. The lack of surveillance programme for mastitis could also be a contributory factor (Dufour *et al.,* 2012; Shittu *et al.,* 2012).

The laboratory findings indicated *Staphylococcus aureus* as the most common bacterial pathogen being implicated in mastitis. This is similar to the findings of (Pitkala *et al.,* 2007; Junaidu *et al.,* 2011; Suleiman *et al.,* 2012; Anayo *et al.,* 2013) who reported *Staphylococcus aureus* to be the most common cause of bovine mastitis. The isolation of streptococci (18.0%) in this work is in agreement with that of (Ankalo and Sterneojo, 2006; Junaidu *et al.,* 2011) who isolated 14.1% and 15.4% respectively from mastitis in lactating cows in some selected commercial dairy farms in Sokoto metropolis and mastitic cow in Kenya. *Escherichia coli* is an environmental pathogen, and *E coli* mastitis is a major disease in cows, previous studies in Nigeria and other part of the world have shown that *E. coli* is an important a etiological agent of clinical mastitis (Junaidu *et al.,* 2011; Mohanty *et al.,* 2013 and Anayo *et al.,* 2013). The percentage of

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occurrence of *E. coli* (4.3%) in this study was in line with what was reported by Ameh *et al.* (1999); Junaidu *et al.* (2011) and Anayo *et al.* (2013), who's reported an incidence of 6.7%, 9.8% and 11.6% respectively, but was slightly different to what was reported by Mohanty *et al.* (2013) which reported a higher prevalence of 21.0%, this could be due to differences in location of the study area and other risk factors.

Biochemically, Staphylococci were identified by conventional methods (Abd-el-Hamid and Mahmoud, 2013; Quinn *et al.*, 2004). All of the one hundred and five (105) strains of *S. aureus* grew on Mannitol Salt Agar (MSA), were gram positive cocci, non-motile, non-spore forming, arranged in grape-like clusters, fermentative and catalase positive. Among them, eighty nine (89) 84.8% were coagulase positive and sixteen (16) 15.2% were coagulase negative staphylococci (CNS) and they produced characteristics golden yellow pigments, so they were considered as *S. aureus.* This is in agreement to what was reported by (Abd-el-Hamid and Mahmoud, 2013; Kataria *et al.*, 2013; Quinn *et al.*, 2004).

Conclusion

This study used laboratory-based diagnostic techniques to isolate and phenotypically characterized *Staphylococcus aureus* from mastitic milk among cattle population in Sokoto metropolis. The study also indicated *S. aureus* to be the most frequently encountered pathogen among the pathogens causing mastitis in Sokoto.

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