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National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng Url:journals.napri.gov.ng

DETECTION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) IN MATURED RED SOKOTO GOATS AND CONTACT PERSONS IN SOKOTO METROPOLIS

[*1]Abdulazeez, N., [2]Abubakar, M. B., [3]Bello, T. K., [4]Oyelowo, B. B. and [5]Tukur, H.
[1]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria
[2]Department of Veterinary Microbiology Faculty of Veterinary Medicine, Usmanu Danfodiyo University of Sokoto, Nigeria
[3]National Animal Production Research Institute, Shika-Zaria, Nigeria
[4]Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University Zaria, Nigeria
[5]Ministry of Animal Health and Fisheries Development, Sokoto, Nigeria
Corresponding Author: taiwokamarbello@gmail.com

ABSTRACT

This study determined the colonization rate of Methicillin resistant staphylococcus aureus (MRSA) Red Sokoto goats and contact persons. Nasal swabs were collected from Sokoto metropolitan abattoir. A total of 100 samples were analyzed. Staphylococcus aureus isolates were properly identified using standard procedure and confirmed as methicillin resistant using oxacillin resistant screening agar base (ORSAB), supplemented with 2g/l of oxacillin. Out of 52 staphylococcus aureus isolates, 17 (32.6%) Methicillin resistant staphylococcus aureus (MRSA) and 35 (67.3%) Methicillin sensitive staphylococcus aureus (MSSA) isolates were detected. 7(26.9%) MRSA and 19 (73%) MSSA were recovered from Red Sokoto Goats, 10 (38.4%) MRSA and 16 (61.5%) MSSA from sahel goats, 12 (33.3%) MRSA and 24 (66.7%) MSSA from Bucks and 5 (31.2% MRSA) and 11 (68.7%) MSSA from Does. In overall antimicrobials susceptibility pattern, MRSA isolates exhibited multidrug resistance pattern and demonstrated high resistance to Ceftazidime (100%), cefuroxime (82.3%) and Ceftriaxone (47%). Moderate susceptibility to cloxicillin (76.5%), ofloxacin (76.5%), erythromycin (58.9%) and high susceptibility to augmentin (82.3%) and gentamycin (94.2%), while majority MSSA isolates demonstrated high susceptibility pattern (>70%). The high prevalence of MRSA is of public health importance as it may play a role in disseminating the organism between animals and human. Therefore, adequate hygienic practices, control of indiscriminate use of antibiotic and frequent screening of the risk population for MRSA are important for both prevention and control of MRSA infection. In conclusion, the study findings revealed a relatively high MRSA colonization rate and unique resistant pattern particularly ceftazidime that is not routinely used in veterinary practice in the study area. The study findings provide a baseline epidemiological information for better understanding of MRSA infection in human and veterinary medicine including food of animal origin in the study area. Further studies are needed to provide insight through molecular characterization of resistant genes.

Keywords: Red Sokoto Goats, *Staphylococcus aureus*, Resistant, Methicillin

INTRODUCTION

Staphylococcus aureus is a known pathogen and has been implicated in a wide range of diseases, ranging from minor skin infections, such as furunclosis and carbunclosis to severe and highly debilitating diseases such as pneumonia, endocarditis and bacteraemia (Zetola *et al*., 2005). *Staphylococcus aureus* causes severe animal diseases, such as suppurative disease, mastitis, arthritis, and urinary tract infection that are associated with numerous virulence factors, such as the production of extracellular toxins and enzymes (Foster *et al*., 1991). Infections associated with *Staphylococcus aureus* has also been reported in various parts of the Nigeria (Ghebremedhin *et al*., 2009; Shittu *et al*.,2011). Immediately after the introduction of methicillin in the 1960s, methicillin resistance emerged in some strains of *Staphylococcus aureus* (Grundmann *et al*., 2006; Sakoulas and Moellering, 2008) and thus the name methicillin–resistant *Staphylococcus aureus (*MRSA). Methicillin resistance is due to a modified penicillin-binding protein PBP2a, encoded by the mecA gene, located on one of six types of staphylococcal chromosomal cassettes (SCCs), usually contain additional genetic material, including genes encoding resistance to multiple classes of antimicrobials (Lambert, 2005). Most animals may be colonized with *Staphylococcus aureus*, but only recently MRSA strains were isolated from several food production animals, including pigs, cattle, chicken and other animals (De Neeling *et al*., 2007; Huijsdens *et al*., 2006; Lee, 2006).

To the best of our knowledge, no study has been carried out and documented to detect the presence of MRSA among goats and contact persons in the study area, hence this study.

MATERIALS AND METHODS

Study Area

The study area is Sokoto metropolis in Sokoto state, Nigeria. The state is located within the Sahel savannah belt and extreme North-western Nigeria between latitude 4^0 to 6^0 North and longitude 11^0 to 130^0 East. It shares boundaries with Zamfara State to East, Niger republic to the North and Kebbi state to the West and South west.

Sample Collection

The study samples were collected at the Sokoto Metropolitan Abattoir. Demographic information of the Animals was obtained from the animal owners. Contact Persons was defined as person with close contact with Goats (in this study, goat owners were classified as contact persons). A total of 100 samples were collected; 95 Goats and 5 contact persons. Sterile cotton-tipped swabs which were pre-moistened in sterile normal saline were inserted into the inner nasal septum of anterior nares of the goats and contact persons, rubbed severally, removed, capped and labeled appropriately. All the samples collected were placed on ice and transported to the laboratory for analysis.

Culture and Isolation of *Staphylococci* Species

All the samples were inoculated on Mannitol salt Agar (Titan Biotech, India) and incubated aerobically at 37^oC for 24 hours. Suspected *S. aureus* with haemolysis on Blood Agar and yellowish appearance on Mannitol-salt Agar were further analyzed using establishe microbiological methods which included colony morphology, gram staining characteristics and biochemical tests [catalase, coagulase and sugar fermentation (Mannitol)] (Bauer *et al*., 1996; Cheesbrough, 2003; Japoni *et al*., 2004).

Antibiotics susceptibility test

Antimicrobial resistance was performed by Kirby- Bauer disk diffusion method using Mueller-Hinton Agar plates according to Bauer *et al* 1996 as Recommended by Clinical Laboratory Standards Institute (CLSI) (CLSI, 2006). The isolates were swabbed onto MuellerHinton Agar (Amershan, England) before the application of the antimicrobial

discs. The isolates were tested against a panel of eight (8) antibiotics; Ceftazidime, Cefuroxime, Gentamicin, Ceftraxone, Erythromycin, Cloxicillin, Oflaxacin, Amoxycilln/Clavulinate. Zones (ZI) of inhibition were measured and recorded after 24 hours of incubation at 35^{o}C, and were interpreted according to the guidelines of the CLSI, 2006.

MRSA Detection using oxacillin resistant screening Agar base (ORSAB) Media

The medium uses aniline blue to demonstrate mannitol fermentation in S*taphylococci*. The dual antibiotic supplement (oxacillin, 2g/l; polymixin B, 50,000 IU/l) and the presence of 5.5%NaCl have the potential to reduce the growth of non staphylococcal organisms and to select for the growth of MRSA. Each specimen was cultured on ORSAB media, with incubation for 48 hours in ambient air at 35°C. The plates were examined at 24 and 48 hours for the presence of mannitol-fermenting colonies, which were blue on ORSAB.

Data Representation and Analysis

Data were analyzed using In vivo stat 3.0 statistical software. Frequencies were obtained and percentages for study variables were calculated. Chi-square at 5% level of confidence was used to performed categorical comparisons and determination of significance. A P value < 0.05 was considered significant for all comparisons.

RESULTS

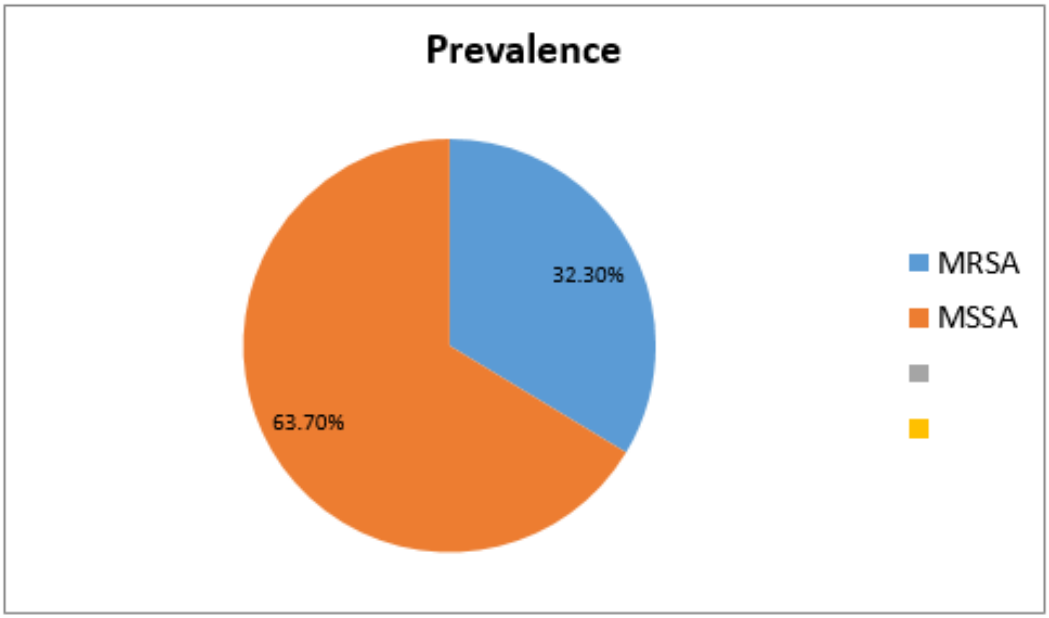


Figure1: *Prevalence of MRSA and MSSA among multiparous goats sampled in Sokoto metropolitan abattoir*

Table 1: Prevalence of *Staphylococci species* among the Goats and contact Persons in Sokoto Metropolitan Abattoir

Species	No. of samples	*S aureus*	*S epidermidis*

Contact persons	5	0	5
Goats	95	52	2
Total	100	52	7

Table 2: Breed Distribution of MRSA and MSSA in apparently healthy Goats sampled in Sokoto Metropolitan Abattoir

Breed	***S aureus***	**MRSA**	**MSSA**
Red Sokoto	26(50%)	7(26.9%)	19(73%)
Sahel	26(50%)	10(38.4%)	16(61.5%)
Total	52	17	35

P=0.24, P>0.05

TABLE 3: Sex distribution of MRSA and MSSA in apparently healthy Goats sampled in Sokoto Metropolitan Abattoir

SEX	***S aureus***	**MRSA**	**MSSA**
BUCK	36(69.2%)	12(33.3%)	24(66.7%)
DOE	16(30.7%)	5(31.2%)	11(68.7%)
TOTAL	52	17	35

Table 4: Antimicrobial resistance profile of bacteria isolated from nasal swab of Goats in Sokoto Metropolitan Abattoir

Genus/species	N	CAZ (30µg) (%)	CRX (30µg) (%)	GEN (10µg) (%)	CTR (15µg) (%)	ERY (30µg) (%)	CXC (5µg) (%)	OFL (5µg) (%)	AUG (30µg) (%)
MRSA	17	17(100)	14(82.3)	1(5.8)	8(47)	7(41.1)	4(23.5)	4(23.5)	3(17.6)
MSSA	35	16(45.7)	7(20)	1(2.8)	1(2.8)	9(25.7)	6(17.1)	5(14.2)	1(2.8)

KEY: MRSA: Methicillin resistant Staphylococcus aureus, MSSA: Methicillin sensitive, Staphylococcus aureus,CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin, CTR: Ceftraxone, ERY: Erythromycin, CXC: Cloxicillin OFL: Oflaxacin, AUG: Amoxycilln/Clavulinate. (Interpreted using Kirby-Bauer chart: Bauer *et al.*, 1996)

Table 5: Multiple drug resistance patterns of Methicillin resistant *Staphylococcus aureus* MRSA isolated from nasal swab of Goats sampled from Sokoto Metropolitan Abattoir.

RESISTANCE PATTERN	NUMBER OF ISOLATES
CAZ-CRX	2
CAZ-CRX-CTR	4
CAZ-CRX-ERY	2
CAZ-ERY-OFL	1
CAZ-CRX-CTR-GEN	1
CAZ-CRX-OFL-AUG	1
CAZ-CRX-CTR-ERY-CXC-OFL	1
CAZ-CRX-CTR-ERY-CXC-AUG	1
CAZ-CRX- CTR -ERY-CXC-OFL-AUG	1

KEY: MRSA: Methicillin resistant Staphylococcus aureus, MSSA: Methicillin sensitive, Staphylococcus aureus,CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin, CTR: Ceftraxone, ERY: Erythromycin, CXC: Cloxicillin OFL: Oflaxacin, AUG: Amoxycilln/Clavulinate. (Interpreted using Kirby-Bauer chart: Bauer *et al.*, 1996)

Table 6: Multiple drug resistance (MDR) indexes of Methicillin resistant *Staphylococcus aureus* MRSA isolated from nasal swab of Goats sampled from Sokoto metropolitan abattoir.

Multiple antibiotics resistance index	Prevalence (%)
0.1	12
0.2	0.0
0.3	18
0.4	29
0.5	18
0.6	0.0
0.7	0.0
0.8	12
0.9	6.0
1.0	6.0

DISCUSSION

The 32.6% prevalence of MRSA recorded in this study is a low MRSA colonization rates as compared to MSSA pattern reported in other studies (Alzhohairy, 2011; Gharsa *et al*., 2012). MRSA colonization rate differs with geographical location and animals sampled in a study in Maiduguri, Nigeria. It was noted that out of the 17 MRSA isolates, 10 were detected in Sahel goats and 7 in Red Sokoto goats, 12 in bucks and 5 in Does while none was detected in contact persons sampled. The level of MRSA colonization in contact person is not comparable to the level reported in other studies that assessed the level of contact persons as a predisposing factor to be 3. In these studies, the level of MRSA reported in contact persons varied with geographical location, type of animal and culture method employed in the study (Graveland *et al*., 2011). In this study, the *S aureus* isolates showed distinctive pattern; low, moderate and high resistance with MRSA exhibiting multiple drug resistant pattern. Both MRSA and MSSA isolate demonstrated high degree of sensitivity to Augmentin and Gentamycin. This implies that these agents are not only still efficacious for staphylococcal infection treatment and management, but also as alternative treatment option for livestock infections due to multidrug resistant *S aureus* in veterinary practice. As obtainable worldwide, indiscriminate use of antibiotics result in selection of resistant bacteria and genetic determinant of resistance can be transferred from animal to human through food stuff (Parreten *et al*., 1998, Schlegelova *et al*., 2004).

The possible explanation for the significant occurrence of MRSA in this study may be due to unrestricted and uncontrolled use of antibiotics in animals and farming, unsatisfactory health status of herds. Secondly, a greater percentage of herds are extensively managed, which exposed them to contaminated environment (Stastkova and Karpiskova 2004). Although surveillance studies are encouraged worldwide to provide epidemiological data on Livestock acquired MRSA, there are limitations, particularly in lack of standardization in the method employed. In addition staphylococcus species that produce positive tube agglutination may be detected as MRSA in low research laboratory in which analysis are based on phenotypic characterization (Morgan, 2008).

CONCLUSION

The study revealed a relatively high MRSA colonization rate and unique resistant pattern particularly ceftazidime that is not routinely used in veterinary practice in the study area. This study hence provides a baseline epidemiological information for better understanding of MRSA infection in human and veterinary medicine including food of animal origin in the study area.

RECOMMENDATIONS

Adequate hygienic practices, control of indiscriminate use of antibiotic and frequent screening of the risk population for MRSA are important for both prevention and control of MRSA infection. Further studies are needed to provide insight through molecular characterization of resistant genes.

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