



Occurrence and Risk Factors Associated with *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* in Sheep Samples from Maiduguri Central Abattoir, Borno State, Nigeria

Abdulrahman Mohammed^{1*}, Lukman Mustapha², Dawud Jidda¹, Adamu Saleh Saidu¹, Sani Mohammed¹, Muhammad Mamman Gashua¹ and Abdulrahman Idris Adamu¹

¹Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.

²Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta. Nigeria.

Corresponding Email: abdulrahmanm@unimaid.edu.ng; Phone: Tel: +2348032844440

Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is an important zoonotic pathogen that has been reported in several domestic animal and avian species, including dogs, cats, horses, sheep, pigs, and poultry. This study assessed the prevalence of Staphylococcus aureus and MRSA among sheep slaughtered at the Maiduguri abattoir, with emphasis on age, sex, and breed distribution. Nasal swabs (n = 200) were collected from Ouda, Yankasa, and Balami breeds. Isolates were identified based on colony morphology, Gram staining, haemolysis, catalase, coagulase, and DNase activities. MRSA was detected by antimicrobial susceptibility test using disc diffusion method. Overall, 87 (43.5%) samples were positive for S. aureus, with no statistically significant differences by age, sex, or breed (p > 0.05). Four isolates (2.0%) were identified as MRSA, all from ewes, suggesting increased occupational risk for farmers and abattoir workers. The findings underscore the need for awareness campaigns promoting the use of protective clothing and highlight the importance of further surveillance of MRSA in other food-producing and companion animal species.

Keywords: Antimicrobial susceptibility; Maiduguri Abattoir; Nasal Swabs; Prevalence; Sheep; *Staphylococcus aureus*

Introduction

Staphylococcus aureus is a common colonizer of the skin and mucous membranes of livestock and humans [1]. The organism is an opportunistic bacterium that has the potential to cause suppurative diseases, mastitis, arthritis, and urinary tract infection when it invades the host due to trauma or weakened immune responses [2]. Some of its strains that possess and express several virulence factors such as extracellular toxins and enzymes present serious threat compared to those lacking them [3, 4]. *S. aureus* is an important cause of food-borne intoxications in humans [5, 6]. Also, it is often one of the highly resistant bacteria associated with nosocomial infections in humans complicating pneumonia and post operative wound infection or septicaemia [7, 8]. Historically, *S. aureus* infections responded to β -lactams; however, the emergence of methicillin-resistant *S. aureus* (MRSA) has presented a critical therapeutic and public health concern [9, 10]. MRSA was first identified in the United Kingdom in 1961 [11] and is now globally distributed showing resistance to most β -lactams, including penicillins and cephalosporins [12]. It is also linked with elevated morbidity and mortality [13] and has been designated by the World Health Organization as a priority pathogen for surveillance [8]. Although MRSA was initially

regarded as a human pathogen, its detection in dairy cows with mastitis in 1972 [14] highlighted its zoonotic potential. In Nigeria, MRSA prevalence in humans has risen markedly, from 18.3% in 2009 to 42.3% in 2013 [15]. Reports have also documented its occurrence in poultry and farm personnel [16]. Identifying reservoirs and transmission pathways is therefore critical for control. This study investigated the occurrence of *S. aureus* and MRSA in sheep at Maiduguri abattoir, with analysis by age, sex, and breed.

Materials and Methods

Study Design, Sample Collection and Preservation

A cross-sectional abattoir-based study was carried out. Two hundred nasal swabs were collected randomly from sheep (Ouda, Yankasa, and Balami) prior to slaughter at Maiduguri Central Abattoir. Sterile swabs were inserted into the anterior nares, rotated against the nasal mucosa, and were aseptically transported to the laboratory at 4 °C for subsequent analysis.

Bacterial Isolation and Identification

Samples were inoculated onto Mannitol Salt Agar and incubated at 37 °C for 24 hours. Pre-

sumptive colonies were characterized by morphology, Gram staining, catalase, coagulase, haemolysis, mannitol fermentation, and DNase activity [17].

Antibiotic Sensitivity Test

All confirmed *S. aureus* isolates were tested for antimicrobial susceptibility to assess methicillin resistance using the disc diffusion technique on Mueller-Hinton agar, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI)[18]. The following antibiotic-impregnated discs were employed in the test: Rifampicin (5µg), Fusidic acid (10µg), Tetracycline (30µg), Erythromycin (15µg), Cefoxitin (30µg), Ciprofloxacin (15µg), Penicillin (2µg), Mupirocin (5µg), Akamycin (30µg), Specromycin (100µg), Kanamycin (30µg), Clindamycin (30µg) and Tobramycin (10µg). The zones of inhibition were measured in mm and compared; interpretation of inhibition diameter for sensitivity or resistance was based on guidelines in CLSI [18].

Data Analysis

Data analysis was performed using SPSS Statistics version 20 (IBM Corp.). Associations between prevalence and risk factors were assessed using chi-square tests and odds ratios. A probability, *p* value < 0.05. was considered statistically significant.

Results

Prevalence of *Staphylococcus aureus*

Of the 200 samples collected, presumptive *Staphylococci* were isolated from 145 samples out of which 87 were coagulase positive given an overall prevalence of 43.5% for *S. aureus* among the sheep samples (Table 1).

Table 1: Prevalence of *Staphylococcus aureus* among sheep (n=200).

Specimen	No. of samples	Presumptive <i>Staphylococci</i>	<i>Staphylococcus aureus</i> (coagulase positive) (%)
Nasal Swabs	200	145	87 (43.5)

Univariate analysis for *Staphylococcus aureus* infection among sheep samples

Of the 200 nasal swabs, 145 yielded presumptive staphylococci, and 87 were confirmed coagulase-positive *S. aureus*, giving an overall prevalence of 43.5%. Prevalence did not differ significantly ($p > 0.05$). by sex (rams 42.9% vs. ewes 43.9%), age group (<2 years: 43.3%; 2–4 years: 44.0%), or breed (Ouda 47.5%, Yankasa 40.7%, Balami 25.0%) (Table 2).

Table 2: Prevalence and Univariate analysis for *Staphylococcus aureus* infection among sheep samples from Maiduguri Central Abattoir

Variables	No. of samples	Positive samples (%)	OR (95% CI)	Chi-square test	P – value
Sex					
Ram	77	33 (42.9)	0.96 (0.54 1.70)	0.021	0.885
Ewe	123	54 (43.9)	Ref.		
Age (years)					
<1.5-2	157	68 (43.3)	0.97 (0.49 1.90)	0.040	0.918
2.5-4	43	19 (44.2)	Ref.		
Breed					
Ouda	101	48 (47.5)	2.72 (0.52 14.11)	1.225*	0.285
Yankasa	91	37 (40.7)	2.06 (0.39 10.75)	0.865*	0.474
Balami	8	2 (25.0)	Ref.		

No. = Number; OR = odds ratio; CI: Confidence Interval; *Fisher's Exact test statistics; n=200; Ref. = reference

Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA)

Out of the 87 coagulase positive *S. aureus*, 4 (2.0%) isolates were determined to be methicillin-resistant consisting of 2 (2.0%) isolates from Ouda and 2 (2.2%) from Yankasa (Table 3). All the 4 (3.3%) isolates were from ewes and of which 2 (1.3%) of the isolates were from the first age group (< 1.5 - 2 years), whereas the remaining 2 animals (4.7%) belonged to the oldest age group (2–4 years) (Table 3).

Table 3:

Percentage distribution of MRSA by Sex, breed and age in Sheep samples from Maiduguri Central Abattoir

Variables	No. of samples tested	Positive MRSA (%)
Sex		
Ram	77	-
Ewe	123	4 (3.3)
Breed		
Ouda	101	2 (2.0)
Yankasa	91	2 (2.2)
Balami	8	-
Age		
<1.5-2	157	2 (1.3)
2.5-4	43	2 (4.7)
Overall Prevalence	200	4 (2.0)

MRSA: Methicillin-resistant *S. aureus*; n=200

DISCUSSION

This study presents the first documented evidence of the occurrence of methicillin-resistant *S. aureus* (MRSA) in sheep from Maiduguri. Out of 200 sheep samples collected, *S. aureus* was detected in 87 (43.5%) and out of this, 4 samples (2.0%) were found to be MRSA. The presence of MRSA in food-producing animals poses a public health risk because of its potential for zoonotic transmission [19]. Transmission may be acquired either through ingestion, direct contact with infected animals [20] or inhalation of respiratory droplets [21]. Considering the fact that small ruminants are usually kept by smallholder farmers in backyards of their houses, this close association could also facilitate the ease in transmission of this organism from these infected animals to humans. People at risk here include the farmers, family members and the community at large; infection may be through person-to-person transmissions. In humans, infections with *S. aureus*, and most especially by the antibiotic-resistant strains have reportedly reached epidemic proportion worldwide [22]. Therefore, in livestock industry today, the challenge posed by MRSA and vancomycin-resistant *S. aureus* (VRSA) strains has increasingly become a challenge in both public health and veterinary medicine [23]. Consistent with our findings, numerous studies have documented varying levels of prevalences of MRSA

in nasal cavities of sheep and other ruminants in the world including Nigeria [24, 25]. However, prevalence from this study was lower than that of Farag *et al.* [26] and van Duijkeren *et al.* [27] who reported MRSA prevalence of 4.5% and 18% in sheep, respectively. Several factors involving, most likely, study designs, sample size, methods of isolations and detection of the bacteria, hygiene status of the farm and antibiotic misuse may account for this disparity in the carriage rates.

In the current study, the occurrence of *S. aureus* and MRSA observed was tested for association with the sheep age group, sex and breed. The sheep were grouped into two age categories viz; < 1.5 - 2 years and 2 - 4 years. The prevalence in the first age group was found to be lower with less likelihood of *S. aureus* occurring compared to that of the second age group, second age group, possibly due to maternal immunity that might not have waned; however, the difference was not statistically significant. Likewise, MRSA prevalence was greater in the second age group than in the first. As far as sex is concerned, there was no significant difference in the prevalences observed, with less likelihood of the disease occurring in ram compared to ewe. Likewise, MRSA was detected only in ewe in this study. Ouda breed recorded the highest prevalence followed by Yankasa and Balami breeds (Ouda>Yankasa>Balami), with more likelihood of *S. aureus* occurring in Ouda and Yankasa compared to Balami. Nevertheless, MRSA prevalence was found to be higher in the Yankasa breed followed by Ouda and undetected in Balami (Yankasa>Ouda>Balami). Generally, the differences among the age groups, sexes and breeds were very small and negligible; however, the level of carriage could be attributed to poor hygienic measures at the animal sources and antibiotics misuse.

In conclusion, background epidemiological information on MRSA among sheep was established in Maiduguri abattoir. *S. aureus* level of carriage was high, however, MRSA was low in this study. Age, sex and breed had no significant association with percentage distribution of *S. aureus* and MRSA. Detection of MRSA in this study suggests ewe breeders are at greater risk of infection. . It is recommended that further studies be conducted to determine the prevalence of MRSA in other food-producing animals, including fish, and companion animals, while farmers and abattoir workers should be sensitized on the need to wear protective clothings to prevent cross transmission of MRSA..

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