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Original Research Article

Haemolytic and antibiogram activities of *Staphylococcus aureus* isolated from cattle with mastitis in Sokoto, Northern Nigeria

[a*]Ahmad, K.H., [b]Abubakar, M.B., [a]Umar, B.N., [a]Salawudeen, M.T., [c]Olorunshola, I.D. & [d]Usman, M.D.

[a]*Department of Veterinary Microbiology, Ahmadu Bello University Zaria,* [b]*Department of Veterinary Microbiology, Usmanu Danfodiyo University Sokoto,* [c]*Department of Veterinary Microbiology, University of Ilorin,* [d]*Department of Veterinary Medicine, Bayero University Kano, Nigeria*

*Corresponding author: khahmad@abu.edu.ng +2348036395435

ABSTRACT
Mastitis continues to be one of the most fatal disease in dairy cattle, and it causes enormous economic losses due to decreased milk production, high treatment costs, and eventual development of antimicrobial resistance. Therefore, this study assessed the *in vitro* haemolytic and antimicrobial activities of *Staphylococcus aureus* isolated from active cases of mastitis in cattle in Sokoto metropolis. Milk samples from various sources in the study area were collected aseptically, placed in an ice-packed container, and transported to the microbiology laboratory of the Department of Veterinary Microbiology, Usmanu Danfodiyo University, Sokoto. The samples were processed according to standard laboratory methods for *S. aureus* isolation and identification and tested for antimicrobial susceptibility using the Kirby-Bauer qualitative disk diffusion method. The diameter zone of inhibitions of each antibiotic used was recorded and interpreted using zone diameter interpretive standards of Clinical and Laboratory Standard Institute. A total of 105 (35 %) *Staphylococcus* species were identified, out of which 86 (81.9 %) were confirmed to be beta-haemolytic *S. aureus* and only 57 (66.3%) had their susceptibility to antimicrobials evaluated. Most of the antimicrobials used were found to be effective, with Gentamicin having the highest level of susceptibility (82.5%). However, reasonable resistance against *S. aureus* was observed in betalactam antimicrobials, revealing 56.1% and 50.9% for Ampicillin and Amoxycillin clavulanic acid, respectively. We recommend the use of antimicrobials only where absolutely necessary and management practices that will promote wholesomeness and control antimicrobial resistance in food animals.

Keywords: antibiogram, cattle, haemolysis, *Staphylococcus aureus*, Sokoto-Nigeria

INTRODUCTION
Mastitis is an inflammatory reaction of mammary glands that results in obvious changes in milk colour and quality. The condition is commonly identified by reddened and swollen udder which can progress to gangrene (Shittu *et al.,* 2008; Garba *et al.,* 2019; Erskine, 2020). The aetiology of mastitis is multifactorial that involve both infectious (virus, bacteria, fungi and algae) and non-infectious causes such as chemical and mechanical injury (Mallikarjunaswamy & Krishnamurthy, 1997; Mishra *et al.,* 2018). However, the pathogenic and commensal bacteria found in animals that are subjected to antimicrobial stress develop survival strategies through different mechanisms (Allen & Stanton, 2014; Grace, 2015; Guetiya *et al.,* 2016).

Staphylococcus aureus is one of the most economically infectious zoonotic agent that affects cattle worldwide (Bradley & Green, 2001; Ismail, 2017). It is important not only because of its widespread dissemination and pathogenicity, but equally due to its capacity to resist antimicrobial effects. Methicillin-resistant *S. aureus* (MRSA) for instance poses a substantial and long-term challenge in the treatment of infections caused by such strains. Resistance is typically conferred by the acquisition of a gene encoding a penicillin-binding protein (PBP2a) with a lower affinity for betalactams (Peacock & Paterson, 2015).

Virulence of *S. aureus* is linked to a number of secreted enzymes, including haemolysin, which is one of the most important virulence factors produced by this microorganism. Haemolysin causes the typical beta-haemolysis, also known as complete haemolysis, though research has revealed that an incomplete haemolytic phenotype has been isolated from clinical cases (Otto, 2014).

The excessive abuse of antimicrobial agents in veterinary and human medicine has led to the emergence of resistance

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microorganisms like *S. aureus* (Thornton, 2010; Paulson & Zaoutis, 2015; Caudell *et al.,* 2017). In most countries, beta lactam antibiotics are the safest therapeutic choice for *S. aureus* infection as resistance to this antimicrobial was rare when penicillin was first used in 1943 (Fleming, 1980). On the contrary, development of multidrug resistance by *S. aureus* is alarmingly increasing (Lowy, 2003; Chamber, 2005) and the effect of such has been documented in some cities in Nigeria, such as Abuja, Ilorin, Jos and Zaria (Ehinmidi, 2003). Therefore, the present study aimed to isolate and characterize *Staphylococcus aureus* from cattle with mastitis in the studied area.

MATERIALS AND METHODS

STUDY AREA

Sokoto lies between longitude 4^{o} 8′E and 6^{o} 54′E and latitude 12^{o}N and 13^{o} 58′N in north-west part of Nigeria. It shares borders with the Niger republic, Kebbi and Zamfara state to the north, west & southwest and east respectively (Junaidu *et al.,* 2011). The research was conducted in microbiology laboratory of the Department of veterinary microbiology, Usmanu Danfodiyo University, Sokoto.

STUDY DESIGN

A convenient sampling technique was used and a total of 300 samples were collected across the sokoto metropolis, with 136 (45.3%), 108 (36.0%) and 56 (18.7%) obtained from Commercial dairy farms, Sokoto central abattoir and Livestock market respectively.

GROWTH CONDITIONS AND IDENTIFICATION OF *S. AUREUS*

All the bacteria including *S. aureus* were isolated and identified using conventional microbiological methods. Briefly, *S. aureus* isolation was done on mannitol salt agar (MSA), most pathogenic bacteria other than the staphylococci are inhibited by the presence of 7.5% Nacl, and they usually ferment the mannitol sugar producing a pH change in the medium from pink to yellow (Markey *et al.,* 2013). Sheep blood agar was used to determine the haemolytic activities of each isolate before further identification tests were performed. Briefly, inoculated plates were incubated aerobically at 37°C for 18-24 hours. Criteria for the identification of *S. aureus* in the MSA were in accordance with the colonial morphology as follows: Golden to yellow colonies that changed the colour of indicator (phenol red) to yellow, indicating mannitol fermentation. Identification of *S. aureus* and other staphylococci was performed by using the following tests: Gram staining, catalase test, coagulase test, mannitol fermentation and ß-haemolysis as was described in earlier study (Songer & Post, 2005; Markey *et al.,* 2013).

ANTIBIOGRAM DETERMINATION USING DISK DIFFUSION TECHNIQUE

The phenotypically confirmed *S. aureus* were tested for their *in vitro* activities against the following oxoid's antimicrobial agents; Ofloxacin (OFX) 5µg, Gentamycin (CN) 10µg, Ampicillin (AMP) 25µg, Cefuroxime (CXM) 30µg, Amoxycillin clavulanic acid (AMC) 30µg, Ceftriaxone (CRO) 30µg, Ciprofloxacin (CIP) 10µg, Streptomycin (S) 25µg, Trimethoprim / Sulfamethoxazole (SXT) 25µg and Erythromycin (E) 10µg. Kirby-Bauer disk diffusion method was used on Mueller-Hinton agar, the zone of inhibitions of each antibiotic disk were recorded and interpreted after 24 hours of incubation at 37°C, using interpretive standards of Clinical and Laboratory Standard Institute (CLSI, 2017).

RESULTS

Higher number 86 representing 81.9% of biochemically defined isolates were confirmed to be beta haemolytic *S. aureus*, while the remaining haemolyses were observed with 13 and 6 isolates exhibited Alpha (α) and Gamma (γ) haemolyses respectively (Table I).

Only 57 (66.3%) identified *S. aureus* isolates were tested for antimicrobial activity due to hitches encountered with the storage. Among the antimicrobial cocktails used, gentamicin had the highest level of susceptibility with 82.46%, followed by ceftriaxone and streptomycin with 73.68% each respectively. However, a significant number of isolates, specifically 56.1% and 50.9%, were found to be resistant to ampicillin and amoxycillin clavulanic acid respectively (Table II).

Table 1: Haemolytic Patterns Observed in *Staphylococcus aureus* Isolated from Cattle with Mastitis in Sokoto Metropolis

S/N	Types of Haemolysis	Number	Percentage (%)
1.	**Alpha (α)**	13	12.38
2.	**Beta (β)**	86	81.90
3.	**Gamma (γ)**	6	5.71
Total		105	100

DISCUSSION

The diseases caused by staphylococci are the results of the synthesis of many virulence factors, including the various haemolysins, which are critical for the *S. aureus* and other virulence species (Ebrahimi *et al.*, 2009; Hongjun *et al.*, 2011). In this study, *S. aureus* isolated were observed to have 12.4%, 81.9% and 5.7% for alpha (α), beta (β) and gamma (γ) haemolytic types of activities respectively. This result is at par with the results of Unakal & Kaliwal, (2010) who reported 20.6%, 75% and 4.4% for alpha, beta, and gamma type of haemolysis respectively. On the other hands, these results differed considerably from the results for alpha, beta, and gamma haemolysis from Ali-Vehmas *et al.* (2001), Mahavir *et al*, (2013), and Parth *et al.* (2016) were 24%, 24%, and 52%, 0%, 100%, and 0%, and 33.96%, 49.06%, and 7.55% respectively. Differences in location, season, growth condition and expertise might all contribute to the disparity.

Table 2: Antibiogram Activity of *Staphylococcus aureus* Isolated from Cattle with Mastitis in Sokoto Metropolis (n = 57)

Anti-microbials	Conc. (µg)	Inhibition zone diameter in millimetres (mm)		
		Resistance (%)	Intermediate (%)	Susceptible (%)
CN	10	3 (5.26)	7 (12.28)	47 (82.46)
CXM	30	8 (14.04)	17 (29.82)	32 (56.14)
CRO	25	11 (19.30)	4 (7.02)	42 (73.68)
CIP	10	13 (22.81)	5 (8.77)	39 (68.42)
S	25	15 (26.32)	0 (0)	42 (73.68)
OFX	5	17 (29.82)	6 (10.53)	34 (59.65)
SXT	25	19 (33.33)	14 (24.56)	24 (42.11)
E	10	24 (42.11)	2 (3.51)	31 (54.39)
AMC	30	29 (50.88)	8 (14.04)	20 (35.09)
AMP	25	32 (56.14)	9 (15.79)	16 (28.07)

Key: Gentamycin (CN), Cefuroxime (CXM), Ceftriaxone CRO),Ciprofloxacin (CIP), Streptomycin (S), Ofloxacin (OFX), Trimethoprim / Sulfamethoxazole (SXT), Erythromycin (E), Amoxycillin clavulanic acid (AMC) and Ampicillin (AMP).

Antibacterial treatment is an essential aspect of dairy cattle mastitis control regimen (Erskine & Wagner, 2003). Bovine specialists also use *in vitro* antibiogram tests of clinical or subclinical mastitis pathogens to direct treatment decisions at the cow or herd stage. Previous research, nevertheless, have struggled to show statistically meaningful links between susceptibility testing outcomes and clinical and/or subclinical patient effects for some antimicrobial agents (Kasravi *et al.,* 2010). Several authors have documented increased resistance of staphylococcal isolates recovered from mastitic ruminants to various antimicrobial agents (Ghaleb, 2006; Nadeem *et al.,* 2013). Antibiotics are commonly given at drying-off to manage subclinical mastitis, and to avoid further infections (Twomey *et al.,* 2000). While effective, this activity has been linked to the growth of antibiotic-resistant strains, as seen in the case of *S. aureus (*Moroni *et al.,* 2004). Therefore, mastitis treatment necessitates bacterial identification and susceptibility testing in order to choose the most effective antimicrobial medication (Gentilini *et al.,* 2000).

Antibiogram test were used in this study to assess *in vitro* antibacterial efficacy against *S. aureus* from mastitic milk. Gentamycin had the highest degree of sensitivity, with 82.5%, led by Streptomycin and Ceftriaxone, both with 73.7%, and Ciprofloxacin, Cefuroxime, Erythromycin, Trimethoprim/Sulfamethoxazole, Amoxycillin, and Ampicillin with 68.4%, 56.1%, 54.4%, 42.1%, 35.1% and 28.1% respectively. The 50-57% resistance of ampicillin and amoxicillin recorded in the current study was lower than the alarming 83-88%, 89% and 100% reported by Singh *et al.,* 2016, Aliyu *et al.,* 2019 and Umaru *et al.,* 2020 respectively. This might be attributed to increased awareness on drug use, which is supported worldwide by multinational organizations like the Fleming Fund and Regional Disease Surveillance Systems Enhancement (REDISSE), among others. Likewise, the 82.5% and 54.4% susceptibility for gentamicin and erythromycin respectively is significantly higher than the 53% reported by Singh *et al.,* 2016 for gentamicin and the 71% resistance reported by Umaru *et al.* (2020) for erythromycin, this could be due to differences in antibiotic manufacturers, expertise, and other factors.

CONCLUSION AND RECOMMENDATION

This study established *S. aureus* as the most prevalent (81.9%) staphylococci causing mastitis in dairy cattle. Similarly, our findings have shown that these bacteria have developed resistance to amoxicillin-clavulanic acid (AMC) and ampicillin (AMP) betalactam antibiotics. We recommend the use of only prescribed medications and measures to improve hygienic practices on our dairy farms in order to reduce sources of contamination and potential development of antimicrobial resistance.

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CONFLICTS OF INTEREST

The authors declare no any conflict of interest

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