

Original Research Article

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## Coagulase - Positive *Staphylococci* Isolated from Cattle in Maiduguri, Nigeria

Martha Echioda-Ogbole<sup>1\*</sup>, James A. Yaza<sup>2</sup>, James A. Ameh<sup>1</sup>, Samuel Mailafia<sup>1</sup>,  
Olatunde H. Olabode<sup>1</sup>, Bridget J. Adah<sup>1</sup>, Godspower R. Okoh<sup>1</sup> and Stella Maduike<sup>1</sup>

<sup>1</sup>Department of Veterinary Microbiology, Faculty of Veterinary Medicine,  
University of Abuja, Nigeria

<sup>2</sup>Department of Veterinary Microbiology, Faculty of Veterinary Medicine,  
University of Maiduguri, Nigeria

\*Corresponding author

### ABSTRACT

*Staphylococci* are Gram positive bacteria capable of causing both localized and life-threatening systemic infections in animals and humans. The present study was aimed at the isolation and identification of various *Staphylococci* species from cattle in Maiduguri Borno State. One hundred (100) nasal swabs were randomly collected from cattle in January 2011, and then processed in accordance with standard methods for bacteria isolation. Colony morphology, assessment on manitol salt agar (MSA), coagulase, catalase and haemolytic tests were among the standard bacteriological methods used in the identification and biochemical characterization of *Staphylococci* species. Isolates were then subjected to an *in-vitro* antimicrobial susceptibility testing using the disc diffusion method. 92 bacterial isolates were obtained, 21 of the isolates were identified as coagulase-positive *Staphylococcus aureus*, 49 as coagulase - negative *Staphylococci*, while 22 isolates were identified as micrococci. All the *Staphylococcus aureus* isolates were positive for mannitol fermentation test and produced beta- haemolysis on blood agar containing 5% sheep erythrocytes. *Staphylococci* species were most susceptible to ciprofloxacin (100%) and ceftazidime (90.5%). In conclusion, this study indicates the occurrence of coagulase positive and negative *Staphylococci* in Bovine nasal mucosa in the study area thus suggest ciprofloxacin and ceftazidime as drug of choice in the treatment of infections and diseases caused by staphylococcus.

### Keywords

*Staphylococci* species, Coagulase, cattle, Antimicrobial testing, Maiduguri

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### Introduction

One of the most important phenotypical features in the classification of *Staphylococci* is their ability to produce coagulase, an enzyme-like protein that clots oxalated or citrated plasma (Ryan and Ray, 2004). Under the family Staphylococcaceae, Genus

*Staphylococcus* is mainly classified as coagulase - positive and coagulase - negative *Staphylococci*. Coagulase may deposit fibrin on the surface of *Staphylococci*, thereby preventing their digestion by phagocytic cells or their destruction within such cells. Its production has traditionally been used to differentiate *Staphylococcus aureus* from

coagulase – negative *Staphylococci* and is considered synonymous with invasive pathogenic potentials (Jin *et al.*, 2004).

Recent taxonomic studies have shown that the genus *Staphylococcus* can be divided into at least 40 species and 17 subspecies, seven species are currently recognised as being coagulase- positive (Jin *et al.*, 2004). *Staphylococci* grow easily on many types of laboratory media, fermenting carbohydrates and producing pigments that vary from white to golden yellow. Some are members of the normal microflora of the skin and mucous membranes of humans and animals, whereas others cause suppuration, abscess formation, pyogenic infections and even life threatening septicemia (Karen, 2013).

Pathogenic *Staphylococci* species often hemolyze blood, coagulate plasma and produce a variety of extracellular toxins and enzymes (Nwankwo and Nasiru 2011). In routine diagnostic microbiology, the major pathogenic *Staphylococcal* species *Staphylococcus aureus* (*S. aureus*) can be differentiated from other species by means of a single test, coagulase or clumping-factor production (Carter *et al.*, 2003). The production of coagulase as an enzyme, fermentation of mannitol and glucose and testing for the presence and types of hemolysis on blood agar plates distinguish *Staphylococcus aureus* primarily from other *Staphylococci* (Carter *et al.*, 2003). Additional diagnostic procedures require to confirm *S. aureus* colonies include microscopic examination following Gram staining and other biochemical tests such as catalase, anaerobic utilization of glucose, anaerobic utilization of mannitol, lysostaphin sensitivity and production of thermostable nuclease (TNase) as well as application of the ready to use identification kits, such as the APi- Staph system (bioMerieux, France) (Silva *et al.*, 2000). The aim of the present study was to

report the occurrence, biochemical characteristics and antimicrobial profile of *Staphylococci* isolates from Bovine nasal swab in Maiduguri.

## **Materials and Methods**

### **Study location**

Maiduguri is located in the arid zone of Borno State covering an area of about 69,436 km<sup>2</sup> and is situated within latitude 11° 50' 46" N and longitude 13° 08' 29" E. It is in the sub-Saharan region with low records of rainfall. The area is in the tropical continental north with dry period of 4-8 months usually October to May, and a short rainy season which starts from June to early October. The state is located in the North Eastern corner of Nigeria and shares common boundaries with Cameroon to the East, Chad to the North East and Adamawa State to the South West. According to the 2005 census, the population is estimated to be 4,558,668 and ranked 12th in the country. Agriculture and livestock husbandry forms the mainstay of the state economy (World Gazetteer, 2007).

### **Sample collection and bacterial isolation**

One hundred (100) nasal swabs were randomly collected from cattle at University of Maiduguri teaching and research farm and immediately transported in a cool thermos to the Veterinary Microbiology Laboratory, University of Maiduguri and processed within 24 hours.

*Staphylococci* were isolated in accordance with standard methods as described by Collee *et al.*, (1989). The swabs were directly inoculated onto prepared plates of mannitol salt agar (Oxiod), and then incubated at 37°C for 24 hours. Following incubation, all plates were examined for colonial morphology and pigmentation, and then recorded for each

different *Staphylococci* isolates. Representative colony of each presumptive *Staphylococci* isolate type per specimen was picked with a sterile wire loop and transferred onto slants of nutrient agar and incubated overnight at 37°C, stored at 4°C for further biochemical characterization and antimicrobial susceptibility testing.

### ***Staphylococci* identification**

Various *Staphylococci* species were confirmed phenotypically on the basis of colony morphology assessment, Gram staining and microscopic appearance, anaerobic acid production from glucose, clumping factor and were tested for oxidase, catalase and coagulase production according to Lancette and tatini (1992). In addition, haemolytic properties of Staphylococcal isolates were studied on blood agar containing 5% sheep blood as described by Cheesbroug (1989).

### **Antibiotic susceptibility testing**

The susceptibility testing of isolates to ten (10) antibiotics was carried out using the disk diffusion method according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2006). The antibiotic (Poly-Test Medical Laboratories) included ofloxacin (5µg), erythromycin (10µg), ciprofloxacin (5µg), clindamycin (10µg), gentamycin (10µg), cephalexin (30µg), cotrimoxazole (50µg), ampicillin (30µg), ceftriaxone (5µg) and augumentin (5µg). Isolates were inoculated unto prepared nutrient agar plates and allowed to dry for 2-5 minute after streaking; sterile forcep was used to place the antibiotic multi- discs on the inoculated plates and then incubated at 37°C for 24h. Zones of bacteria inhibition were measured to the nearest millimetre using a ruler and interpreted as susceptible, intermediate and resistant as indicated by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006).

### **Results and Discussion**

A total of 92 bacterial isolates of different species were isolated from one hundred (100) cattle in this study. Of these, 21 isolates were confirmed phenotypically as *Staphylococcus aureus*, all the *S. aureus* strains were positive for coagulase test, with vary degree of reactions ranging from 2+, 3+ and 4+. Forty nine (49) Staphylococcal isolate were found to be coagulase negative *Staphylococci* (CoNS), while 22 bacteria isolates were identified as Micrococci species.

The coagulase gene has been a target for PCR genotyping where size and DNA restriction endonuclease site polymorphism within the *coa* gene is been use for PCR-RELP analysis (Hookey *et al.*, 1998). Generally, molecular typing of *Staphylococcus aureus* has been reported as most preferred method for most clinical laboratories because of its ease, speed and a precise result (Montesinos *et al.*, 2000). However, this is not within the scope of our study. Hence, the limitation of this study was the inability to subject isolates to molecular work such as PCR genotyping of *coa* gene of the isolated strains of *Staphylococcus aureus*, thus recommend for future work.

The 21 coagulase positive *Staphylococcus aureus* (CoPS) were positive for mannitol fermentation and coagulase tests, and produces β haemolysis on blood agar which contained sheep erythrocytes. Whereas the 49 coagulase negative *Staphylococci* (CoNS) were found negative for the above tests. This finding is in line with report of Arshad *et al.*, (2006), who collaborate production of coagulase, DNAase and the fermentation of mannitol as important characteristics of *Staphylococcus aureus*. In clinical microbiology, the production of coagulase a major virulence factor is the principal criterion used in the diagnosis of strains of *Staphylococcus aureus* (Carter *et al.*, 2003).

So far, *S. aureus* remains the most important pathogenic species of Staphylococcus, it is an organism physicians should be buddy with.

The occurrence of CoNS in this study was higher (53%) than CoPS. This finding could be attributed to the fact that CoNS are part of the normal microbiota of the body with striking predilection for skin and mucous membranes and are generally considered to be of low virulence. However, sometimes they

can cause infections often associated with implanted devices, such as joint prostheses, shunts and intravascular catheters by colonizing these biomaterials, especially in very young, old and immunocompromised patients (Karen, 2013).

CoNS have also established themselves as important pathogens showing increasing trends towards antibiotics resistance in the last decade (Smolinski *et al.*, 2003).

**Table.1** Cultural and biochemical characteristics of *Staphylococci* and *Micrococci* isolated from cattle in Maiduguri

Growth on MSA	Colony colour	Gram staining	Coagulase	Catalase	Hymolysis	Isolates	No
+	yellow	+	+	+	+	<i>S. aureus</i>	21
+	pink	+	-	+	-	CoNS	49
+	yellow	+	-	+	-	Micrococci	22

**Table.2** *In – vitro* antibiotic susceptibility testing of *Staphylococcus aureus* isolated from cattle in Maiduguri n=21

Antibiotic	No susceptible (%)	Resistant (%)
Ciprofloxacin	21 (100)	0 (0)
Gentamycin	6(28.6)	15(71.4)
Ceftriaxone	19(90.5)	2(9.5)
Cotrimoxazole	15(71.4)	6(28.6)
Ampicillin	7(33.3)	14(66.7)
Clindamycin	16(76.2)	5(23.8)
Augumentin	17(81.0)	4(19.0)
Ofloxacin	13(61.9)	8(38.1)
Cephalexin	3(14.3)	18(85.7)

Biochemically, *Staphylococci* isolates were catalase and citrate positive, oxidase negative, strains of *S. aureus* were coagulase positive, whereas other *Staphylococci* isolates were negative table 1. The production of catalase, an enzyme which breaks down hydrogen peroxide to water and oxygen by *Staphylococci* distinguished them from *Streptococci*.

Result of antibiotic susceptibility testing did show that *Staphylococcus aureus* species were susceptible to ciprofloxacin (100%), ceftriaxone (90.5%), augmentin (81%) and clindamycin (76.2%). Our study observed a high percentage of cephalexin resistance (85.7%) and gentamycin (71.4%) to *Staphylococci* isolates as shown in table 2.

In this study, there was complete (100%) susceptibility of *Staphylococcus aureus* species to ciprofloxacin. This result collaborate the reports of Nwankwo and Nasiru (2011) that reported high level of susceptibility of *Staphylococci* to ciprofloxacin and showed that the flouroquinolones are effective in the treatment of *Staphylococcus aureus* infections including Methicillin resistant strains in Kano, North West Nigeria.

The high sensitivity of *Staphylococcus aureus* to these drugs could be attributed to the fact that the quinolones are new generation antibiotics and have not been abuse. In conclusion, this study reports the occurrence of coagulase positive *Staphylococcus aureus* from apparently healthy cattle in the study area and recommends ciprofloxacin and ceftriaxone as drugs of choice for the treatment of Staphylococcal infections.

### **Conflict of interest**

All authors hereby declare that they have no conflict of interest

### **Acknowledgement**

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