

Prevalence of *Staphylococcus Lugdunensis* Nasal Colonization among Apparently Healthy Ruminants and Their Handlers in Maiduguri Borno State, Nigeria

Samson Amali Onyilokwu¹, Shuaibu Gidado Adamu^{2*}, Samuel Mailafia³,
Naphtali Nayamanda Atsanda², Abdulyeken Olawale Tijjani²,
Fatima Adamu Lawan¹, Jasini Athanda Musa.¹, James A. Ameh³

¹Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

²Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

³Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Abuja, Nigeria.

Abstract: A study was carried out to determine the Prevalence of Microbial characteristics and antibiotic susceptibilities of *staphylococcus lugdunensis* nasal colonization among apparently healthy ruminants and their handlers in Maiduguri, Borno State, Nigeria. A total of six hundred (600) nasal swab samples were aseptically collected using simple random technique from the ruminants and their human handlers at the Maiduguri livestock market. 150 samples each were collected from cattle, sheep, goats and their handlers respectively. 195 (32.62%) were presumptively identified as coagulase negative *Staphylococcus* species. *Staphylococcus lugdunensis* nasal colonization was 9(4.62%) of 195, 5(55.56%) of 9 isolates were positive for clumping factor, however, all the 9 isolates were tube coagulase negative, but positive for DNase, Omithine Decarboxylase (ODC) test, betta – hemolysis and synergistic hemolysis. 4(44.4%) of the 9 isolates were positive for *mecA* virulence gene, 2(22.2%) positive for *Staphylococcus* Protein A (SPA) and 3(33.3%) were positive for slime (Biofilm) production. The antibiotic susceptibility pattern of these isolate were found to be highly variable. With the forging characteristics shown by *Staphylococcus lugdunensis* above, the organism can easily be misidentified with *Staphylococcus aureus*.

Keyword: *Staphylococcus*, farm animals, antibiotic, susceptibility profiles, Maiduguri

I. Introduction

Staphylococcus lugdunensis, is a gram positive cocci in clusters, belonging to the coagulase negative Staphylococci family (CoNS), with a variable properties of strains been positive for clumping factor (bond coagulase) which can be detected on slide based test for coagulase detection. *S. lugdunensis* can easily be isolated from routine media such as blood agar, Muller – Hinton agar and Manitol salt agar; depending on the media used, Beta-hemolysis may be seen with it characteristic odor as it usual indicator. *Staphylococcus lugdunensis*, a seemingly harmless (CoNS), has recently emerged as an important human and animal pathogen (Frank *et al.*, 2008; Betty, 2010). *S. lugdunensis* has clinical characteristics that resemble those of the coagulase positive *Staphylococcus aureus* (Dundar *et al.*, 2012) with potentials to be opportunistic pathogen (VanderMee - Marquret *et al.*, 2003). *S. lugdunensis* is an unusually virulent CoNS that often causes aggressive and rapidly progressive infections (Tan *et al.*, 2008).

Recently, *S. lugdunensis* has been reported as a highly virulent coagulase–negative, *Staphylococci* species implicated in serious infective endocarditis and joint infections with clinical incidence that is likely to be underreported. More than 80 case of *S. lugdunensis* endocarditis have been reported, primarily involving native left sided valves (Frank *et al.*, 2008; Stephen *et al.*, 2014). Meanwhile, a review of *S. lugdunensis* endocarditis estimated its mortality to be 50% with mortality rates higher with *S. lugdunensis* than other CoNS (Babu *et al.*, 2011, Stephen *et al.*, 2014). *S. lugdunensis* has also been shown to be associated with serious infections such as breast abscesses (Hellbacher *et al.*, 2006), peritonitis associated with continuous ambulatory peritoneal dialysis (Greig and Wood, 2003), septic arthritis, meningitis and ventriculo-peritoneal shunt infections (Kaabia *et al.*, 2012), prosthetic joint infections, and other infections such as toxic shock syndrome, skin and soft tissue cellulites and spondylodiscitis. *Staphylococcus lugdunensis* has also been implicated in urinary tract, central nervous system and surgical wound infections (Hellbacher *et al.*, 2006, Stephen *et al.* 2014).

S. lugdunensis can be misidentified as *S. aureus* because of the similar morphological appearance with yellow pigmentation, weakly positive DNase test and complete hemolysis when cultured on blood agar. In

addition, infections with this tube coagulase – negative. *S. lugdunensis* tend to run a more severe course, which resemble that of *S. aureus* infections rather than that caused by other coagulase – negative *Staphylococci*.

In light of the foregoing and given the pathogenic potential and wide spectrum of clinical disease associated with *S. lugdunensis*, this study aimed to isolate, phenotypically characterize and determine the antibiotic susceptibility profiles of *S. lugdunensis* isolated from the nasal colonization among apparently healthy ruminants and their Human handlers in Maiduguri, Borno State Nigerian.

II. Materials and Methods

The study was conducted in Maiduguri livestock market (Kasua - nshanu) jointly with Maiduguri central abattoir where the animals are brought for sale and slaughtered for consumption. Maiduguri is located in the arid zone of Borno State with an area of about 69436 km² and lies within latitude 10-13⁰N and longitude 12-15⁰E. It lies within the Savannah with low records of rainfall. It has two seasons, the dry season between Octobers to May followed by a short rainy season from early June to early October. The state is located in the North Eastern part of Nigeria bounded to Chad republic in the North East, republic of Cameroon in the East and Adamawa state to the South West. It has a population of about 4,558,668 and ranked 12th in the country according to the 2005 national census before the advent of Boko- Haram insurgency.

2.1 Sample Collection

A total of six hundred (600) nasal swab samples were collected under aseptic conditions for bacteriological examinations from ruminants (cattle, sheep and goats) and their human handlers in Maiduguri Livestock market and the central abattoir. All the samples were collected using sterile swab with broth medium from apparently healthy cattle (n=150), sheep (n=150), goats (n=150) and handlers (n=150) respectively regardless of the sex and age.

The samples were collected by gently inserting the sterile swab sticks into each nostril and rotated in a circular manner on both nares of the study subject to ensure adequate content with the mucus membrane of the nostrils and the tip is at the nasal osteum level. The samples were immediately transported to the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri in a Stuart transport medium. Analysis of all these samples commenced almost immediately less than 2 hours.

2.2 Bacteria Isolation

The nasal swab samples collected were systematically and aseptically cultured on Manitol salt agar (MSA) (Oxoid, UK) using streak plate method and incubated aerobically at 37⁰C for 24 – 48hrs in 5 to 7% CO₂. Presumptive *S. lugdunensis* colonies were further sub cultured onto fresh Manitol salt agar (MSA) and 5% Sheep blood agar (Oxoid, UK) in order to obtain pure colonies of the organisms and the hemolytic pattern of the bacteria. The presence of growth, colour, average colony size, margin surface elevation, opacity and consistency of colonies produced by the isolate on Manitol salt agar and 5% Sheep blood agar (Oxoid, UK) were noted including the hemolytic characteristics.

2.3 Identification of the Isolated Species

All the presumptive colonies were subsequently subjected to standard biochemical Laboratory tests as Gram staining, catalase test, modified oxidase test, rapid slide agglutination test for clumping factor, tube coagulase production test using human plasma and acid production from various sugars. Further test includes DNase (Salubris, Turkey), Slime (Biofilm) formation according to the method of Bannerman and Paecok, (2007), synergistic hemolysis and Omithine Decarboxylase (ODC) were carried out to identify *S. lugdunensis* and finally analysis in *Staphylococcal* API test Kits (Biomereux, UK).

2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of all the isolates were determined by Kirby- Bauer disc-diffusion technique using Muller – Hinton agar (Oxoid, England) approved by the clinical Laboratory Standard Institute CLSI/NCCL, (2010). The antibiotics used for the test includes, Genlamycin, Penicillin, Oxacillin, Chloramphenicol, Ciprofloxacin, Clindamycin, Sulphanmethaxazole, Streptomycin, Tetracycline, Rifampicin, Vancomycin and Co-trimoxazole. All the antibiotics were produced and obtained by Oxoid, England. These tests were all conducted using Muller-Hinton agar (MHA) (Remel, England) according to the protocol of (CLSI/NCCLS (2008). All the isolates identification were further confirmed by polymerase chain reaction amplification and sequence analysis of 16S-rRNA (ribosomal Ribonucleic Acid).

The ability of the organism to produce biofilm was performed and measured as described by Monk *et al.* (2008)

III. Results

A total of 9 (4.61%) *S. lugdunensis* were isolated of all the 195 CoNS isolates. Of these 9 *S. lugdunensis* isolates, 2 (22.2%) were from sheep, cattle and human handlers, 3 (33.33%) were isolated from goats. A total of 7 isolates (77.78%) should hemolysis on 5% sheep blood agar within 24 hours while 2 (22.22%) isolates show no hemolysis after 48hrs of incubation period. These 7 isolates also demonstrated synergistic hemolytic activity. The results in this study show that 6 isolates of 9 *S. lugdunensis* isolates were strongly slide coagulase positive while the remaining 3 isolates weak slide coagulase positive, however, it is worthy of note that all the 9 isolates (4.62%) where tube coagulase negative.

All the 9 *S. lugdunensis* isolates in this study showed DNase positive, ODC positive and Manitol fermented. Biofilm (slime) formation was observed in 6 (66.67%) of the 9 isolates with 4 from human handlers and 2 from cattle none from sheep and goats. 16S-rDNA gene sequencing was used to verify, identify and authenticate all the isolates as *S. lugdunensis*. All the 9 isolates (4.62%) were susceptible to all the antimicrobial agents tested with varying degree of zone of inhibition.

Table 1: Species distribution of *S. lugdunensis* from cattle, sheep, goats and their handlers in Maiduguri, Borno State

Source	Number of Sample	No. CoNS	Isolate (%) <i>S. lugdunensis</i> isolate (%)
Cattle	150	52 (26.7)	2 (22.22)
Sheep	150	48 (24.6)	2 (22.22)
Goat	150	43 (22.1)	3 (33.34)
Human handlers	150	52 (26.7)	2 (22.22)
Total	600	195 (100)	9 (100)

Table 2: Prevalence of virulence genes of *S. lugdunensis* isolated from cattle, sheep, goats and handlers in Maiduguri, Borno State.

Source	Number of Isolates	MecA gene (%)	StaphProtein A (SPA) gene
Cattle	2	2	0
Sheep	2	0	0
Goat	3	0	0
Human handlers	2	2	1
Total	9	4	1

Table 3: Antimicrobial Susceptibility of *S. lugdunensis* isolated from cattle, sheep goats and their handlers – in Maiduguri, Borno State Nigeria. N (%)

Source	No.	Isolate	No. Resistant Isolate											
			Gent.	Pen.	Chl.	Cip.	Cld.	Sul.	Str.	Tet.	Rif.	Van.	Cot.	
Cattle	(150)	2(22.22)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Sheep	(150)	2(22.22)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Goat	(150)	3(33.34)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Handlers	(150)	2(22.22)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Keys:

Gent.: Gentamycin; Pen.: Penicillin; Chl.: Chloramphenicol; Cip.: Ciprofloxacin; Cld.: Clindamycin; Sul.: Sulphametaxazole; Str.: streptomycin; Tet.: Tetracycline; Rif.: Rifampicin; Van.: Vancomycin; Cot.: Cotrimaxazole

IV. Discussion

A total of 195 (32.5%) CoNS species isolated from 600 nasal swabs samples in all from the three ruminant and their human handlers. Out of this 195 Isolates, 9 (4.6%) were confirmed to be *S. lugdunensis* slightly higher than the (3.0%) prevalence rate reported by Vivian, 2016, but lower than the 6% reported by Haile *et al.*, 2002 when they recovered the Isolates from urine cultures. Similarly, the prevalence rate (4.6%) in this study is also lower than the (7.3%) reported by Betty (2010) when they isolated *S. lugdunensis* from sterile body fluids and wounds. However, the result of this study is in contrast to the frequency estimated from a previous work in which *S. lugdunensis* represented (1.3%) of all *Staphylococcal* clinical isolates reported by Kawamura (1998). Since *S. lugdunensis* is as most virulent and destructive as *S. aureus*, it is therefore very important if it is suspected during clinical course of infection, particularly in sterile sites, the prompt identification to the species level will amount to life saving (Frank *et al.*, 2008). Major possible means of differentiating *S. lugdunensis* from other CoNS and *S. aureus* is by a negative tube coagulase test, a positive PYR reaction and ODC positive activity. It becomes important to differentiate because *S. lugdunensis* can easily be misidentified as *S. aureus* due to some certain characteristic of *S. lugdunensis* that mimics that of *S. aureus* such as weakly positive DNase, test for clumping factor can be positive, and the colonial morphology that often resembles that of *S. aureus* (Hellbacher *et al.*, 2006).

The upper respiratory tract has been recognized as a major ecological site for various bacteria that can migrate to a neighboring sterile site and cause mild to severe infection and invasive diseases (Kassis *et al.*, 2009). Studies have shown that the upper respiratory tract of man and animals may be an important reservoir of multiple antibiotic resistant CoNS with *S. lugdunensis* inclusive has been largely unreported in ruminants and their human handlers. Meanwhile, Ruminants animals in this part of the country have remained a very important sources of lively hood due to the huge income yield hence the presence and identification of this pathogen possess a serious public health and economic concern. The clinical relevance of *S. lugdunensis* was first described when it was implicated as a causative organism in endocarditis (Etienne *et al.*, 1989). It was further recognized as pathogenic organism in epidermoid cyst when in 2006, the culture results of 152 patients with epidermoid cyst over a 7-year period were reported (Stephen *et al.*, 2014). Since then, many authors have described *S. lugdunensis* as the most virulent coagulase – negative *Staphylococcus* species (CoNS) with percentage of 90% among all Isolates associated with clinically significant infections (Tan *et al.*, 2006, Dundar *et al.*, 2012). Infections caused by *S. lugdunensis* are well documented in Microbiology infectious disease, Cardiology and in Pre and Post operational journals with Skin and Soft tissue been consistently shown to be the most common site of clinically significant *S. lugdunensis* infection (Stephen *et al.*, 2014). In addition, they cause a wide range of diseases such as boils to mastitis in bovine, peritonitis, septicemia, Otitis media and urinary tract infections both in human and animals. In this study therefore, the prevalence of *S. lugdunensis* from cattle, sheep, goats and their handlers in Maiduguri Livestock marker was determined.

In the present study however, all the 9 *S. lugdunensis* isolates were positive for DNase and ODC which is in agreement with the report of other previous studies. In this same study 6 (66.67%) out of 9 *S. lugdunensis* Isolates were strongly coagulase positive while all the isolates examined were tube coagulase negative. Infections caused by biofilm (slime) formation CoNS are difficult to treat due to the high levels of resistance to antimicrobial agents (Frank *et al.*, 2008 Dundar *et al.*, 2012). Meanwhile, biofilm (slime) produced by CoNS is found in both nosocomial and commensal isolates. Hebert (1990) in his study, reported that 4 (11%) of 38 clinical isolates of *S. lugdunensis* were positive for slime production. In another study by Koksai *et al.* (2006), he reported 4 (22%) of 18 *S. lugdunensis* isolates positive for slime production in our study 6 (66.67%) of 9 isolates were positive for slime production with 4 (44.4%) from human handlers and 2 (22.2%) from cattle Isolates. However, there was no isolates from goat and sheep that was found to be positive for slime production in this study. In different studies carried out by other workers Hebert (1990), Freney *et al.* (1988), Bannerman and peacock (2007), Frank *et al.* (2007), Tan *et al.* (2008), *S. lugdunensis* was reported to have showed delayed moderate to wide Zone of hemolysis within 48 to 72 hours; in the present study, 7 isolates (77.78%) of *S. lugdunensis* demonstrated complete hemolysis and synergistic hemolytic activity within 24 to 48 hrs. However, 2 (22.2%) isolates of *S. lugdunensis* showed no hemolysis even after 48 hrs.

Unlike most CoNS, *S. lugdunensis* has been generally reported as being susceptible in vitro to most antibiotics (Dundar *et al.*, 2012, Tan *et al.*, 2008, Frank *et al.*, 2008). Frank *et al.*, (2007) reported that all the *S. lugdunensis* isolated from clinical specimen were susceptible to 10 antistaphylococcal antimicrobial agents with one isolate found to be resistant to Trimethoprim Sulfamethoxazole (Dundar *et al.*, 2012, Frank *et al.*, 2007). In another study, 15 bloodstream isolates of *S. lugdunensis* were reported to be susceptible to many common antimicrobials including penicillin (Dundar *et al.*, 2012). In the present study, all the 9 (100%) isolates of *S. lugdunensis* were susceptible to all the antimicrobial agents used including oxacillin, penicillin and Vancomycin. The findings in this study tend to differ from other previous work which suggested that penicillin resistance is rare in *S. lugdunensis* due to lack of B – lactamase and the *mecA* gene as reported by Mateo *et al.* (2005). Here some *S. lugdunensis* isolates possess *mecA* and SPA virulent gene yet they were susceptible to not only penicillin but also Oxacillin and Vancomycin. However, the susceptibility of these 9 isolates to all the antimicrobial agents used may be due to the few numbers (9) of isolates.

V. Conclusion

Before now, no case of *Staphylococcus lugdunensis* has ever been demonstrated in ruminants and their human handlers in North eastern Nigeria. This is the first case report of *S. lugdunensis* in ruminants and their handlers in the North eastern Nigeria. The major finding that the workers emphasize in this study is the aggressive nature of the infections caused by this *S. lugdunensis* relative to those from other CoNS, mimicking infections due to *S. aureus*. *S. lugdunensis* is being described as the most virulent CoNS among all isolates associated with clinically significant infections (Tan *et al.*, 2006). Jay (2007) in his work described *S. lugdunensis* as a dangerous wolf in a sheep's clothing. In a related development Johnson (2008) described it as a Lion among CoNS – while Dundar *et al.* (2012) described it to be as virulent and destructive as *S. aureus*. If such a pathogen so horribly described can be found and isolated from the nasal region, then it must be a serious public health concern. The present study indicates moderate nasal colonization rate of *Staphylococcus lugdunensis* (33.34%) among goats. Considering the pathogenic potential as mentioned above, broad spectrum

of clinical infection and the current recommendation to use *S.aureus* interpretative breakpoints for identification of *S.lugdunensis* by clinical Laboratory standards institute is essential.

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