

Species of Coagulase- Negative Staphylococci Isolated from Anterior Nare and Milk of Ruminant Animals and Contacts Persons in Maiduguri, Nigeria

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Abstract: Coagulase negative staphylococci (CNS) are primary cause of mastitis in ruminant animal, and can either be transmitted by contagious or by environmental means. Therefore, we speculate whether there are similarity in the species distribution of CNS colonising the anterior nare and milk of ruminant animals that can serve as source of infection. The study examined the species of CNS from the anterior nare of ruminant animals and contact persons. A total of 75 CNS isolates were analysed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), *mecA* gene detection by PCR assay and antibiotic susceptibility testing according to CSLI guidelines. Overall, four CNS spp were identified, *S.sciuri* predominate 71 (96%) and were recovered from all samples analysed, while one specie each (1.3%) of *S.lentus*, *S.condementii* and *S.xylosus* (1.3%) respectively. No *mecA* gene was detected in the CNS spp identified. The CNS spp demonstrated low to moderate resistance pattern (15-30%) to all antibiotics tested. In conclusion, the study provided a baseline epidemiological information on CNS spp colonizing the ruminants ruminant animal and contact persons. In addition, non-detection of *mecA* gene and high sensitivity pattern demonstrated by the CNS species.

Keywords: Coagulase Negative Staphylococci, Species, Ruminant Animals, Maiduguri

1. Introduction

Coagulase negative staphylococci (CNS) are emerging as pathogenic agents in both human and veterinary field. It is seen as the reservoir and responsible for horizontal transmission of methicillin resistance genes to susceptible staphylococci resulting in MRSA and MRCNS [7]. CNS, is the major causative agent of mastitis in ruminant animals resulting in significant economic losses to livestock farmer [5,13]. Wide variety of CNS species responsible for mastitis includes *S.chromogenes* and *S.epidermidis* that are more adaptive to the mammary gland, while others like *S. equorum*, *S.sciuri*, *S.fleuretti*, *S.cohnni*, *S.devriesei*, *S.xylosus*,

S.arlettae and *S.succinc* are known to have environmental transmission pattern [2,7,11].

In Nigeria, *S.aureus* is the most common of the gram-positive bacteria isolated in both human and veterinary laboratories. The CNS identification is seldom carried out probably due to the lack of basic facilities and research materials. However, molecular techniques like MALDI-TOF, offers a fast, accurate and species specific identification [6] but not applicable in resource limited laboratories.

In the northeastern Nigeria, epidemiological information gap exist on the species of CNS colonizing ruminant animals. Therefore, this is the first report to examine the species of CNS from the anterior nare of ruminant animals and contact persons. The goals of the study, was to address some salient

epidemiological questions, (i) whether there are similarity in the nasal CNS spp of ruminant animals and those isolated from mastitis cases in some published works (ii) the presence of *mecA* gene among the CNS isolates and (iii) the determine the degree of antibiotic resistance among the CNS spp.

2. Methodology

2.1. Samples/Isolates Collection

Nasal swabs of ruminant animals and contact person were collected at the Maiduguri Metropolitan council between January and June 2012. The University of Maiduguri, Faculty of Veterinary Medicine institutional review board approved the study and Borno state Veterinary department authorized the specimens collections. As documented in previous published work[9] a total of 510 nasal swabs and milk samples were collected from 12 ruminant animals breed and contact persons, 87(17.1) and 75(14.7%) were identified as *S.aureus* and CoNS isolated respectively.

2.2. CNS Species Identification and Antimicrobial Susceptibility Testing

The CNS species –specific test was performed by MALDI-TOF as previously described[6], and the analysis was carried out at the institute of medical microbiology, University Hospital Muenster, Germany. The *mec A* gene detection was carried out by PCR assay [15]. Antibiotic susceptibility pattern was carried out according CSLI guidelines [3] on Mueller-Hinton Agar. The following antibiotics were tested, penicillin, ampicillin, gentamycin, tetracycline, erythromycin, cotrimoxazole, ciprofloxacin, ofloxacin, and clindamycin. The antibiotic susceptibility was determined according to the agent breakpoint.

3. Result

Of the 75 CNS isolates analysed, (table 1), 21(28%) were recovered from cattle, 18(24%) from camel, 7(9.3%) each from goat and sheep and one(1.3%) from contact person. Demographic information showed that 70(93.3%) of the ruminants animals grazed extensively, while local remedies were used for the treatment of veterinary diseases (90.7%). Majority (n=72, 96%) of the CNS isolates were identified as *S.sciuri*, and recovered from all the sampled analysed., with *S.lentus* from cattle and *S.condementii* and *S.xylosus* from goat. No methicillin resistance gene(*mecA*) was detected in the CoNS idenfied. Overall, all the CNS spp demonstrated high sensitivity of 100% with *S.lentus*, *S.condementii* and *S.xylosus*, while *S.sciuri* resistance percentage ranged between 15-30% to all antibiotics tested.

Table 1. Demographic and bacteriological variables of the samples analysed in Maiduguri.

Samples(n=75)	Frequency(%)
Cattle	21(28)
Sheep	7(9.3)
Goat	21(28)
Camel	18(24)
Milk(cattle)	7(9.3)
Contact person	1(1.3)
Grazing	
Extensive	70(93.3)
Intensive	5(6.7)
Medication	
Antibiotic usage	7(9.3)
Local Remedy	68(90.7)
Coagulase negative staphylococci	
<i>S. sciuri</i>	72(96)
<i>S.lentus</i>	1(1.3)
<i>S.condementii</i>	1(1.3)
<i>S.xylosus</i>	1(1.3)

Table 2. Distribution of CNS within the samples analysed.

	cattle	sheep	goat	camel	Human	Milk	Total
<i>S.sciuri</i>	20	7	19	18	7	1	72
<i>S.lentus</i>	1						1
<i>S.condementii</i>			1				1
<i>S.xylosus</i>			1				1
Total	21	7	21	18	7	1	75

Table 3. Antibiotic resistant pattern(%) of CNS isolates.

	<i>S.sciuri</i>	<i>S.lentus</i>	<i>S.condementii</i>	<i>S.xylosus</i>
Pen	20	-	-	-
amp	30	-	-	-
gen	30	-	-	-
tet	25	-	-	-
ery	15	-	-	-
sxt	30	-	-	-
cpx	-	-	-	-
ofx	-	-	-	-
cld	-	-	-	-

Pen-penicillin, amp-ampicillin, gen-gentamycin, tet-tetracycline, sxt-contrimoxazole, cpx-ciprofloxacin, ofx-ofloxacin, cld-clindamycin

4. Discussion

CNS are emerging as bacterial pathogens of public health

concern in both human and medicine field. In veterinary field, it is primarily identified as the major cause of subclinical and chronic masitiis with consequential negative impacts like high

mortality, cost of animals treatment, and economic loss. Presently, CNS are seen as the reservoir of methicillin resistance gene that can be horizontally transferred to other susceptible staphylococci[7]. This is of public health concern because it is responsible for the emergence of MRSA, livestock-associated methicillin-resistant *S.aureus* (LAMRSA) and methicillin-resistant coagulase negative staphylococci(MRCNS). In Maiduguri, Mai-siyami et al[9] reported MRSA nasal carriage prevalence of 34.6% among ruminant animals and contact persons. The prevalence may be considered to be relatively high, due to lack of preexisting epidemiological data for comparison and documented predisposing risk factors. Therefore, the findings of this study provides a baseline epidemiological information on the CNS colonizing the anterior nares and milks of ruminant animals and contact in the study area.

The demographic variables of the ruminant animals as documented in the study questionnaire- extensive grazing practices and the use of local remedies for the treatment of veterinary diseases affirmed the common practices among the nomadic in the northern part of Nigeria.

Overall, *S.sciuri* predominates the total CNS spp identified($n=72$, 96%) and were recovered from all the samples analysed, while one species each(1.3%) of *S. lentus*, *S.condementii* and *S.xylosus*. In a similar study conducted in Switzerland, Huber et al[7] reported *S.sciuri* prevalence of 63.5%, which was also recovered from all the samples analysed, while *S.lentus* accounted for 15.7%. However, some studies have reported the recovery of *S.lentus* from other animals species such as horses[10], cattle sheep, and goat[15,16].

To collaborate our study speculation that there are similarity between nasal carriage CNS spp and those isolated from mastitis cases. Piessens et al[11], reported that *S.equorum*, *S.sciuri*, *S.heamolyticus* and *S.fleuretti* were predominant in the environment and milk samples. While studies have identified *S.sciuri*, *S.xylosus* and *S.cohnii* as environmental spp, which are capable of causing intramammary infection, especially in compromised animals[4,11,12, 15]. In contrast, several studies have observed that the level of preventive measures does not necessarily translate to reduction in the prevalence of CNS infection[14,16]. But, only few studies have identified the possible reservoir of CNS infection[11]. In this study, only one species of *S.xylosus* was identified, which has been identified as one of the primary reservoirs of CNS infection that can be contracted from environment, superficially from sawdust stock[11].

Methicillin resistant staphylococci (MRCNS) are emerging as a public health concern in veterinary field. The methicillin resistance genes have been reported to evolve in coagulase negative staphylococci and transferred horizontally to susceptible staphylococci, *S.sciuri* and *S.fleuretti* are known as natural reservoir of *mecA* gene [7]. The first report of MRCNS was in chicken[8], while other studies have reported it in ruminant animals like cattle sheep and goat [10,15]. The high prevalence of MRCNS reported among large scale livestock in Europe and US, have consequential impact on

food safety and potential spread of *mecA* genes to *S.aureus*(MRSA). In this study, no *mecA* genes was detected in all CNS species identified. Similarly, high sensitivity pattern was demonstrated by the CNS spp tested. The reason for such findings may be due to low level of antibiotic usage compared to high usage of local remedies as documented in the study. In Europe and US, high prevalence of MRCNS and antibiotic resistance in CNS isolates have been reported[7], that may be attributable to indiscriminate antimicrobial incorporation in animal feeds and also as growth promoter.

Though this study has provided a baseline epidemiological information, nevertheless there are limitations in the study, (i) number of CNS isolates analysed were relatively few (ii) non data on CNS spp from mastitis cases for comparison.

In conclusion, this study findings has provided a baseline epidemiological information on nasal carriage CNS spp among ruminant animals and contact persons. In addition, it revealed predominance of *S.sciuri*, non-detection of *mecA* gene and high sensitivity pattern. However, further studies are needed to evaluate the environmental effect on the distribution of CNS species.

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