



## Occurrence of *Staphylococcus piscifermentans* among Apparently Healthy Ruminants and Their Handlers in Maiduguri, Borno State, Nigeria

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### ABSTRACT

This study was conducted to determine the occurrence and antimicrobial susceptibility of *Staphylococcus piscifermentans* nasal colonization among apparently healthy ruminants and their handlers in Maiduguri, Borno State, Nigeria. A total of six hundred and five (605) samples were collected aseptically using sterile swab sticks (Oxoid, UK) using a simple random technique from the ruminants and their human handlers at the Maiduguri central abattoir and livestock market (Kasuwan shanu). One hundred and fifty-one (151) samples were collected from each species (cattle, sheep and goat), while 152 samples were collected from animal handlers. The samples were immediately subjected to standard bacteriological analysis. A total of 195 (32.2%) coagulase-negative *Staphylococcus* species (CoNS) were presumptively identified; out of the 195 identified CoNS, 9 (4.62%) was *Staphylococcus piscifermentans* nasal colonization. The results revealed that out of 9 isolates, *S. piscifermentans* was highly isolated 4(8.3%) from sheep, followed by cattle and human handlers 2 (3.8%) respectively, and the least was from goats 1 (2.3%). Meanwhile, all the 9 (4.62%) isolates were negative for both tube and slide coagulase test. However, they were positive for DNase, Ornithine Decarboxylase (ODC) test, beta – hemolysis and synergistic hemolysis. 4(44.4%) of the 9 isolates were positive for *Staphylococcus* Protein A (SPA) virulence gene, none 0(0%) for *mecA* virulence gene, and 5(55.6%) were positive for slime (Biofilm) production. The antibiotic susceptibility pattern of these isolates was found to be highly variable to all the used drugs. Therefore, it is concluded that *Staphylococcus piscifermentans* isolated from these ruminants and their handlers contain virulence genes that could be responsible for pathogenicity. There is a need for more studies using higher technologies in other parts of the country to reveal the thorough status of the disease in Nigeria.

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### INTRODUCTION

*Staphylococcus piscifermentans* are Gram-positive, catalase-positive, Oxidase-negative, facultative anaerobic cocci organisms found in singles pairs and clusters. It belongs to the Coagulase Negative *Staphylococcus* species (CoNS) **Onyilokwu et al., (2016)**. It is a rare strain that is biochemically and phylogenetically most closely related to

*Staphylococcus carnosus carnosus*; *Staphylococcus carnosus utilise* and *Staphylococcus condiment* (**Shubham et al., 2018**). In addition, CoNS like *S. condimenti*, *S. equorum*, *S. piscifermentans* and *S. succinus* belonging to the microbiota of spontaneously fermented foods (**Shubham et al., 2018**).

They are common colonizers of both animals and humans' anterior nares and ear canals (**Surkha et al., 2011**). While Stetina reported the isolation of *S.*

*piscifermentans* from faeces of healthy dogs (Stetina et al., 2005), Yasser, however, reported the isolation of the same organisms from teeth of an apparently healthy cow in a dairy farm (Yasser et al., 2018). Coagulase-negative staphylococcus species have long been considered opportunistic pathogens and were rarely reported to cause severe infections. However, due to the combination of the increase in the number of hospitalized immunocompromised patients, CoNS have become the major cause of nosocomial bloodstream infections. They are found to account for 9% of nosocomial infections (Bodonaik and Moonah, 2006). Hajar and Hamid (2013) recently reported the isolation of *Staphylococcus piscifermentans* from human brain abscess samples. In a related work, Chinwe et al., (2018) reported the isolation of the *S. piscifermentans* from ready-to-eat meat in Enugu, Southeastern Nigeria.

This rare coagulase-negative germ of *Staphylococcus* species was originally isolated from fermented fish, fermented fish sauce, and fermented shrimp in Thailand (Hajar and Hamid, 2013; Shubham et al., 2018). *Staphylococcus piscifermentans*, a non-pathogenic Gram-positive *Staphylococcal* species, are used in combination with *S. census* and *S. condimental* as starter cultures for fish fermentation and other food processing Industries (Shubham et al., 2018). Hajar and Hamid also reported the isolation of the organism as lactic acid bacteria strain *Staphylococcus piscifermentans* from Malaysian traditional fermented shrimp called the Cincaluk (Hajar and Hamid, 2013).

*Staphylococcus piscifermentans* can easily be isolated from routine media such as blood agar, Muller-Hinton agar and Mannitol salt agar, depending on the media used with its characteristic odour as its usual indicator. Compare with other CoNS, *Staphylococcus piscifermentans* is generally sensitive to antibiotics used against *Staphylococci*. It is then often possible to use ampicillin, penicillin, and methicillin to treat possible infections caused by this organism. On record, various *Staphylococcus* species of both coagulases are positive and negative (*S. schleiferi*, *S. sciuri*, *S. simulans*, *S. xylosus*, *S. lentus*, *S. aureus* and *S. epidermidis*) have already been isolated from apparently healthy ruminants and their handlers. Additionally, *S. piscifermentans* has also been described and isolated from tuna candy products (Hwang et al., 20103). However, this is the first time *S. piscifermentans* are isolated from ruminants and their handlers in Maiduguri, Borno State, and the study area.

Despite the risk associated with animal farming and the emergence of CoNS as a potential pathogen

causing disease, the business of livestock farming continues to thrive, most especially in Northern Nigeria and particularly in Maiduguri, the study area. There is a paucity of information on *S. piscifermentans* nasal colonization in ruminants and their handlers in Maiduguri, Borno State, Nigeria. The health risks posed by *Staphylococcus piscifermentans* nasal colonization in ruminants and their human handlers in the study area may be significant and need to be investigated. The epidemiological information on *S. piscifermentans* nasal colonization among apparently healthy ruminants and their handlers in Maiduguri will form baseline data to guide public health intervention programmes and empirical treatments to suspected cases of *S. piscifermentans*.

The study was to determine the occurrence, distribution, virulence markers and antimicrobial susceptibility profile of *S. piscifermentans* nasal colonization among apparently healthy ruminants and their handlers in Maiduguri, Borno State, Nigeria.

## MATERIALS AND METHODS

### Ethical approval:

The samples collected for this study were nasal swabs from the nasal carriageway of ruminants (cattle, sheep and goat) and their handlers at the Maiduguri central abattoir and livestock market (Kasuwan shanu). The samples collected for this study were nasal swabs from the nasal carriageway of ruminants (cattle, sheep and goat) and their handlers at the Maiduguri central abattoir and livestock market (Kasuwan shanu). Ethical approval was not collected because we seek the consent of the owner of the animals before the samples were collected. Standard sample collection for microbiological procedures was strictly observed.

### Study area:

The study was conducted in Maiduguri livestock market (Kasuwan - shanu) 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 km<sup>2</sup> 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 October and 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 the Republic of Niger in the West, Chad republic in the North East and the republic of Cameroon in the South East as shown in figure 1 below. 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 the Boko- Haram insurgence in 2009 to date ( Fig.1).

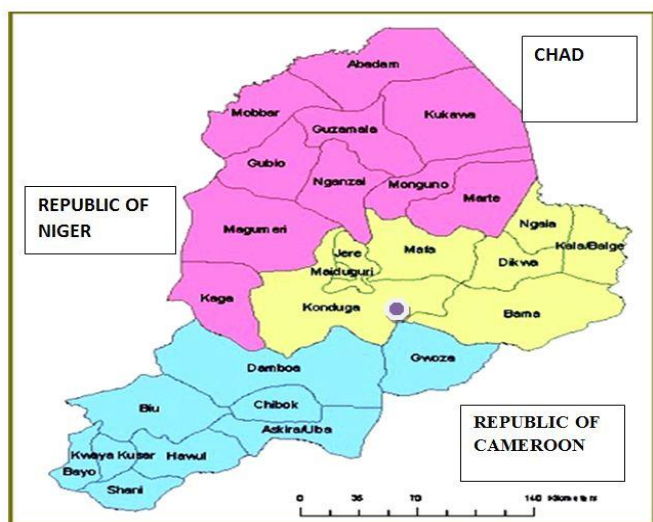


Fig.1: Map of Borno State showing Maiduguri at the centre (with yellow colour) where the Sample was collected.

**Sample collection:**

A total of six hundred and five (605) nasal swab samples were aseptically collected for bacteriological examinations from ruminants (cattle, sheep and goats) and their handlers in the Maiduguri Livestock market and the central abattoir. All the samples were collected using a sterile swab (Oxoid, UK) with broth medium from apparently healthy cattle (n=151), sheep (n=151), goats (n=151) and handlers (n=152), respectively. Four hundred (400) samples were collected from the abattoir; the 205 samples were collected from the livestock market the following day using simple random sampling. 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 animals and their handlers to ensure adequate content with the mucus membrane of the nostrils and the tip is at the nasal ostium level. In a Stuart transport medium, the samples were immediately transported to the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine University of Maiduguri. Analysis of all the samples commenced immediately less than 2 hours after the collection.

**Bacterial isolation:**

The nasal swab samples collected were aseptically and systematically cultured on Mannitol salt agar (MSA) (Oxoid, UK) using streak plate method and incubated aerobically at 37 °C for 24 – 48hrs in 5 to 7% CO<sub>2</sub>, according to the method of **Shubham et al., (2018)**. Presumptive *S. piscifermentans* colonies were further sub-cultured onto fresh mannitol 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 mannitol salt agar and 5% Sheep blood agar (Oxoid, UK) were noted including the haemolytic characteristics.

**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 Ornithine Decarboxylase (ODC) were carried out to identify *S. piscifermentans* and finally analysis in *Staphylococcal* API test Kits (Biomereux, UK).

**Antimicrobial susceptibility testing:**

Kirby determined antimicrobial susceptibility testing of all the isolates- Bauer disc-diffusion technique using Muller – Hinton agar (Oxoid, England) approved by the Clinical Laboratory Standard Institute CLSI/NCCL, (2014). The antibiotics used for the test include Gentamycin, Penicillin, Oxacillin, Chloramphenicol, Ciprofloxacin, Clindamycin, Sulphamethaxazole, Streptomycin, Tetracycline, Rifampicin and Vancomycin. 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 (2014). All the isolates identification was further confirmed by polymerase chain reaction amplification and sequence analysis of 16S-rRNA (ribosomal Ribonucleic Acid). The organism's ability to produce biofilm was performed and measured as described by **Monk et al., (2008)**.

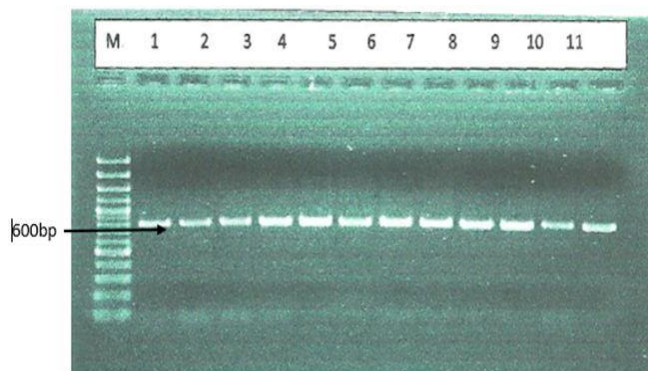


Fig. 2: Amplification of *Staphylococcus piscifermentans* from nasal colonization swab samples of cattle, Sheep, Gaots and Animal handles. 16S-rRNA genes amplification yields 600bp fragment (Lane's 4-10) to confirm *S. piscifermentans* isolates.

- Lane M: DNA molecular marker (100bp ladder).
- Lanes 1-3: positive control marker.
- Lane's 4-10 products of *S. piscifermentans* s isolates.
- Lanes 11-12 control

**Statistical analysis:**

The results of the total *S. piscifermentans* isolated from the nasal carriageway among the apparently healthy ruminants and their handlers were analysed using Statistical Package for Social Sciences (SPSS) version 21.0 statistical software and the Chi-square test at P< 0.5 was considered significant.

**RESULTS**

A total of 9(4.62%) *S. piscifermentans* were isolated from all the 195 coagulase-negative *Staphylococcus* isolates. Out of these 9(4.62%) *S. piscifermentans* isolates, 2 (3.8 %) from cattle and human handlers, 4(8.3%) were from sheep 1(2.3%) isolated from goats.

Table1: *Staphylococcus piscifermentans* species isolated from cattle, sheep, goats and animal handlers.

Source	Number of samples	No of the CONS isolated	Number of <i>S. piscifermentans</i>
Cattle	151	52(26.7)	2(3.8%)
Sheep	151	48 (26.6%)	4(8.3%)
Goat	151	43(22.0%)	1(2.3%)
Handlers	152	52(26.7%)	2(3.8%)
Total	605	195(32.2%)	9 (4.6%)

All the 9 (4.62%) *S. piscifermentans* were negative for both slides and tube coagulase test, but positive for Esculin, Nitrate reduction, Methyl- red, Bacteriocin activity, DNase, ODC, Urease and mannitol fermented. However, none of the 9 *S. piscifermentans* isolate showed biofilm (slime) formation.16S-rDNA gene sequencing was used to verify, identify and authenticate all the isolates obtained from phenotypic and biochemical tests. All the 9 *S. piscifermentans* isolates were susceptible to all the antibiotics tested with varying degrees of the zone of inhibition except for tetracycline.

Table2: Prevalence of virulence genes of *S. piscifermentans* isolated from cattle, sheep, goats and animal handlers.

Source	No. of <i>S. piscifermentans</i> isolate	Virulence Genes	
		mec A	SPA
Cattle	2	0	2
Sheep	4	0	0
Goats	1	0	0
Animal handlers	2	0	2
Total	9	0	4

Table3: Antimicrobial susceptibility of *Staphylococcus piscifermentans* isolated from Cattle, Sheep, Goats and their handlers

Source	No.of samples	No.of Isolates	TET	PE N	GEN	CIP	ME	CH	VR	KN	S	AM P	E
Cattle	151	2(3.8)	2(100)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Sheep	151	4(8.3)	4(100)	00	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Goats	151	1(2.3)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Animal Handlers	152	2(3.8)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

**DISCUSSION**

Out of the 605 nasal swabs samples collected from the ruminants (cattle, sheep and goats) and their handlers, a total of 195 (32.5%) CoNS species were isolated. The (32.5%) overall occurrence of CoNS species isolated from the nasal carriageway of apparently healthy ruminants and their handlers in the present study is higher than the 3.2% reported in South

Africa by Nyenje *et al.*, (2015). It is also higher than the 9.4% reported by Chinwe *et al.*, (2018) and 28% reported by Mashak *et al.*, (2015) in Teheran Province, Iran from vended RTE meats. The differences could be due to differences in the sources of samples and the geographical location of the samples. Environmental conditions such as the dusty path to the livestock market and even in the abattoir before slaughter in the study area may have also greatly contributed to the

high occurrence of this organism *Staphylococcus piscifermentans* and hence the higher prevalence being reported.

Out of these 195 isolates, 9 (4.6%) were confirmed to be *S. piscifermentans*, slightly higher than the 1(4.2%) prevalence rate reported by **Chinwe et al., (2018)** from vended Ready to Eat (RTE) meats and 2 (0.8%) by **Yasser et al., (2018)** isolated from bovine intramammary infections and teat apex colonization. However, the overall prevalence rate of 9(4.6%) of *S. piscifermentans* in this study is lower than the total 10 isolates identified as *Staphylococcus piscifermentans* based on biochemical and molecular characterization by **Shubham et al., (2018)** associated with traditional fermented fish products of Northeast India.

Coagulase-negative *Staphylococcus* species, though regarded as recently being recognized, especially in patients with underbelly devices, are among the most commonly isolated bacteria in the clinical microbiology laboratory **Von Eiff and Heilmann, (2002)**. However, there has not been any report of a case of an outbreak of disease or infection caused by *S. piscifermentans* to our knowledge. The present study showed no statistical differences between *S. piscifermentans* isolation rate among the four sources of samples. Major possible means of differentiating *S. piscifermentans* from other CoNS and *S. aureus* is by a negative coagulase test, a positive PYR reaction and ODC positive activity. It becomes important to differentiate because *S. piscifermentans* can easily be misidentified as *S. aureus* due to some certain characteristic of *S. piscifermentans* that mimics that of *S. aureus* such as haemolysis on blood agar plate after 24 h growth 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 migrating to a neighbouring 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. piscifermentans* inclusive has been largely unreported in ruminants and their human handlers. However, Ruminants animals in this part of the country have remained a very important source of livelihood due to the huge income yield. Hence, the presence and identification of this pathogen possess serious public health and economic concern.

Economically, **Ali et al., (2009)** stated the use of *S. piscifermentans* as a lactic acid bacteria (LAB) that produce lactic acid as the result of carbohydrate fermentation that is widely used as a starter culture in

the dairy, meat and vegetable fermentation industries. *S. piscifermentans* are also useful in flavour development and food preservation **Ali et al., (2009)**. It is also said to produce a variety of compounds with antimicrobial activity, such as organic acids, hydrogen peroxide and bacteriocin (**O-Sullivan et al., 2002**). The isolation of *S. piscifermentans* in this study confirmed the nasal carriageway as the predilection site of this organism. It indicates that *S. piscifermentans* can be harvested from this site and used for the above-mentioned benefits. The clinical relevance of *S. piscifermentans* was first described when it was isolated from faeces of healthy dogs (**Stetina et al., 2005**), human brain abscess samples (**Hajar and Hamid, 2013**) and teeth of an apparently healthy cow in a dairy farm (**Yasser et al., 2018**).

In the present study, all the 9 *S. piscifermentans* isolates were positive for ODC, DNase, esculine and urease, which agrees with the report of other previous studies. In this same study, all the 9 *S. piscifermentans* isolates were tube and slide coagulase-negative, which agrees with **Stetina et al. (2005)**. Meanwhile, all the 9 *S. piscifermentans* isolates were negative for lactose, mannose, oxidase activity, xylose and maltose, which are in agreement with the report of **Stetina et al. (2005)**. Unlike most CoNS, *S. piscifermentans* has been generally reported as being susceptible in vitro to most antibiotics (**Tan et al., 2008; Frank et al., 2008; Dundar et al., 2012**).

In the present study, all the 9 (100%) isolates of *S. piscifermentans* were susceptible to all the antimicrobial agents used including Oxacillin, penicillin and Vancomycin except tetracycline. Some *S. piscifermentans* isolates possessed SPA virulent gene but no *mecA* gene yet was susceptible to penicillin and Oxacillin, streptomycin, and gentamycin erythromycin, ampicillin, kanamycin, ciprofloxacin, chloramphenicol, and methicillin. However, the susceptibility of these 9 isolates to all the antimicrobial agents used may be due to the few numbers (9) of isolates. The resistance of *S. piscifermentans* to tetracycline could be associated with the frequent use of Oxytetracycline by veterinarians to treat animal diseases and the rampant drug abuse and misuse use of tetracycline by the public. The result agrees with the report of **Habeb and Mohammad (2010)**.

This confirmed that extensive use of antimicrobial agents in animal husbandry contributes to developing antibiotic-resistant microorganisms in animals and humans (**Barber et al., 2003; Martín et al., 2006; Normanno et al., 2007; Allen, 2014**). Based on these reservoirs, resistant strains or resistance genes may be distributed to humans through the food chain (**Martín et al., 2006; Phillips et al., 2004; Verraes et al., 2013**).

## CONCLUSION

The present study confirmed the presence of *Staphylococcus piscifermentans* in cattle, sheep and goats' as 4.6%. *Staphylococcus piscifermentans* were demonstrated at a high rate in sheep 8.3% and the least was in goats 2.3%. This is the first case report of *S. piscifermentans* in ruminants and their handlers in Northeastern Nigeria. The study also revealed that all except tetracycline could be used as a drug of choice to treat any case of *Staphylococcus piscifermentans* infestation. Finally, the present study revealed *Staphylococcus piscifermentans* isolated from ruminants and their handlers in Northeastern Nigeria possess SPA virulence genes.

## Declaration of Conflicting Interests

The authors revealed that there was no potential conflicts of interest.

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