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Full Length Research Paper

Identification and Enumeration of *Staphylococci species* isolated from Livestock in North-eastern Nigeria.

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Identification and enumeration of pathogens is a vital tool for disease detection and control, as well as, for risk assessment in both humans and animals. This paper reports the identification and enumeration of some *Staphylococci species* in livestock in north-eastern Nigeria. A total of 1,320 samples comprising blood, lung, trachea, nostril, and cloacae, aseptically collected from livestock in some parts of north-eastern Nigeria between April 2013 and January 2014, were inoculated onto culture media for isolation of staphylococci. Isolates were characterized by Gram stain, and biochemical tests including catalase, coagulase, and staphylase. Ribosomal and DNA-binding proteins extracted from some representative isolates of the presumptive staphylococci using ethanol, formic acid, and acetonitrile, were characterized by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS), using MALDI Biotyper developed by Bruker Daltonik (Germany). Isolates were subjected to antimicrobial resistance test using disk diffusion test method. Out of 28 picked-up Isolates characterized, *S. aureus* (28.6%), *S. sciuri* (32.0%), *S. simulans* (14.3%), *S. cohnii* (7.0%), *S. hyicus* (3.6%), and *Enterococcus faecalis* (3.6%) were identified; whilst 10.7% of the isolates were not reliable identification (NRI). All the species isolated were found to exhibit multiple antimicrobial resistances: tetracycline, penicillin, and ampicillin resistances were most commonly observed. Livestock in north-eastern Nigeria were found to harbour staphylococci commonly isolated from humans; implying a grave danger of pathogen cross-transmission. MALDI-TOF-MS used in this study was the first in animal studies from Nigeria, and appeared to be a very useful tool for rapid and accurate characterization of staphylococci to specific species level.

Keywords: Staphylococci, Enumeration, Livestock, Nigeria, Antimicrobial, Resistance, MALDI-TOF-MS.

INTRODUCTION

Staphylococcus species are a ubiquitous, gram-positive bacterial species, found mainly on the skin and mucosal surfaces of humans and animals as commensals, and normal microflora (Chajęcka-Wierżchowska *et al.*, 2014; Wedley *et al.*, 2014). Staphylococci are routinely enumerated in a wide variety of ready - to - eat foods (Chajęcka-Wierżchowska *et al.*, 2014), and more often in raw foods (Ademola and Effiong, 2013). *Staphylococcus aureus* (*S. aureus*), and other coagulase-positive staphylococci (CoPS), and coagulase-negative staphylococci (CoNS), have more often been isolated from food animals and humans (Nemeghaire *et al.*, 2014; Kolar *et al.*, 2010; Adegoke, 1986), and from pets, particularly dogs (Wedley *et al.*, 2014; Fazakerley *et al.*, 2010). Staphylococci have been reported to cause a wide range of disease conditions in animals and humans (Sasaki *et al.*, 2012), ranging from nosocomial, pyoderma, otitis, osteomyelitis, pneumonia, arthritis, septicemia, deep tissue abscesses, and wound infections (Vanderhaeghen *et al.*, 2010; Jensen and Lyon, 2009; McCaig *et al.*, 2006), to toxin-mediated diseases and food poisoning (Kadariya *et al.*, 2014).

Routine identification and enumeration of staphylococci are usually carried out by traditional methods based on the use of selective media such as Mannitol Salt Agar (MSA) and Baird-Parker Agar (BPA), and identification of suspicious colonies by colony morphology, Gram stain and biochemical reactions. However, these methods have been cumbersome and time-consuming, frequently giving ambiguous results (Gekenidis *et al.*, 2014). Fast and accurate method for identification and biotyping of bacteria organisms to species specific levels has been developed by Bruker Daltonik (Biswas and Rolain, 2013; Carpaij *et al.*, 2011; Alatoon *et al.*, 2011; Barbuddhe *et al.*, 2008; Carbonelle *et al.*, 2007): The Matrix-Assisted Laser Desorption Ionization -Time of Flight Mass Spectrometry (MALDI-TOF-MS) technique is based on unique ribosomal protein profiles matched to reference database and identified accordingly (Carpaij *et al.*, 2011). The resulting finger prints of the profiled proteins can be reproduced and mostly independent of culture medium, incubation temperature, and growth state (Dingle and Butler-Wu, 2013; Dieckmann *et al.*, 2010).

There is a scarcity of published information on the enumeration of staphylococci in animals in north-eastern Nigeria. Most researches were focused on humans; and those involving animals previously were centered in South-

western Nigeria (Ajuwape and Akinyede, 2000; Adegoke, 1986; Adegoke *et al.*, 1985; Adegoke and Ojo, 1982). Enumeration of pathogenic microorganisms is said to be a necessary tool for quantitative risk assessment (Rodrigues-Lazaro *et al.*, 2007). Therefore, this paper aimed at identifying and enumerating staphylococci in livestock in north-eastern Nigeria.

MATERIALS AND METHODS

Samples and sampling

Samples consisting of blood (300), lung (340), and trachea (300) samples from cattle, sheep, goats, pigs, and chicken; and nasal swabs (300) from cattle, sheep, goats, and pigs, and cloacal swabs (80) from chicken totalling 1,320 were aseptically collected on livestock farms and markets, and abattoirs/slaughter slabs in some parts of north-eastern Nigeria, between April 2013 and January 2014. Sample size was determined according to Shah (2011) for animal studies. The nasal mucosae of both nostrils in cattle, sheep, goats, and pigs, and the cloacae in chicken were respectively swabbed gently using sterile swab stick. Samples of lungs and trachea in each of the animals, where possible at abattoirs/slaughter plants, were swabbed immediately on exposure, after slaughter. The swab sticks were returned into their cases as fast as possible after swabbing; blood samples were aseptically collected from the jugular vein in cattle, sheep, and goats, and from the ear vein in pigs, and the radial vein in chicken, using sterile hypodermic syringes and needles, into sterile EDTA bottles. Bottles were swirled gently to mix the blood with the salt so as to prevent clotting. Samples were transported in an airtight ice container to the laboratory within 24 hours of collection for inoculation.

Isolation and identification

All the media used for the isolation and identification were purchased from Oxoid (Basingstoke, Hampshire, UK), unless otherwise stated. Each sample was homogenized in 250 µl of sterile normal saline, added to 2 ml nutrient broth No 2, and incubated aerobically for 24 hrs at 37°C. The enriched broth was vortexed and then streaked onto Blood Agar (BA) supplemented with 5% sheep blood (Kolar *et al.*, 2010). One to two growth or colonies from each plate was further sub-cultured onto MSA, and incubated overnight at 37°C (Wedley *et al.*, 2014). All streakings were done using 5 µl sterile disposable loops. Where present, isolates morphologically resembling staphylococci, and yielding

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small to medium, pink or yellow or golden yellow colonies on MSA, and white or yellow colonies on BA were selected from all plates using a 5 µl sterile disposable loops, and sub-cultured onto Columbia Blood Agar (CBA) plates, and incubated aerobically overnight at 37°C. All fresh cultures on CBA were subjected to Gram stain, and biochemical tests including catalase and coagulase using rabbit coagulase plasma (Pro-Lab Diagnosis Inc. Wirral, UK), and to Staphylase test (Pro-Lab Diagnostics, Wirral, UK), to identify staphylococci (Schmidt *et al.*, 2014).

Identification by MALDI-TOF-MS

Characterization of the staphylococci isolates was carried out on 28 picked-up representative isolates.

Extraction of Ribosomal and DNA-binding proteins

The ribosomal and DNA-binding proteins were extracted using previously described methods (Schmidt *et al.*, 2014; Alatoon *et al.*, 2011).

The MALDI-TOF-MS Typing procedure

Each target plate (MSP 96 well polished steel; Bruker Daltonik GmbH, Leipzig, Germany) was spotted in duplicate with 1 µl aliquots of the DNA-binding proteins extracts, and allowed to air dry for 15 minutes. Following the manufacturer's instructions, each sample spot was then overlaid with 2 µl of matrix solution (saturated solution of α-cyano-4-hydroxy-cinnamic acid (HCCA)(Bruker, Bremen, Germany) in 50% acetonitrile with 2.5% trifluoroacetic acid) and again air dried for 15 minutes. After air-drying, the target plate was inserted into the mass spectrometer for analysis. The bacterial test standard (*E. coli* DH5 alpha, Bruker, Bremen, Germany) was used for calibration before each experiment and included in duplicate on each target plate. Identification of the organism being tested was then automatically determined using the MALDI Biotyper 2.0 (Bruker Daltonics, Bremen, Germany) software programme with default settings: Laser frequency of 20 Hz within a mass range from 2000 to 20,000 Da, acceleration voltage of 20 kV, IS2 voltage maintained at 18.6 kV, and the extraction delay time of 200 ns. The mass peak profiles (spectral profile) of the unknown organism were then matched to a reference database, and scores generated based on similarity. A positive identification to species level was made for single result (> 2.0) or duplicate results (> 1.8) (Carpaij *et al.*, 2011).

Determination of Antimicrobial Resistance of the isolates

Disk diffusion test was performed on fresh cultures of the isolates from CAB plates according to the Clinical Laboratory Standards Institute (CLSI, 2014). Two Mueller Hinton agar plates with 5% defibrinated horse blood were inoculated with each isolate homogenised in saline (0.5 McFarland Standard) for semi-confluent growth using a cotton swab and rotary plating device. Antimicrobial disks obtained from Oxoid® (Basingstoke, Hampshire, UK) were then applied onto the surface of the plates, using an antimicrobial disk dispenser. Ten antimicrobials including chloramphenicol (30 µg), penicillin G (10 IU), enrofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), clindamycin (2 µg), ampicillin (10 µg), and vancomycin (5 µg) were used. The plates were incubated aerobically at 35°C for 16 to 18 hours for all disks other than vancomycin, which was incubated for 24 hours. The diameter of the zone of inhibition for each antimicrobial disk was measured with a ruler, and recorded in millimetres. The CLSI (2014) zone diameter interpretive standards were used to interpret the results.

Statistical analysis

Data were analysed using SPSS software package (SPSS 20.0 for Mac, SPSS Inc, Chicago, Illinois). Difference in isolation rate was determined using one way ANOVA, and P value < 0.05 was considered significant.

RESULTS

Isolation rate of bacteria in livestock in north-eastern Nigeria

Out of 1,320 samples collected and cultured, 219 (16.6%) were found positive for bacteria (Table 1). The rate of bacterial isolation was higher from cloaca: 27/80 (33.8%), followed by nostril: 74/300 (24.7%), lung: 58/340 (17.1%), and trachea: 50/300 (16.7%), and the least from blood: 10/300 (3.3%) specimens. Higher rate of isolation was observed in samples obtained from chicken: 55/270 (4.2%), followed by cattle: 52/270 (3.9%), sheep: 44/270 (3.3%), and goats: 44/270 (3.3%), and the lowest in samples from pigs: 24/240 (1.8%).

Table 1: Rate of Bacterial Isolation from Livestock in parts of North-eastern Nigeria

Types of Sample tested		Source of sample/number positive (%)					Total
		Cattle	Sheep	Goats	Pigs	Chicken	
Nasal	300	26(8.7)	18(6.0)	16(5.3)		14(4.7)	74(24.7)
Cloaca	80	-	-	-	-	27(33.8)	27(33.8)
Blood	300	0(0)	1(0.3)	1(0.3)	5(1.7)	3(1.0)	10(3.3)
Lung	340	14(4.1)	9(2.6)	20(5.9)	3(0.9)	12(3.5)	58(17.1)
Trachea	300	12(4.0)	16(5.3)	7(2.3)	2(0.7)	13(4.3)	50(16.7)
Total	1320	52 (3.9)	44 (3.3)	44 (3.3)	24 (1.8)	55 (4.2)	219 (16.6)

One-way ANOVA: $F = 1.971$; $P = 0.140$ ($P > 0.05$); not significant. There is no significant difference amongst the species of animals in rates of bacterial isolation.

Table 2: MALDI-TOF-MS Characterization of Representative Isolates of Presumptive Staphylococci Isolated from Livestock in North-eastern Nigeria (N = 28)

Species Identified	Sources and number of isolates (%) ^a					Total
	Cattle	Sheep	Goats	Pigs	Chickens	
<i>S. aureus</i>	2 (7.1)	5 (17.9)	nil	1 (3.6)	nil	8 (28.6)
<i>S. sciuri</i>	1 (3.6)	2 (7.1)	2 (7.1)	nil	4 (14.3)	9 (32.1)
<i>S. cohnii</i>	nil	2 (7.1)	nil	nil	nil	2(7.1)
<i>S. simulans</i>	3 (10.7)	nil	nil	1 (3.6)	nil	4 (14.3)
<i>S. hyicus</i>	nil	nil	nil	1 (3.6)	nil	1 (3.6)
<i>E. faecalis</i>	nil	nil	1 (3.6)	nil	nil	1 (3.6)
NRI ^b	2 (7.1)	nil	nil	nil	1 (3.6)	3 (10.7)

^a Percentage of identified *Staphylococcus species* calculated from the 28 picked-up isolates; ^b NRI = Not Reliable Identification. One-way ANOVA: $F = 1.424$; $P = 0.240$ ($P > 0.05$); not significant. There is no significant difference in the frequencies of isolation of staphylococci species amongst the animal species.

MALDI-TOF-MS Characterization of Staphylococci Isolated from Livestock in North-eastern Nigeria

Table 2 shows the results of MALDI-TOF-MS performed on 28 picked-up representative isolates of presumptive staphylococci isolated from livestock in north-eastern Nigeria. It was observed that 28.6% of the isolates were identified as *S. aureus*, 32.1% as *Staphylococcus sciuri* (*S.*

sciuri), 7.1% as *Staphylococcus cohnii* (*S. cohnii*), 14.3% as *Staphylococcus simulans* (*S. simulans*), 3.6% as *Staphylococcus hyicus* (*S. hyicus*), and 3.6% as *Enterococcus faecalis* (*E. faecalis*); whilst 10.7% were not reliable identification (NRI). High rate of isolation of *S. aureus* was observed in sheep (17.9%), followed by cattle (7.1%), and chicken (3.6%). High rate of *S. sciuri* isolation was observed in chicken (14.3%) isolates, followed by in

Table 3: Antimicrobial Resistance Patterns of the *Staphylococci species* Isolated from Livestock in North-eastern Nigeria

Antimicrobial	<i>Staphylococci species</i> isolated and % resistant					
	<i>S. cohnii</i> (n = 8)	<i>S. simulans</i> (n = 9)	<i>S. hyicus</i> (n = 2)	<i>E. faecalis</i> (n = 4)	NRI (n = 1)	<i>S. aureus</i> (n = 1)
T	100.0	89.9	100.0	100.0	0.0	100.0
C	0.0	22.2	50.0	0.0	0.0	0.0
CIP	50.0	66.7	100.0	75.0	0.0	100.0
TS	37.5	22.2	0.0	0.0	0.0	0.0
CDA	62.5	55.6	100.0	50.0	100.0	100.0
GM	25.0	0.0	0.0	0.0	0.0	100.0
P	100.0	100.0	100.0	100.0	100.0	100.0
AP	100.0	77.8	100.0	100.0	100.0	100.0
VAN	62.5	22.2	50.0	0.0	100.0	0.0
ENR	0.0	11.1	0.0	0.0	0.0	0.0

tetracycline, C: chloramphenicol, CIP: ciprofloxacin, TS: trimethoprim/sulfamethoxazole, CDA: clindamycin, GM: gentamicin, P: penicillin, AP: ampicillin, VAN: vancomycin, ENR: enrofloxacin

goats and sheep (7.1% each), and cattle (3.6%) isolates. *S. cohnii* was isolated from sheep (7.1%) samples only, and *S. hyicus* was isolated from pig (3.6%) samples only. *S. simulans* was isolated from cattle (10.7%) and chicken (3.6%) samples; while, *E. faecalis* was isolated from goats (3.6%) samples only. However, 3 (10.7%) of the presumptive staphylococci isolates: 2 from cattle (7.1%) and 1 from chicken (3.6%), were not reliably identified by the MALDI biotyper.

Antimicrobial resistance determination of the staphylococci isolated from livestock in northeastern Nigeria

The antimicrobial resistance characteristics of the isolates were determined and results are presented in tables 3 and 4. The patterns of resistance demonstrated by the isolates (table 3) showed that *S. aureus* exhibited 100% resistances against tetracycline, penicillin and ampicillin, 62.5% against clindamycin and vancomycin, and moderate resistance (50.0%) against ciprofloxacin. *S. sciuri* exhibited high resistances against penicillin (100%), tetracycline (88.9%), ampicillin (77.8%), and ciprofloxacin (66.7%), and moderately resistant to clindamycin (55.6%). *S. cohnii* were highly (100%) resistant to tetracycline, ciprofloxacin, ampicillin, penicillin, and clindamycin, and moderately resistant to chloramphenicol (50.0%) and vancomycin (50.0%); whilst *S. simulans* were resistant to tetracycline

(100%), ampicillin (100%), penicillin (100%), ciprofloxacin (75.0%), and clindamycin (50.0%). On the other hand, *S. hyicus* exhibited 100% resistances against ampicillin, penicillin, clindamycin, and vancomycin; whilst, *E. faecalis* exhibited 100% resistances against tetracycline, ampicillin, penicillin, ciprofloxacin, clindamycin, and gentamicin. The NRI isolates were 100% resistant to trimethoprim/sulfamethoxazole, tetracycline, ampicillin, clindamycin, penicillin, and vancomycin, and 66.7% resistant to gentamicin and ciprofloxacin.

The antimicrobial resistance profiles of the isolates shown in table 4 indicate that *S. aureus* and *E. faecalis* exhibited multiple antimicrobial resistances against 6 antimicrobials respectively; *S. sciuri* and *S. simulans* were resistant to 5 antimicrobials respectively, *S. cohnii* exhibited resistances against 7 antimicrobials, and *S. hyicus* was resistant to 4 antimicrobials; Whilst the NRI isolates were resistant to 8 out of the ten antimicrobials tested.

DISCUSSION AND CONCLUSION

DISCUSSION

Several studies (Gekenidis *et al.*, 2014; Biswas and Rolain, 2013; Barbuddhe *et al.*, 2008) have described the MALDI-TOF-MS as a method for typing methicillin-resistant *S*

Table 4: Antimicrobial Resistance Profiles of the *Staphylococci* species Isolated from Livestock in North-eastern Nigeria

<i>Staph.species</i>	Number Tested	Antimicrobial phenotypes*
<i>S. aureus</i>	8	T, AP, P, CIP, CDA, VAN
<i>S. sciuri</i>	9	T, AP, P, CIP, CDA,
<i>S. cohnii</i>	2	T, AP, P, CIP, CDA (C, VAN)
<i>S. simulans</i>	4	T, AP, P, CIP (CDA)
<i>S. hyicus</i>	1	AP, P, CDA, VAN
<i>E. faecalis</i>	1	T, AP, P, CIP, CDA, GM
NRI	3	T, TS, AP, P, CIP, CDA, GM, VAN

*Isolates exhibited Intermediate Resistances against the antimicrobials in parentheses

aureus, and determination of the epidemiologic relatedness of vancomycin-resistant strains, and for epidemiologic and infection prevention. Although, Multiplex Polymerase Chain Reaction (mPCR) remains the rapid method to discriminate isolates to specific species level, and for identifying *S. aureus* based on the *nuc* gene, it was however found to be very expensive, also requiring great expertise; and some *S. aureus* strains are becoming too difficult to be identified (Biswas and Rolain, 2013) using the mPCR. Fortunately, the recent discovery of MALDI-TOF-MS, which is a rapid and reliable method that can characterize both CoPS and CoNS to specific species levels within a very short time is a boost to the microbiology laboratory; and is able to replace the conventional and molecular biology techniques (Schmidt *et al.*, 2014; Carpaj *et al.*, 2011; Dubois *et al.*, 2010). In this study, about 89% of the staphylococci isolates were identified and characterized to specific species level using the MALDI-TOF-MS. This finding is in concordance with that of Carbonnelle *et al* (2007) who reported 95% identification and characterization of unknown clinical strains of *Staphylococcus spp.* to specific species level. Similarly, Schmidt *et al* (2014) identified and characterized 79% of staphylococci isolated from dogs to specific species level by the MALDI-TOF-MS technique. This rapid bacterial profiling using the MALDI-TOF-MS has also found application in clinical microbiology, where characterization of certain species proved more accurate than with the polymerase chain reaction (Dingle and Butler-Wu, 2013). In this study, duplicate spots were processed for each isolate, but there was no identification for three isolates using the MALDI-TOF-MS. An interesting finding is that for one of the isolates (isolate number 01 from cattle samples), nothing matched of the over 5,000 strains profiles in the database; suggesting that the isolate may be a new animal *Staphylococcus species* occurring in

livestock in north-eastern Nigeria that is yet to be characterized. Addition of more highly characterized reference isolates to the database may help increase the species level identification of unknown bacteria. Another interesting finding observed in the present study is that isolates presumptively identified using the traditional methods as *S. aureus* from pigs was confirmed as *S. hyicus* using the MALDI-TOF-MS technology. This may infer that pigs are not readily colonized by *S. aureus* in this region of Nigeria; which is beneficial to pig farmers, handlers and consumers. However, more studies that will corroborate this finding using large sample sizes from pigs are imperative.

Although, *S. sciuri*, *S. simulans*, *S. hominis*, and *S. cohnii* were mainly human-associated staphylococcal species (Weist *et al.*, 2006), they have been isolated from livestock as rare colonizers of the animal body (Petinaki and Spiliopoulo, 2015; Schmidt *et al.*, 2014; Huber *et al.*, 2011). The isolation of these human-associated staphylococci in animals in this study is the first report in north-eastern Nigeria; and suggests a potential cross-transmission from humans to animals. Because of the inadequacies of the traditional identification methods to identify the isolates to the species level, MALDI-TOF-MS was incorporated into the work flow of this study, for the first time to successfully characterize staphylococcal populations in a group of livestock in Nigeria, and was found to be rapid and reliable for the identification and characterization of animal staphylococci to the specific species level.

Antimicrobial resistance is a significant cause of morbidity and mortality, hence rapid and accurate detection of antimicrobial resistance is important for prevention and management of infections in animals. In the present study, multiple antimicrobial resistances were common amongst the isolates. From the findings of the present study, it was

observed that *S. aureus* isolates were more multidrug resistant compared to *S. cohnii*, *S. sciuri*, *S. hyicus*, and *S. simulans* isolates. This could suggest a higher selective pressure due possibly to frequent use of antimicrobials in animals without prescription. Similarly, Lee (2006) reported multidrug resistance in *S. aureus* species isolated from sheep, cattle, pigs, and chickens from over 2000 specimens. Corroborating with the findings in the present study also, Mai-Siyama *et al* (2014) reported multidrug resistant staphylococci isolated from cattle, sheep, and goats slaughtered for human consumption at Maiduguri abattoir in Borno state, Nigeria. Interestingly, in the present study, apparent resistance against vancomycin was observed amongst some *S. aureus*, *S. cohnii*, and *S. hyicus* isolates by the disk diffusion test (zone diameter \leq 11 mm). These isolates might probably display the characteristic profile of superbugs (Batabyal *et al.*, 2012); although vancomycin was only introduced recently in northern Nigeria for use in human treatments (Suleiman *et al* 2012). However, according to Clinical Laboratory Standards Institute (CLSI, 2014), resistance against vancomycin can only be confirmed through the determination of minimum inhibitory concentration (MIC); this was not done in this study.

The high resistances observed against tetracycline, penicillin, ampicillin, ciprofloxacin, and clindamycin by most of the isolates in this study calls for restraint in the routine use of these antimicrobials in animals, except on prescription. One significant finding from the resistance patterns of some isolates observed in this study was the same degree of resistance against penicillin and ampicillin. This infers that ampicillin can replace penicillin for detection of penicillin-resistance, just as it is used in place of oxacillin in the detection of oxacillin-resistance in *S. aureus* (CLSI, 2012). Also, of interest was the resistance against same groups of antimicrobials by *S. sciuri* and *S. simulans* isolates in this study. Furthermore, isolates in this study exhibited similar trend of resistance profiles against β -lactam (penicillin and ampicillin), and non- β -lactam (tetracycline, clindamycin, and ciprofloxacin) antimicrobials in a fashion similar to those reported by previous studies (Lee, 2003; Suleiman *et al.*, 2012). The resistance against non- β -lactam antimicrobials observed in this study may possibly be due to mutation and/or overexpression of associated membrane proteins such as activated protein C (APC), which resistance have been mostly attributed by point mutation in the nucleotide G-A or factor V gene (Katy and Roepe., 2014). This suggests that antimicrobial resistance may not necessarily be due to overuse of antimicrobials only; but some genetic factors may be playing a role.

CONCLUSION

The present study reports the presence of *Staphylococcus aureus* and other staphylococci species including *S. sciuri*, *S. cohnii*, *S. simulans*, and *S. hyicus* in cattle, sheep, goats, pigs, and chicken from north-eastern Nigeria, with *S. aureus* prevalence higher in sheep than in the other animals. The frequency of isolation of *S. sciuri* was higher, followed by *S. aureus*, and *S. simulans*. The low frequency of isolation of *S. aureus* than *S. sciuri* in this study may indicate rare carriage of *S. aureus* in healthy animals in this region, or possible mutation of *S. aureus*; or *S. sciuri* may be mimicking *S. aureus*. Studies using larger sample size may be carried out to dispute or corroborate this assertion.

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7.0 Declaration of conflict of interest

No conflict of interest to declare.

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