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Characterization of *Mammaliicoccus sciuri* and Mannitol-Fermenting Staphylococci from Small Ruminants and Chickens in the Federal Capital Territory, Nigeria

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Abstract

Mammaliicoccus and *Staphylococcus* species are Gram-positive bacteria found on the skin and mucous membranes of some mammals. This study aimed to identify, determine the species distribution, and assess the antimicrobial profile of mannitol-fermenting non- *S. aureus* species from chickens, sheep, and goats in the Federal Capital Territory, Nigeria. Forty-seven isolates from chickens (n = 15), sheep (n = 18), and goats (n = 14) were screened using the Staph Latex Kit (Labmann, UK) and identified using the Analytical Profile Index Kit. Antimicrobial susceptibility testing (AST) was performed using disk diffusion and E-test methods. *Mammaliicoccus sciuri*, *Staphylococcus intermedius*, and *Staphylococcus xylosus* were the three species identified. *Mammaliicoccus sciuri* was found to be the most predominant species with 79% (n=37) prevalence, followed by *Staphylococcus intermedius* with 15% (n = 7), while *S. xylosus* was the least common with 4.25% prevalence. The AST results showed complete sensitivity of all isolates (100 %) to cefoxitin, penicillin, gentamicin, kanamycin, rifampicin, spectinomycin, and ciprofloxacin. Isolates were, however, resistant to trimethoprim (48.93%), tetracycline (15%), erythromycin (9%), and amikacin (4%). Ten percent of the isolates exhibited multidrug resistance. This study documents a high occurrence of *Mammaliicoccus sciuri* in small ruminants and chickens. Periodic AST should be conducted to determine the level of antimicrobial use in food animals and to facilitate effective monitoring and reporting of AMR in animals.

Key words: *Mammaliicoccus*, *Staphylococcus*, chickens, sheep. Goats, antimicrobial resistance

INTRODUCTION

Mammaliicoccus is a recently reassigned genus under the family *Staphylococcaceae* (Madhaiyan *et al.*, 2020). The novel genus was formally referred to as the *Staphylococcus sciuri* group, including *S. vitulinus*, *S. lentus*, *S. fleurettii*, and *S. stepanovicii* (Adesoji *et al.*, 2024). *Mammaliicoccus* and *Staphylococcus* are commensal bacteria found on the skin and mucous membranes of humans and animals (de Moura *et al.*, 2023). Although both species are considered to be of low virulence, they are increasingly becoming pathogenic and are reported to be implicated in life-threatening animal and human infections (Bora, 2018). They have established themselves as important pathogens, exhibiting increasing trends towards antibiotic resistance (May *et al.*, 2014).

Mammaliicoccus sciuri is the most common species of the genus *Mammaliicoccus* and has been reported to cause mastitis and metritis in cattle (Schnitt *et al.*, 2021), septicemia in poultry and wild animals such as tigers, buffaloes, and elephants (Singh *et al.*, 2024). Staphylococci are a diverse group of bacteria that can cause a wide spectrum of diseases in mammals, resulting in an increased healthcare burden (Frey *et al.*, 2013). Aside *S. aureus*, which is reported to be mostly incriminated in cases of mastitis and skin infections, other species of staphylococci such as *S. epidermitis*, *S. intermedius*, *S. hyicus*, and *S. delphini* have been reported to cause mastitis and pyoderma in cows, horses, pigs, goats, and dogs (Frey *et al.*, 2013). *S. xylosus* and *S. gallinarum* have been reported to be associated with endophthalmitis and pyelonephritis in chickens (Vela *et al.*, 2012). These infections are

sometimes very difficult to treat because staphylococci rapidly develop resistance to many antimicrobial agents to which they were once susceptible to (Echioda-Ogbole et al., 2025). The development of resistance to antimicrobial agents by Staphylococci are due to a number of mechanisms, including the production of beta-lactamase enzymes which hydrolyses the amid bond of beta-lactam antibiotics thereby inactivating the drug (Torimiro and Olusayo, 2017), acquisition of chromosomal *mecA* gene carried on staphylococcal cassette chromosomemec (SCCmec) which encodes a low-affinity penicillin binding proteins (PBP2a), associated with methicillin and oxacillin resistance (Zhu et al., 2017). *Mammaliicoccus sciuri* is a known reservoir for the *mecA* gene and a potential carrier of other antimicrobial and virulence genes to *S. aureus* (de Moura et al., 2023).

Mannitol fermentation is a species signature of *S. aureus*, use in low-resource settings along with the tube coagulase test (TCT) for the identification of the organism. Although these traditional test methods have been reported to identify *S. aureus* (Echioda-Ogbole et al., 2018), their performance and results are subjective and vary from setting to setting (Bello and Qahtani, 2006). Studies have shown that some coagulase-negative staphylococci (CoNS) species can ferment mannitol and produce golden-yellow colonies typical of *S. aureus* due to the high salt content of the Mannitol salt agar (Thakur et al., 2017). There is a dearth of information on mannitol-fermenting non-*S. aureus* species in the study area. Hence, this study was undertaken to identify Staphylococci species other than *S. aureus* from chickens, sheep, and goats in the Federal Capital Territory, Nigeria.

MATERIALS AND METHODS

Sample collection

Simple random sampling was used to collect 684 samples from chickens, sheep, and goats from livestock markets in the six Area Councils of the FCT. The samples consist of 228 nasal swab samples from sheep and goats each, and 228 trachea swabs from chickens. A sterile swab stick was inserted into the nostrils of sheep and goats, and the trachea of chickens, and then gently rubbed against the mucosal surface for approximately 4 to 5 seconds. Individual swab sticks were placed in sterile cryo-vials containing Mueller-Hinton broth (Oxoid, UK) supplemented with 6.5% Sodium Chloride (NaCl), appropriately labeled, and placed in a flask

containing icepacks. They were then immediately transported to the Department of Veterinary Microbiology laboratory, University of Abuja, for processing.

Bacteria isolation and identification

All bacteriological culture media (Oxoid, Basingstoke, Hampshire, UK) were prepared according to manufacturers' instructions. Each sample was analyzed individually by inoculating it into 5ml of Muller Hinton broth and incubating at 37°C for 24 hours. A loopful of about 10-µl of the inoculum from Mueller Hinton broth (Oxoid, England) was streaked onto prepared plates of Mannitol salt agar (Oxoid, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 hours. Following incubation, all cultured plates were examined for colonial morphology and pigmentation. Smooth, shiny, convex, distinct golden yellow colonies were picked with a sterile wire loop and transferred onto nutrient agar plates incubated at 37°C for 24 hours to obtain a pure culture. The pure isolates were inoculated on nutrient agar slants, incubated at 37°C for 24 hours, and then stored in the refrigerator at 4°C for further characterization.

Biochemical identification of isolates

Preliminary identification of the isolates was done based on Gram reaction (microscopic appearance) and biochemical characterization based on catalase, oxidase, and coagulase tests as described by Cheesbrough (2016). Isolates were further screened using the Staph Latex Kit (Labmann, UK).

Identification of mannitol-fermenting *Staphylococcus* species

The Analytic Profile Index (API) Kit, a standardized system utilizing miniaturized biochemical tests and a specially adapted database, was employed for the identification of Staphylococcus, Micrococcus, and Kocuria. The test strip consists of 20 microtubes containing dehydrated substrates including D-glucose, D-fructose, mannose, maltose, lactose, D-trhalose, mannitol, xylitol, melibiose, nitates, Vogesproskauer, raffinose, xylose, saccharase, methyl-α-D-glucopyranoside, N-acetylglucosamine, Arginine DiHydrolase, and Urease. Forty-seven mannitol-fermenting non-*S. aureus* isolates were characterized using the API Kit (BioMerieux, Marcy-l'Etoile, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method of Kirby-Bauer with some modifications and in accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2018). The following antibiotic disks with their corresponding concentration were tested: Cefoxitin (30 µg), Penicillin (10 units), Gentamicin (10 µg), Amikacin (30µg), Erythromycin (15 µg), Clindamycin (2 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Trimethoprim (25µg), Rifampicin (5 µg), Spectinomycin 100µg, Fusidic acid (10 µg), Ciprofloxacin (5 µg), Kanamycin (30 µg). Minimum inhibitory concentrations (MICs)

for Vancomycin, Teicoplanin, Linezolid, and Tigecycline were determined using E-test strips (BioMerieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. The results were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2020).

RESULTS

Forty-seven mannitol-positive isolates were identified as *S. sciuri* (*M. sciuri*), *S. intermedius*, and *S. xylosus*. Of the three species, *M. sciuri* was found to be the most predominant with 79% prevalence (n = 37), followed by *S. intermedius* with 15% (n = 7), while *S. xylosus* was the least common with 4.25% prevalence (Table 1).

Table 1: Prevalence of mannitol-fermenting non-*S. aureus* species and their percentage identity range

| <i>Staphylococcus</i> species | Number | Percentage (%) | S. index (%) |
|-----------------------------------|--------|----------------|--------------|
| <i>Mammaliicoccus sciuri</i> | 37 | 79 | 98.30 |
| <i>Staphylococcus intermedius</i> | 7 | 14 | 94 |
| <i>Staphylococcus xylosus</i> | 2 | 4.25 | 91 |

Key: %= percentage, S. index= similarity index

Of the 37 *M. sciuri* species, 10 (27%) were recovered from chickens and goats each, while 17 (46%) were from sheep. *S. xylosus* was isolated only from chickens (n = 2), giving a prevalence of 4.3%, while the distribution of *S. intermedius* in chickens, sheep, and goats are 29% (n = 2), 43% (n = 3) and 29% (n = 2), respectively.

The Antimicrobial susceptibility testing results showed complete sensitivity of all isolates (100 %) to cefoxitin, penicillin, gentamicin, kanamycin, rifampicin, spectinomycin, and ciprofloxacin. Isolates were, however, resistant to trimethoprim (48.93%), tetracycline (15%), erythromycin (9%), amikacin (4%), with 2% resistance to chloramphenicol and mupirocin (Table 2).

Table 2: Antimicrobial susceptibility of mannitol-fermenting *Staphylococcus* species from food animals in FCT

| Antimicrobial Agent | Disk Concentration (µg) | Sensitive n (%) | Resistant n (%) |
|---------------------|-------------------------|-----------------|-----------------|
| Cefoxitin | 30 | 47 (100.0%) | 0 (0.0%) |
| Penicillin | 10 | 47 (100.0%) | 0 (0.0%) |
| Gentamicin | 10 | 47 (100.0%) | 0 (0.0%) |
| Kanamycin | 30 | 47 (100.0%) | 0 (0.0%) |
| Amikacin | 30 | 45 (96.0%) | 2 (4.0%) |
| Erythromycin | 15 | 43 (91.0%) | 4 (9.0%) |
| Clindamycin | 2 | 47 (100.0%) | 0 (0.0%) |
| Chloramphenicol | 30 | 46 (98.0%) | 1 (2.0%) |
| Tetracycline | 30 | 39 (83.0%) | 8 (17.0%) |
| Trimethoprim | 25 | 24 (51.0%) | 23 (49.0%) |
| Rifampicin | 5 | 47 (100.0%) | 0 (0.0%) |
| Spectinomycin | 100 | 47 (100.0%) | 0 (0.0%) |
| Ciprofloxacin | 5 | 47 (100.0%) | 0 (0.0%) |
| Mupirocin | 200 | 46 (98.0%) | 1 (2.1%) |

KEYS: % = percentage positive, µg = microgram

Eight (8) resistance phenotypes were observed among the other *Staphylococci* isolates, and 5 (10.63%) of the isolates exhibited multidrug resistance. Three (20%) of the multidrug-

resistant isolates were from chickens, while 2 (11.11%) were from sheep. None of the isolates from goats were multidrug resistant (Table 3).

Table 3: Antimicrobial resistance profiles of mannitol-fermenting *Staphylococcus* species from food animals in FCT

| Resistance Pattern | Chickens (n) | Sheep (n) | Goats (n) |
|--------------------|--------------|-----------|-----------|
| EM, TET, TMP | 2* | 0 | 0 |
| EM | 1 | 0 | 0 |
| EM, C, TET, TMP | 1* | 0 | 0 |
| TMP | 2 | 14 | 4 |
| MUPH | 1 | 0 | 0 |
| EDB | 1 | 0 | 0 |
| TET | 0 | 1 | 2 |
| AMC, TMP, TET | 0 | 2* | 0 |

KEYS: * = multidrug resistant, Em = erythromycin, TET = tetracycline, TMP = Trimethoprim, C = chloramphenicol, MUPH = high level mupirocin resistance, AMC = amikacin

DISCUSSION

In this study, *Mammaliicoccus sciuri* was found to be the most predominant mannitol-fermenting non-*S. aureus* specie identified, followed by *S. intermedius*, while *S. xylosus* was the least common. All three strains were detected in chickens, while two species (*S. sciuri* and *S. intermedius*) were detected in sheep and goats, as *S. xylosus* was not detected in either sheep or goats in this study. This study differs from that of Mamza *et al.* (2020) in northeastern Nigeria, who reported *S. sciuri* in sheep and goats with a lower prevalence of 3.6 and 7.2%, respectively. In other part of the world, both *S. sciuri* and *S. xylosus* have been reported from different food animals such as chickens (Nemeghaire *et al.*, 2014), sheep (Wesołowska *et al.*, 2023) and goats (Egyir *et al.*, 2022). *Staphylococci* species other than *S. aureus* (SOSA), such as *S. xylosus*, *S. intermedius*, *S. cohnii*, *S. epidermidis*, *S. hyicus*, *S. lentus*, *S. haemolyticus*, and *S. lugdunensis* are reported more in pigs in Nigeria than in small ruminants and chickens (Lawal *et al.*, 2021).

Although *M. sciuri* is a primary animal bacterial species, its clinical relevance is increasing due to its pathogenic potential, and it has been reported to be associated with fatal infections in animals (Sands *et al.*, 2022) and in humans (Jesumirhewe *et al.*, 2024). *S. intermedius* is a coagulase positive *Staphylococcus* frequently misidentified as *S. aureus* and has been isolated from cases of dog bites in humans (Wang *et al.*, 2013). All three staphylococcal species isolated in this study have been reported to be part of the normal floral of the skin and mucous membranes of different animal species, but with serious zoonotic potentials (Chen *et al.*, 2017). A recent study by Battalia *et al.* (2023) involving genomic analysis of *S. xylosus* identified a number of loci in the organism with similar homology to known virulence factors of *S.*

aureus, which shows that *S. xylosus* has more pathogenic potential than *M. sciuri*.

The AST results in this study showed complete sensitivity of all the isolates to ceftiofur, penicillin, gentamicin, kanamycin, rifampicin, spectinomycin, ciprofloxacin, vancomycin, teicoplanin, linezolid, and tigecycline. This finding is similar to that reported by Zhou *et al.* (2017), who reported high susceptibility of staphylococcal isolates from goats in China. The high susceptibility of isolates in this study could be attributed to the fact that the chickens, sheep and goats sampled in this study are home grown, owned by small holder farmers who may not have used antimicrobials, and thus, shows that the animals have not been exposed to ceftiofur, penicillin, gentamicin, kanamycin, rifampicin, spectinomycin, ciprofloxacin, vancomycin, teicoplanin, linezolid and tigecycline antibiotics. However, 48 % of the isolates were found to be resistant to trimethoprim, tetracycline resistance was also seen in 15% of the isolates, while erythromycin and amikacin resistance were observed in 9 % and 4% of the isolates, respectively. In this study, 10.63% of the isolates exhibited multidrug resistance; three of the multidrug-resistant isolates were from chickens, while 2 were from sheep. None of the isolates from goats were multidrug-resistant. Resistance to these agents could be attributed to misuse of the antimicrobial agents in the study area or due to environmental exposure (Panyako *et al.*, 2022). Clinical isolates of *M. sciuri* have been previously reported to be multidrug-resistant, carrying antimicrobial resistance genes against major classes of antibiotics such as oxazolidinones, phenicols, and aminoglycosides (Li *et al.*, 2016).

CONCLUSION

In conclusion, this study has shown that *M. sciuri*, *S. intermedius*, and *S. xylosus* are predominant mannitol-fermenters found in the

upper respiratory tracts of small ruminants and chickens. *Mammaliococcus sciuri* was found to be most common with 79 % prevalence, followed by *S. intermedius* with 15 % prevalence, while *S. xylosus* was the least common with 4.2 % prevalence.

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CONFLICT OF INTEREST

All authors hereby declare that they do not have any conflict of interest.

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