




# Isolation and Characterization of Coagulase Positive, Methicillin and Multi-Drug Resistant *Staphylococcus* and *Mammaliicoccus* Species from Wound of Patients Attending Federal Medical Centre, Yola, Adamawa State, Nigeria

Olumuyiwa Adeyemo<sup>1\*</sup> , Olufemi Okunye<sup>2</sup>, Francisca Nwaokorie<sup>3</sup> , Omri Kamet<sup>4</sup> 

1. Department of Biotechnology, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa State, Nigeria
2. Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria
3. Department of Medical Laboratory Science, College of Medicine, University of Lagos, Lagos State, Nigeria
4. Department of Microbiology, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa State, Nigeria

## ABSTRACT

**Background and Aim:** The treatment of bacterial infections especially of the family Staphylococcaceae is a major health burden that has led to economic losses, high morbidity, and mortality rates globally. This study aimed at the isolation and characterization of coagulase-positive, methicillin, and multi-drug-resistant Staphylococci isolated from wounds of patients.

**Materials and Methods:** Forty-five wound swabs were collected using sterile swab sticks. Isolation was done by streaking technique on mannitol salt agar. The isolates obtained were screened for methicillin and multi-drug resistance using the disk diffusion method. Biochemical and molecular characteristics using the 16S rDNA gene were used to identify the selected isolates.

**Results:** Four Staphylococci isolates resistant to methicillin (0.00 mm to 9.0 mm) were selected out of thirty-one. Reactions to catalase and coagulase productions were positive. Three out of the four isolates were identified using their 16S rDNA genes and they were found to be closely related to other family members of Staphylococcaceae at GenBank. The fourth isolate was identified using its biochemical characteristics as *Staphylococcus* spp. HFS4. The three identified isolates were *M. sciuri* HFS1 (ON340756), *M. sciuri* HFS3 (ON340770), and *S. haemolyticus* HFS2 (ON358435). The four isolates were resistant to more than five antibiotics that cut across all the classes of antibiotics. The multiple antimicrobial indices were between 0.5 and 0.8.

**Conclusion:** There is a need for regular antimicrobial resistance surveillance within the hospital environment, earlier detection and correct prescription of potent antimicrobials will check the spread of Staphylococcus infections and their virulent genes.

**Keywords:** Antimicrobial surveillance, Coagulase Positive Staphylococci, Methicillin and Multi-drug resistance, *Mammaliicoccus sciuri*, *Staphylococcus haemolyticus*

Received: 2023/01/15

Accepted: 2023/06/19;

Published Online: 2023/09/27

**Corresponding Information:** Olumuyiwa Adeyemo, Department of Biotechnology, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa State, Nigeria Email: [omadeyemo@mautech.edu.ng](mailto:omadeyemo@mautech.edu.ng)



Copyright © 2023, This is an original open-access article distributed under the terms of the Creative Commons Attribution-noncommercial 4.0 International License which permits copy and redistribution of the material just in noncommercial usage with proper citation.

Use a device to scan and read the article online



Adeyemo O, Okunye O, Nwaokorie F, Kamet O. Isolation and Characterization of Coagulase Positive, Methicillin and Multi-Drug Resistant *Staphylococcus* and *Mammaliicoccus* species Isolated from Wound of Patients Attending Federal Medical Centre, Yola, Adamawa State, Nigeria. Iran J Med Microbiol. 2023;17(4):414-22.

**Download citation:** [BibTeX](#) | [RIS](#) | [EndNote](#) | [Medlars](#) | [ProCite](#) | [Reference Manager](#) | [RefWorks](#)

**Send citation to:**  [Mendeley](#)  [Zotero](#)  [RefWorks](#)

## 1. Introduction

*Staphylococcus* is a prominent genus that has been implicated in opportunistic infections in both humans and animals (1). The genus is classified into two main groups, coagulase positive and negative *Staphylococcus*. Coagulase is an enzyme and one of

the basic virulent factors found in *Staphylococcus aureus* and other species of Staphylococci. The enzyme is responsible for plasma clotting and it is used to identify *S. aureus* (2). *S. aureus* can cause gastroenteritis due to the production of enterotoxins.

This bacterium is regarded as one of the most frequent food-borne pathogens (3). They have been recognized as pathogenic Gram-positive bacteria of public health concerns. The family is made up of ten genera. Recently, the genus *Mammaliococcus* was taken out of the family and assigned with five species previously under *Staphylococcus*. The type species of the newly created genus is *M. sciuri* (4). The reclassification of the five species was based on phylogenomic analyses due to the perception that the five were heterotypic synonyms. Hence, the five species were taxonomically reassigned to the new genus *Mammaliococcus* gen. nov (4) Members of the genus *Staphylococcus* and *Mammaliococcus* are normal microbial flora of the skin and mucosa parts of humans and animals as well as soil. Staphylococcal infections could affect everywhere including organs in the human body, leading to surface and deep abscesses, respiratory as well as urinary tract infections (5). Staphylococci and Mammaliococci species have been regarded as a group of bacteria with a tendency to cause debilitating infections when the opportunity arises (6). Most of these infections are associated with methicillin and multidrug resistance (7).

Antimicrobial resistance (AMR) has become a worldwide threat to human and animal health, with tedious and expensive treatment and possible mortality (3). Not long after the clinical application of methicillin as an antibiotic-resistant, it emerged, as a result of methicillin-resistant gene *mecA*, found on Staphylococcal cassette chromosome *mec* (SCC*mec*), a genetic mobile element (8, 9). The global occurrence of methicillin-resistant Staphylococci has become a threat to public health. This could be due to the ease with which genes for resistance are acquired among the family members of Staphylococcaceae. The formation of biofilm by the strains of *S. aureus* has been mentioned to be an important factor in the development of infections as well as resistance to methicillin by *S. aureus* (10) The interaction between animals, wild or domesticated, and man could be another factor for the spread of the resistance genes (11).

Methicillin-resistant *M. sciuri* has been reported in ruminants and New World camelids, harboring antimicrobial-resistant genes that are found in other closely related and unrelated species (12). *Mammaliococcus* species are thought to be involved in the transmission of resistant genes and serve as reservoirs for these genes found among the members of the family Staphylococcaceae, especially the well-known *S. aureus*. The abuse of antibiotics to treat microbial infections in human and animal has been mentioned to increase antibiotic resistance among the Staphylococci. Methicillin-resistant Staphylococci are

known to be resistant to virtually all classes of antibiotics. When a bacterium is resistant to at least one member of three or more classes of antibiotics, such a bacterium is said to be multi-drug resistant (2, 13, 14). The report of Bardasheva *et al.* (15) showed that coagulase-positive and negative *S. haemolyticus* and *S. epidermidis* were both methicillin and multidrug-resistant.

The success of antibiotic treatment of methicillin-resistant *Staphylococcus* is relatively dependent on promptness to avoid nosocomial infections. Strains of *S. haemolyticus* are known to cause infections in some hospital patients such as those on dialysis, diabetics, and post-surgery patients as well as the development of resistance to many antibiotics in bedridden individuals (16, 17). This study was carried out to isolate and characterize methicillin and multidrug-resistant Staphylococci isolated from wounds of patients with cases of wound infection at Federal Medical Center, Yola, Adamawa State, Nigeria, and the possible presence of some virulent indicators.

## 2. Materials and Methods

### Samples Collection and Isolation of *Staphylococci* Species

Forty-five clinical samples were collected from patients using sterile cotton swabs within Federal Medical Centre, Yola, Adamawa State, Nigeria. The samples were transported on ice packs to the General Laboratory, Department of Biotechnology, Modibbo Adama University, Yola, Adamawa State, Nigeria. Isolation was done by streaking on Mannitol Salt Agar. The plates were incubated at 37°C for 18 to 24 h aerobically. Individual colonies suspected to be Staphylococci were sub-cultured on freshly prepared Mannitol Salt Agar (MSA) and incubated at 37°C for 18 – 24 h. The pure cultures of the isolates were recovered and sub-cultured on nutrient agar slant and incubated at 37°C for 18 – 24 h. These pure culture slants were kept in the refrigerator (Haier Thermocool) at 4°C until they were needed for other analyses.

### Screening for Methicillin and Multi-Drug Resistant *Staphylococcal* Species

Suspected Staphylococci isolates were screened for methicillin resistance using the disk diffusion method as described by Hudzicki (18). Staphylococcal isolates that were positive for methicillin resistance were further screened for multi-drug resistance using a Gram-positive multi-drug antibiotic disc (Biomark Laboratories, India). Both methicillin and multi-drug resistant screening were carried out on Mueller Hinton Agar. Cefoxitin antibiotic disc (Rapid Lab, UK)

was used to screen for methicillin-resistant while antibiotic disc comprising Vancomycin (VAN) 30 µg, Ceftazidime (CPZ) 10 µg, Cephalexin (CP) 1.5 µg, Ampicillin (AMP) 10 µg, Meropenem (MEM) 10 µg, Erythromycin (ERY) 5 µg, Tetracycline (TET) 30 µg, Cotrimoxazole (COT) 25 µg, Cefuroxime (CRX) 10 µg, Gentamicin (GEN) 10 µg, Ciprofloxacin (CIP) 5 µg, and Augmentin (AUG) 30 µg were used for multi-drug antibiotic screening. The results were measured using a metric ruler and interpreted as either resistance (R), intermediate (I), or sensitive (S) using the CLSI standard (19).

#### Determination of Multiple Antibiotic Resistance Indices of the Four Staphylococci Isolates

The multiple antibiotic resistance index of each of the isolates *M. sciuri* HFS1, *S. haemolyticus* HFS2, *M. sciuri* HFS3, and *Staphylococcus spp.* HFS4 was determined by dividing the number of antibiotics each of the isolates was resistant to by the total number of antibiotics applied in the study as described by Krumperman (20).

#### Sugar Fermentation and Biochemical Tests

Biochemical characteristics and sugar fermentation using arabinose, xylose, inositol, mannitol, fructose, lactose, maltose, glucose, sucrose and galactose, catalase, urease, citrate, TSI, oxidase, and indole tests were carried out to have a presumptive identity of the isolates (21).

#### DNA Extraction

Deoxyribonucleic acid (DNA) of the four selected Staphylococci that were methicillin and multi-drug resistant were extracted using an overnight growth culture in nutrient broth. A DNA extraction kit (model 24700) manufactured by Norgen, Canada was used according to the instructions of the manufacturer.

#### Amplification and Partial Sequencing of 16S rDNA Gene

The 16S rDNA was amplified using the primers are ST16-F-5'-TTGCTTCTCTGATGTTAGCG-3', ST16-R-3'-AATCATTGTCCCACCTT-5' (22). The mix consisted of NEB one Taq master mix with standard buffer (catalog No MO4825) 10 µL, genomic DNA 1.0 µL, forward and reverse primers each 1.0 µL and nuclease-free water (catalog No E476) 7.0 µL were prepared to run the PCR. The initial denaturation was done at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1.0 min. The annealing took place at 50°C for 30 sec while the extension took place at 68°C for 1.0 min and the final extension at 68°C for 1 min.

The integrity of the PCR amplicon was visualized on a 1 % agarose gel (CCL-AG500, Cleaver Scientific Ltd).

A molecular ladder of 100 bp (NEB's fast DNA ladder) was added to serve as a standard. The bands were stained with EZ-Vision Blue light DNA Dye. The amplicon was purified by PCR clean-up and then sequenced in an automated DNA sequencer using the dideoxy chain termination method (23).

#### Construction of the phylogenetic tree

The sequences of the 16S rDNA gene of three out of four (one failed to amplify) selected Staphylococci were compared with similar sequences of Staphylococci strains deposited at GenBank using the Entrez search engine at the National Center for Biotechnology Information (NCBI). The similarity in sequences of the isolated Staphylococci and deposited Staphylococci strains at GenBank was done using the BLAST program. Additionally, multiple sequence alignment and phylogenetic tree construction were done with molecular evolutionary genetics analysis (MEGA X) (24).

The 16S rDNA gene sequences of three Staphylococci were deposited at GenBank with assigned accession numbers.

#### Amplification of Coagulase *coa*, Methicillin *mec A*, and Enterotoxigenic *SEA* Genes

Two genes, *coa*, and *SEA* were amplified using the respective primers *coa* F 5'-ATAGAGATGCTGGTACAGG-3', *coa* R 3'-GCTTCCGATTGTTTCGATGC-5' for coagulase gene, and SEA-A2-5'-ATTAACCGAAAGTTCTGTAGA-3', SEA-U2-3'-TTGCGTAAAAGTCTGAATT-5' for the enterotoxigenic gene in the different reaction mix. The amplicon of each gene was observed on agarose gel of 1.0 % with a molecular ladder of 100 bp (NEB's fast DNA ladder) (CCL-AG500, Cleaver Scientific Ltd) while the staining of the bands was done with EZ-Vision Blue light DNA Dye.

#### Statistical Analysis

Data collected in this study were analyzed using simple mean  $\pm$  standard deviation (Mean $\pm$ SD) and presented using bar charts.

### 3. Results

#### Isolation and Identification of the Staphylococci Species

Thirty-one suspected Staphylococci isolates were recovered from wound samples collected from patients attending Federal Medical Center, Yola, Adamawa State, Nigeria. They were mannitol fermenters based on their cultural characteristics on mannitol salt agar. Four of the isolates were coagulase-positive, resistant to methicillin, and resistant to not less than five antibiotics tested that cut across all the classes of antibiotics (Table 1).

**Table 1.** Screening for Multi-Drug Resistant Staphylococci isolates

Bacterial strain	ERY (15 µg)	TET (30 µg)	COT (25 µg)	CRX (10 µg)	GEN (10 µg)	CIP 5 µg	AMP 10 µg	MEM 10	AUG 30 µg	CP 1.5 µg	CPZ 10 µg	VAN 30 µg
1. <i>M. sciuri</i> HFS1	19.4±.4	18.9±0.6	16.7±0.4	R	16.9±0.4	21.9±0.6	R	R	R	8.1±0.3	R	13±0.8
2. <i>S. haemolyticus</i> HFS2	R	R	R	R	25.9±0.8	R	R	R	R	23.9±0.6	R	R
3. <i>M. sciuri</i> HFS3	8.9±0.6	R	R	R	7.1±0.8	7.0±0.8	9.9±0.4	R	R	R	R	15.9±0.8
4. <i>Staphylococcus</i> sp. HFS4	19.9±0.4	9.7±1.0	R	7.7±0.8	R	9.0±0.4	R	R	R	R	R	11.9±0.4

**Key:** R = Resistant, ERY = Erythromycin, TET = Tetracycline, COT = Cotrimoxazole, CRX = Cefuroxime, GEN = Gentamicin, CIP = Ciprofloxacin, AMP = Ampicillin, MEM = Meropenem, AUG = Augmentin, CP = Cephalexin, CPZ = Ceftazidime and VAN = Vancomycin, ≤ 9.0 mm = Resistant, 10.0-13.0 mm = Intermediate and ≥ 14.0 mm = Susceptible

The four presumptive Staphylococci isolates were identified using their biochemical and molecular characteristics (Table 2, Figure 1). Biochemically, the four isolates *M. sciuri* HFS1, *S. haemolyticus* HFS2, *M. sciuri* HFS3 and *Staphylococcus* spp. HFS4 exhibited characteristics of the Staphylococci with little differences among them. They all fermented most 6-C sugars and were unable to utilize the 5-C sugars such as xylose, inositol, and arabinose (Table 2). All four isolates HFS1, HFS2, HFS3, and HFS4 were unable to ferment sucrose, inositol, and xylose while only HFS2 and HFS4 utilized arabinose and lactose as their carbon and energy sources, respectively. However, all four isolates metabolized galactose, glucose, maltose, fructose, and mannitol as their carbon and energy sources (Table 2). Isolates HFS1, HFS2, HFS3, and HFS4 were positive for catalase, and coagulase and were able to utilize citrate. All the isolates except HFS4 were positive for TSI. However, none of the isolates metabolize urea. Likewise, they were all negative in reaction to oxidase and indole tests (Table 2).

All four isolates HFS1, HFS2, HFS3, and HFS4 were identified using the sequences of the 16S rDNA gene. But only three HFS1, HFS2, and HFS3 gave satisfactory amplification that enabled them to be identified using

the computer program Basic Local Alignment Search Tool (BLAST) at NCBI. Isolates HFS1 and HFS3 were identified to belong to the genus *Mammaliicoccus* and they were tagged *Mammaliicoccus sciuri* HFS1 (ON340756) and *M. sciuri* HFS3 (ON340770) while HFS2 was identified to be a strain of *Staphylococcus haemolyticus*, hence it was tagged *Staphylococcus haemolyticus* HFS2 (ON358435). All three sequenced isolates clustered closely with other species of Staphylococci (Figure 2).

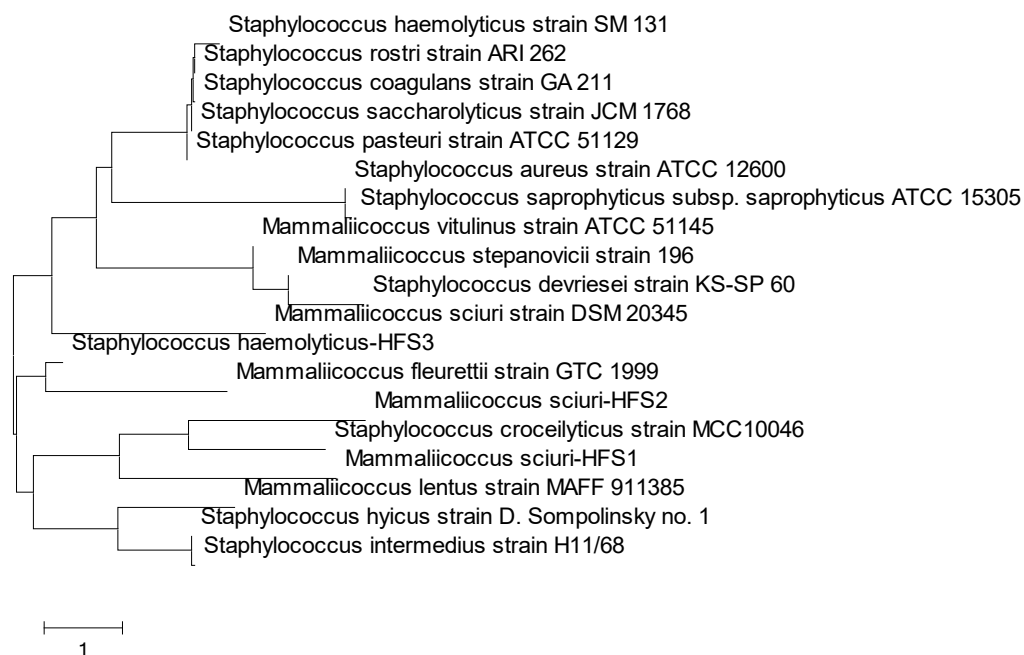
**Methicillin and Antimicrobial Resistance Screening**

The four methicillin-resistant isolates had zones of inhibition that ranged between 0.0 mm to 9.0 mm with cefoxitin and they were coagulase-positive strains, *S. haemolyticus* HFS2, *Staphylococcus* spp. HFS4, *M. sciuri* HFS1, and *M. sciuri* HFS3 screened for multidrug resistance using a multidisc antibiotic (Biomark Laboratories, India) that contained twelve different antibiotics that cut across the major classes of antibiotics such as cephalosporin, fluoroquinolone, carbapenems, glycopeptides, aminoglycosides, and penicillin.

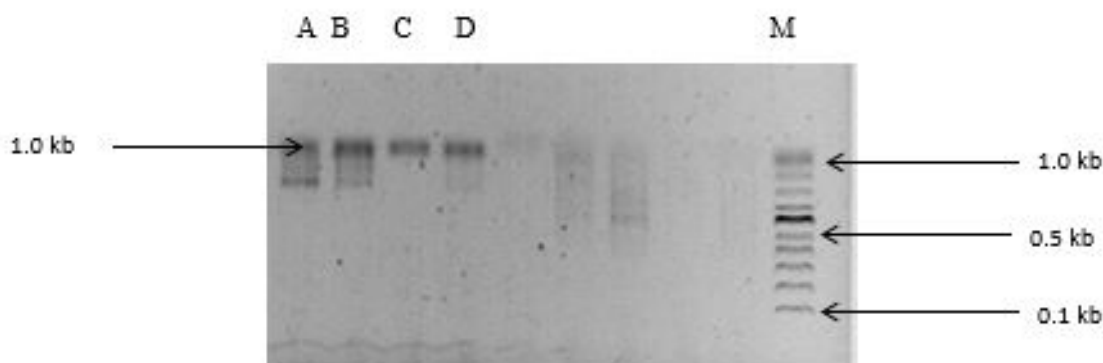
**Table 2.** Biochemical characteristics and sugar fermentation of the Staphylococci isolates

Bacterial strain	Gala	Sucro	Gluco	Malt	Lact	Fruct	Mannit	Inosit	Xylo	Arabino	Cata	Coagu	Citrat	Urea	TSI	Oxi	Indole
1. <i>M. sciuri</i> HFS1	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
2. <i>S. haemolyticus</i> HFS2	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
3. <i>M. sciuri</i> HFS3	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
4. <i>Staphylococcus</i> spp. HFS4	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve

Keys: Gala = Galactose, Sucro = Sucrose, Gluco = Glucose, Malt = Maltose, Lact = Lactose, Fructo = Fructose, Mannit = Mannitol, Inosit = Inositol, Xylo = Xylose, Arabino = Arabinose, Cata = Catalase, Coagu = Coagulase, Citrat = Citrate, TSI = Triple sugar iron and Oxi = Oxidase, +ve = Positive reaction and -ve = Negative reaction



**Figure 1.** Phylogenetic tree showing the taxonomic relationship of *Mammaliicoccus sciuri*-HFS1, *M. sciuri*-HFS2, and *Staphylococcus haemolyticus*-HFS3 with other *Staphylococci* isolates at GenBank



**Figure 2.** Agarose gel electrophoresis of PCR product of 16S rDNA gene amplicon of 1.0 kb of isolates A, B, C, and D. NEB fast ladder (M) 0.1 kb was the standard marker used

A = *M. sciuri* HFS1      B = *S. haemolyticus* HFS2      C = *M. sciuri* HFS3      D = *Staphylococcus* spp. HFS4

**Table 3.** Multi-drug resistance pattern of the four *Staphylococci* isolates

Bacterial strain	Resistance phenotype	Class of antibiotics
<i>M. sciuri</i> HFS1	CRX, AMP, MEM, AUG, CP, CPZ	Penicillins, Carbapenems, Cephalosporins,
<i>S. haemolyticus</i> HFS2	ERY, TET, COT, CRX, CIP, AMP, MEM, AUG, CPZ, VAN	Macrolides, Penicillins, Sulfonamides, Cephalosporins, Carbapenems, Glycopeptides, Fluoroquinolones
<i>M. sciuri</i> HFS3	ERY, TET, COT, CRX, GEN, CIP, MEM, AUG, CP, CPZ	Macrolides, Cephalosporins, Sulfonamides, Carbapenems, Aminoglycosides, Fluoroquinolones,
<i>Staphylococcus</i> spp. HFS4	COT, CRX, GEN, CIP, AMP, MEM, AUG, CP, CPZ	Sulfonamides, Cephalosporins, Penicillins, Fluoroquinolones, Carbapenems,

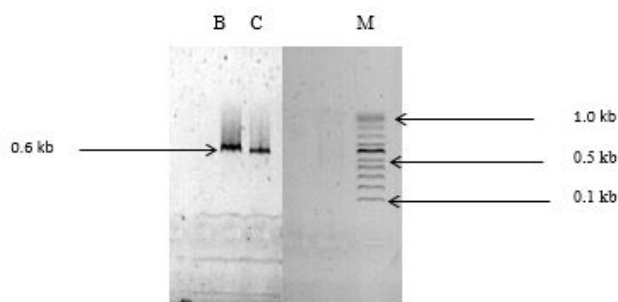
**Key:** ERY = Erythromycin, TET = Tetracycline, COT = Cotrimoxazole, CRX = Cefuroxime, GEN = Gentamicin, CIP = Ciprofloxacin, AMP = Ampicillin, MEM = Meropenem, AUG = Augmentin, CP = Cephalexin, CPZ = Ceftazidime and VAN = Vancomycin

**Table 4.** Multiple Antibiotics Resistance (MAR) Index of the four Staphylococci isolates

Bacterial strain	Number of antibiotics tested (b)	Number of antibiotics resistant to (a)	MAR index (a/b)
<i>M. sciuri</i> HFS1	12	6	0.50
<i>S. haemolyticus</i> HFS2	12	10	0.80
<i>M. sciuri</i> HFS3	12	10	0.80
<i>Staphylococcus</i> sp. HFS4	12	9	0.75

### Amplification of Coagulase *coa* and Enterotoxigenic *SEA* Genes

Amplification of the coagulase gene, *coa* was observed in *S. haemolyticus* HFS2 and *M. sciuri* HFS3, but not in the remaining two isolates, though all four strains had a positive reaction to the coagulase biochemical test (Figure 3, Table 2). None of the isolates studied had their enterotoxigenic gene *SEA* amplified after four attempts running the PCR, showing that they all lack this particular gene. The presence of *coa* gene has been a measure of virulence in *S. aureus*. The amplicon size of about 600 bp was generated for the *coa* gene in *S. haemolyticus* HFS2 and *M. sciuri* HFS3.



**Figure 3.** Agarose electrophoresis PCR product (cropped) for coagulase (*coa*) gene (600 bp) of *S. haemolyticus* HFS2 and *Mammaliicoccus sciuri* HFS3 and isolates A and D (not amplified, but biochemically positive). Lane M is NEB fast ladder

B = *S. haemolyticus* HFS2 C = *M. sciuri* HFS3

## 4. Discussion

All four isolates HFS1, HFS2, HFS3, and HFS4 were unable to ferment sucrose, inositol, and xylose while only HFS2 and HFS4 utilized arabinose and lactose as their carbon and energy sources respectively. However, they were able to metabolize galactose, glucose, maltose, fructose, and mannitol as their carbon and energy sources. The reported observations in this study in sugar fermentation, enzymes catalase and coagulase production as well as oxidase and indole reaction are similar to the earlier report by Gotz *et al.* (5). The utilization of any carbon source and production of specific enzymes are under the influence of genes controlling the reaction and

environmental factors. Similarly, all four strains were negative for indole and oxidase tests Gotz *et al.* (5). These two biochemical tests are used to presumptively biochemically identify Staphylococci isolates (25).

The amplicon of the 16S rDNA was found to be around 1.0 kb. The use of PCR amplification of 16S rDNA specific primers has been mentioned to identify Staphylococcal isolates up to the species and subspecies level (22), who reported an amplicon size of 1.4 kb for 16S rDNA gene of the Staphylococci isolated by them.

Methicillin and multi-drug resistant strains of Staphylococci are becoming a public health burden due to the tediousness and cost of treatment. They are responsible for nosocomial infections which have led to an increase in morbidity and mortality rates (2). Sands *et al.* (26) reported the isolation of methicillin-resistant strains of *S. epidermidis*, *S. haemolyticus*, and *M. sciuri* that are resistant to routinely prescribed antibiotics. The methicillin resistance could be due to the possession of different *mec* genes present in the staphylococcal cassette chromosome *mec* (SCC*mec*), a genetic mobile element (International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (9).

Another factor that has been mentioned to be responsible for the spread of methicillin and antimicrobial resistance genes is the ease with which the genes are acquired among the family members of Staphylococcaceae as well as wide distribution of species of *Staphylococcus* and *Mammaliicoccus* among human and animals (9). *M. sciuri* is a reservoir for the *mecA* gene though it may not be expressed in the strain when acquired and mutated within the promoter region, it could be expressed in *S. aureus* (5) Species of *Mammaliicoccus* have been implicated to carry genes that make them to be resistant to many antibiotics (6, 27). Bardasheva *et al.* (15) had previously mentioned the resistance of coagulase-positive *S. haemolyticus* and *S. epidermidis* to be methicillin and multidrug-resistant. High resistance of Staphylococcal species to ampicillin, tetracycline, ciprofloxacin, and erythromycin has been mentioned by Osada *et al.* (27), Marincola *et al.* (7), Sadiq *et al.* (28).

The multiple antimicrobial resistance indices for the four isolates studied ranged from 0.5 to 0.8. A similar report of high indices has been recently reported by Mir *et al.* (29), though with *Salmonella* sp., while Asante *et al.* (30) reported a high index of 0.8 with *Staphylococci* species associated with nosocomial infections. The high values of indices are indications of high-risk contamination which could be responsible for multi-drug resistant strains observed in this study. The values we report in this study are higher than 0.2, which is regarded as the standard (19). The observations we are reporting in this study are similar to a previous report by Gotz *et al.* (5).

#### Sugar Fermentation and Biochemical Characteristics of the *Staphylococci*

Biochemically, the four isolates *M. sciuri* HFS1, *S. haemolyticus* HFS2, *M. sciuri* HFS3 and *Staphylococcus* spp. HFS4 exhibited characteristics of the *Staphylococci* with little differences among them. They all fermented most 6-C sugars and were unable to utilize the 5-C sugars such as xylose, inositol arabinose, and mannitol (15). These observations are similar to the previous report by Gotz *et al.* (5). All four isolates were catalase and coagulase enzyme producers making them potential virulent and pathogenic strains (15, 31). Coagulase for instance is produced by *Staphylococci* to initiate blood plasma clotting in host plasma (31).

#### Amplification of Coagulase *coa* and Enterotoxigenic *SEA* Genes

Amplification of the coagulase gene, *coa* was seen in *S. haemolyticus* HFS2 and *M. sciuri* HFS3, but not in the remaining two isolates, though all four strains had a positive reaction to the coagulase biochemical test. The positive reaction to the coagulase enzyme test could be attributed to the presence of genes for the enzyme in these strains while the negatives recorded in the PCR amplification could be due to experimental error or nonbinding of the primers to the specific gene. None of the isolates studied had their enterotoxigenic gene *SEA* amplified after four attempts running the PCR, showing that they all lack this particular gene. The presence of *coa* gene has been a measure of virulence in *S. aureus*. In this study, an amplicon size of about 600 bp was generated for the *coa* gene in *S. haemolyticus* HFS2 and *M. sciuri* HFS3. Varied values of amplicon sizes that ranged between 681 bp to 891 bp have been earlier reported

by Sadiq *et al.* (28). A similar study carried out in India and the United Kingdom showed that the amplicon size of the *coa* gene could range between 510 bp and 1000 bp when same primers were used (31).

## 5. Conclusion

This study showed that *Staphylococci* species of *Staphylococcus* and *Mammaliococcus* genera are methicillin, multidrug-resistant, and coagulase as well as catalase positive. They are potential pathogens with multiple genes that have been reported to be resistant to most commonly available drugs in developing countries like Nigeria. All four isolates studied *M. sciuri* HFS1, *S. haemolyticus* HFS2, *M. sciuri* HFS3, and *Staphylococcus* spp. HFS4 were resistant to not less than five antibiotics that cut across most classes of antibiotics. The multiple antimicrobial indices ranged from 0.5 to 0.8. Isolation of these four strains from clinical samples is a great public health concern, as their presence indicates a high-risk area and may worsen antimicrobial resistance in immune-compromised patients. These may lead to an economic burden on treatment, high morbidity and mortality rates, and death. Therefore, there is a need for regular antimicrobial surveillance within the hospital, timely detection of methicillin and multi-drug resistant *Staphylococci*, and administration of a cocktail of more potent antibiotics in the treatment of wounds and other patients within the hospital environment.

## Acknowledgment

All the authors sincerely appreciate the support received from the management of Federal Medical Centre, Yola, (FMC, Yola) the staff of the Medical Microbiology Department, FMC, Yola, the technical staff of the Biotechnology Department, Modibbo Adama University, Yola.

## Funding

The authors jointly funded this research.

## Conflict of Interest

No conflict of interest was declared by the authors.

## Reference

- Bierowiec K, Korzeniowska-Kowal A, Wzorek A, Rypuła K, Gamian A. Prevalence of Staphylococcus Species Colonization in Healthy and Sick Cats. *Biomed Res Int.* 2019;2019:4360525. [DOI:10.1155/2019/4360525] [PMID] [PMCID]
- Lienen T, Schnitt A, Hammerl JA. *Mammaliococcus* spp. from German Dairy Farms Exhibit a Wide Range of Antimicrobial Resistance Genes and Non-Wildtype Phenotypes to Several Antibiotic Classes. *Biol.* 2022;11(2):152. [DOI:10.3390/biology11020152] [PMID] [PMCID]
- Shivaee A, Rajabi S, Farahani HE, Imani Fooladi AA. Effect of sub-lethal doses of nisin on *Staphylococcus aureus* toxin production and biofilm formation. *Toxicon.* 2021;197:1-5. [DOI:10.1016/j.toxicon.2021.03.018] [PMID]
- Madhaiyan M, Wirth JS, Saravanan VS. Phylogenomic analyses of the *Staphylococcaceae* family suggest the reclassification of five species within the genus *Staphylococcus* as heterotypic synonyms, the promotion of five subspecies to novel species, the taxonomic reassignment of five *Staphylococcus* species to *Mammaliococcus* gen. nov., and the formal assignment of *Nosocomiicoccus* to the family *Staphylococcaceae*. *Int J Syst Evol Microbiol.* 2020;70(11):5926-36. [DOI:10.1099/ijsem.0.004498] [PMID]
- Götz F, Bannerman T, Schleifer KH. The Genera *Staphylococcus* and *Macrococcus*. *Prokaryotes.* 2006:5-75. [DOI:10.1007/0-387-30744-3\_1] [PMCID]
- Nemeghaire S, Argudín MA, Feßler AT, Hauschild T, Schwarz S, Butaye P. The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. *Vet Microbiol.* 2014;171(3-4):342-56. [DOI:10.1016/j.vetmic.2014.02.005] [PMID]
- Marincola G, Liong O, Schoen C, Abouelfetouh A, Hamdy A, Wencker FDR, et al. Antimicrobial Resistance Profiles of Coagulase-Negative Staphylococci in Community-Based Healthy Individuals in Germany. *Front Public Health.* 2021; 9:684456. [DOI:10.3389/fpubh.2021.684456] [PMID] [PMCID]
- Barber M. Methicillin-resistant staphylococci. *J Clin Pathol.* 1961;14(4):385-93. [DOI:10.1136/jcp.14.4.385] [PMID] [PMCID]
- International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of Staphylococcal Cassette Chromosome mec (SCCmec): Guidelines for Reporting Novel SCCmec Elements. *Antimicrob Agents Chemother.* 2009;53(12):4961-7. [DOI:10.1128/AAC.00579-09] [PMID] [PMCID]
- Shivaee A, Sadeghi Kalani B, Talebi M, Darban-Sarokhalil D. Does biofilm formation have different pathways in *Staphylococcus aureus*?. *Iran J Basic Med Sci.* 2019;22(10):1147-52.
- Belhout C, Elgroud R, Butaye P. Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Other Methicillin-Resistant Staphylococci and *Mammaliococcus* (MRNaS) Associated with Animals and Food Products in Arab Countries: A Review. *Vet Sci.* 2022;9(7):317. [DOI:10.3390/vetsci9070317] [PMID] [PMCID]
- Schauer B, Szostak MP, Ehricht R, Monecke S, Feßler AT, Schwarz S, et al. Diversity of methicillin-resistant coagulase-negative *Staphylococcus* spp. and methicillin-resistant *Mammaliococcus* spp. isolated from ruminants and New World camelids. *Vet Microbiol.* 2021;254:109005. [DOI:10.1016/j.vetmic.2021.109005] [PMID]
- Sadigh-Eteghad S, Dehnad A, Shanebandi D, Khalili I, Razmarayii N, Namvaran A. Identification and characterization of a *Streptomyces* sp. isolate exhibiting activity against multidrug-resistant coagulase-negative Staphylococci. *Vet Res Commun.* 2011;35(8):477-86. [DOI:10.1007/s11259-011-9491-9] [PMID]
- Mapara N, Sharma M, Shriram V, Bharadwaj R, Mohite KC, Kumar V. Antimicrobial potentials of *Helicteres isora* silver nanoparticles against extensively drug-resistant (XDR) clinical isolates of *Pseudomonas aeruginosa*. *Appl Microbiol Biotechnol.* 2015;99(24):10655-67. [DOI:10.1007/s00253-015-6938-x] [PMID]
- Bardasheva A, Tikunov A, Kozlova Y, Zhirakovskaia E, Fedorets V, Fomenko N, et al. Antibiotic Resistance and Pathogenomics of Staphylococci Circulating in Novosibirsk, Russia. *Microorganisms.* 2021;9(12):2487. [PMID][PMCID] [DOI:10.3390/microorganisms9122487]
- McClure JA, Conly JM, Obasuyi O, Ward L, Ugarte-Torres A, Louie T, et al. A Novel Assay for Detection of Methicillin-Resistant *Staphylococcus aureus* Directly From Clinical Samples. *Front Microbiol.* 2020;11:1295. [DOI:10.3389/fmicb.2020.01295] [PMID] [PMCID]
- Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, et al. Whole-Genome Sequencing of *Staphylococcus haemolyticus* Uncovers the Extreme Plasticity of Its Genome and the Evolution of Human-Colonizing Staphylococcal Species. *J*



- Bacteriol. 2005;187(21):7292-308. [PMID] [PMCID] [DOI:10.1128/JB.187.21.7292-7308.2005]
18. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009;15:55-63.
  19. Wayne P. Clinical and Laboratory Standards Institute (CLSI) method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard-8th ed, CLSI document M07-A8, USA; 2009.
  20. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983;46(1):165-70. [PMID] [PMCID] [DOI:10.1128/aem.46.1.165-170.1983]
  21. Fawole M, Oso B. Characterization of bacteria: Laboratory manual of microbiology. Spectrum Book Ltd, Ibadan, Nigeria. 2004:24.
  22. Shanehbandi D, Baradaran B, Sadigh-Eteghad S. Occurrence of Methicillin Resistant and Enterotoxigenic *Staphylococcus aureus* in Traditional Cheeses in the North West of Iran. Int Sch Res. 2014;2014:129580. [DOI:10.1155/2014/129580] [PMID] [PMCID]
  23. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol. 1975; 94(3):441-8. [PMID] [DOI:10.1016/0022-2836(75)90213-2]
  24. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 2018;35(6): 1547-9. [DOI:10.1093/molbev/msy096] [PMID] [PMCID]
  25. Fernandes Queiroga Moraes G, Cordeiro LV, de Andrade Júnior FP. Main laboratory methods used for the isolation and identification of *Staphylococcus* spp. Rev Colomb Cienc Quim-Farm. 2021;50(1):5-28. [DOI:10.15446/rcciquifa.v50n1.95444]
  26. Sands K, Carvalho MJ, Spiller OB, Portal EAR, Thomson K, Watkins WJ, et al. Characterisation of *Staphylococci* species from neonatal blood cultures in low- and middle-income countries. BMC Infect Dis. 2022;22(1):593. [DOI:10.1186/s12879-022-07541-w] [PMID] [PMCID]
  27. Osada M, Aung MS. Prevalence and Antimicrobial Resistance of *Staphylococcus aureus* and Coagulase-Negative *Staphylococcus/Mammaliococcus* from Retail Ground Meat: Identification of Broad Genetic Diversity in Fosfomycin Resistance Gene fosB. Pathogens. 2022;11(4):496. [PMID] [PMCID] [DOI:10.3390/pathogens11040469]
  28. Sadiq A, Samad M, Saddam, Basharat N, Ali S, Roothullah, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Slaughter Houses and Meat Shops in Capital Territory of Pakistan During 2018-2019. Front Microbiol. 2020;11: 577707. [DOI:10.3389/fmicb.2020.577707] [PMID] [PMCID]
  29. Mir R, Salari S. Determination of frequency, multiple antibiotic resistance index and resistotype of *Salmonella* spp. in chicken meat collected from southeast of Iran. Vet Med Sci. 2022;8(1):229-36. [DOI:10.1002/vms3.647] [PMID] [PMCID]
  30. Asante J, Hetsa BA, Amoako DG. Multidrug-Resistant Coagulase-Negative *Staphylococci* Isolated from Bloodstream in the uMgungundlovu District of KwaZulu-Natal Province in South Africa: Emerging Pathogens. Antibiotics. 2021;10(2):198. [DOI:10.3390/antibiotics10020198] [PMID] [PMCID]
  31. Javid F, Taku A, Bhat MA, Badroo GA, Mudasir M, Sofi TA. Molecular typing of *Staphylococcus aureus* based on coagulase gene. Vet World. 2018;11(4): 423-30. [DOI:10.14202/vetworld.2018.423-430] [PMID] [PMCID]