Original Research Article

Genetic diversity of the methicillin-resistant *Staphylococcus aureus* isolates circulating in Fako Division, South West Region, Cameroon.

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of hospital- and community-associated infections impacting national and global health. In recent years, MRSA has been detected in livestock species and their products. WHO has MRSA among the top 24 priority pathogens in 2024, posing the greatest threats to human health.

Aim: To characterise the SCCmec elements and investigate the genetic diversity of MRSA strains recovered from different sources in Fako.

Study Design: A laboratory-based cross-sectional study.

Place and Duration: Department of Microbiology and Parasitology, Faculty of Science, University of Buea, and Laboratory for Emerging Infectious Diseases, University of Buea, between June 2022 and September 2024.

Methodology: The study characterised 76 MRSA strains from diverse sources in Fako to identify the SCCmec types by conventional and multiplex PCR assay. Additionally, the *mecA* gene amplicons of some strains was sequenced. The sequences were analysed and uploaded to the NCBI-Genbank.

Results: SCCmec type IV was most prevalent (57.9%), followed by type V (21.1%). All the sequences revealed high sequence identity when compared to those available on NCBI using the BLASTN. The sequences showed significant genetic similarity, ranging from 99.1% to 100% for the nucleotides and 98.6% to 100% for the amino acids. Compared with reference sequences retrieved from GenBank, similarity ranged from 98.2% to 100% and 97.9% to 100%, respectively.

Conclusion: Detecting diverse SCCmec types in the MRSA strains denotes the complexity of MRSA epidemiology in Fako. Our findings indicate a risk for the transmission of MRSA across different sources, thus highlighting the need for a One Health approach to MRSA prevention and control. GenBank assigned the accession numbers OP042343 to OP042352 for HA-MRSA, OP042356 to OP042361, OP042363, and OP716861 to OP716868 for LA-MRSA, and OP716859 to OP716860 for CA-MRSA were assigned.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), SCCmec (staphylococcal cassette chromosome *mec*), CA (Community-associated), LA (Livestock-associated), HA (Hospital-associated),

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* causes significant health burden worldwide. While people who carry the pathogen may not have visible symptoms, it can lead to health issues like skin and soft tissue infections, pneumonia, bacteremia, surgical site infections and sepsis, especially for individuals with comorbidities [1]. MRSA resistance to methicillin and most β-lactam antibiotics is mediated by a *mecA* gene, which encodes a penicillin-binding protein, PBP2a. The *mecA* gene is carried on a mobile genetic element, the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) [2, 3], defined as DNA fragments encoding a set of virulence and resistance factors that initiate their transition and incorporation into new host DNA [4, 5, 6]. The emergence of methicillin-resistant strains of staphylococci is due to the acquisition and insertion of the SCCmec into the chromosomes of susceptible strains [7]. Fourteen major variants of SCC*mec*, type I to XIV, have been distinguished according to the International Working Group on the Classification of SCCmec elements, IWC-SCC [8].

The fourteen SCCmec types are characterised by the arrangements of the *mec* gene complex and cassette chromosome recombinase (*ccr*) gene complex [8]. Five classes of *mec* gene complex (A - E) have been identified with many subclasses in various MRSA isolates [2]. The *ccr* gene complex encodes DNA recombinase enzymes that catalyse the mobility of the SCCmec by excision or insertion into several integration sites. Three *ccr* genes have been reported (*ccrA*, *ccrB*, and *ccrC*), with multiple allotypes for each gene forming eight complexes [8, 9]. Combining five *mec* genes and eight *ccr* gene complexes is essential in SCCmec nomenclature and the assignment of new types [3].

The characterisation of SCCmec element types by multiplex PCR (M-PCR) technique developed by Zhang *et al.* (2005) guaranteed the identification of variant *mec* elements; unfortunately, it can only detect SCCmec elements I – V [10]. Both Okuma and colleagues in 2002 and Youssef and colleagues in 2022 have used a traditional method to type *mec* and *ccr* in separate PCRs for the unknown SCCmec types (VI to XIV), which has proven to be an effective method [8, 11].

Understanding the different types of MRSA strains is crucial for effective surveillance and control measures. This includes identifying the bacteria's genetic makeup, drug resistance, and transmission patterns. This knowledge can help trace the spread of MRSA across different environments and locations and evaluate the effectiveness of prevention strategies. It can also aid in understanding the evolution of the bacteria and the origin of infections.

MRSA is associated with clonal spread and diversity, and different typing methods have been used for epidemiological surveillance [12, 13], ranging from PCR-based typing systems [7, 14]; to, PCR-based methods [12, 13], to sequence-based methods, phage typing, and DNA microarrays [12 - 13, 15, 16 - 17]. Different studies have used various methods to investigate the genetic diversity of MRSA. Methods such as Multilocus sequence typing (MLST), Pulsed-field gel electrophoresis (PFGE), SCCmec typing, whole-genome sequencing, and accessory gene regulator typing have been used in studies from Japan [18], Malaysia [19 - 20], Iran [9, 15, 21], and Cameroon [14, 22] to identify different strains of MRSA and report their prevalence and diversity.

In developed countries, the clonal relatedness of MRSA isolates has been widely analysed, compared to scanty information from developing countries [23 - 24]. In Africa, the epidemiology of MRSA has dramatically increased and has been documented in the past few years [8, 25]. In Cameroon, few studies have been published on MRSA genetic diversity [14, 22, 24]. This study investigated the genetic diversity of MRSA strains from hospitals, livestock, and communities in Fako Division, South West Region Cameroon, by SCCmec typing and sequencing the *mecA* gene.

2. MATERIAL AND METHODS

2.1 Description of study area

The study was conducted in the Fako division of the South West Region (SWR) of Cameroon, having many social and economic amenities and educational institutions. Fako Division has six health districts: Buea, Limbe (I, II, and III), Tiko and Muyuka health districts [26]. Samples were collected from four health districts Buea and Limbe (I, II, and III) considered to be the largest and most residential during the socio-political crisis period which also involved the study period. Buea, the capital of the SWR of Cameroon, is located at 4°09'97.20" N and 9°14'27.60" E. Buea is situated on the eastern slope of Mount Cameroon, an active volcano standing about 4010 meters high, and spans an area of 870 km² [27]. As a historic and cosmopolitan town, Buea had an estimated population of around 300,000 in 2013, comprising diverse demographics [28]. The town experiences two seasons (dry and rainy) with an equatorial climate and annual rainfall ranging from 3000 mm to 5000 mm. Despite the high rainfall, Buea faces significant water shortages, especially during the dry season, partly due to its rapidly growing population as at least 7,000 people relocate to Buea each year, according to the municipal council statistics [27]. Additionally, the town's drainage and waste management systems remain underdeveloped [29]. Limbe, formerly known as Victoria, is a seaside city in the SWR of Cameroon, founded in 1858 by British missionary Alfred Saker. Limbe is located on

the eastern slope of Mount Cameroon at 4°02'41.00" N, and 9°21'48.00" E, it had an estimated population of 120,000 in 2010, which grew to 130,000 by 2013 [30]. The city covers an area of 596 km² with a population density of 218 persons per km² [31]. Limbe is a popular international tourist destination, known for its beautiful beaches, historical monuments, botanic garden, and wildlife center [31]. The city experiences an equatorial climate with high rainfall, an average annual temperature of 26°C, and humidity above 82.5%. Despite its high rainfall, the population density is uneven, particularly intense in urban areas, due to the demand for essential services like water, electricity, and housing [31].

2.2 Study design

This study was a laboratory-based cross-sectional study of MRSA strains recovered from varied samples and locations in the Fako Division. The samples included swabs from multiple sources: for HA, wound and nasopharyngeal swabs, and urine samples of admitted patients; for LA, meat, meat-handlers hands, working surfaces and butchery equipment swabs of meat retail shops and abattoirs; and ready-to-eat food samples. More details of the MRSA strains involved in this study have been previously described in various articles [32-35].

2.3 Collection of study samples

Prior to sample collection, normal saline was prepared, divided into 1.5ml Eppendorf tubes, and sterilised. The sterile normal saline tubes and the necessary swab tubes were placed in a cool box at 4°C, along with informed consent sheets, and taken to the field. Pre-moistened cotton swabs [36] were used to collect swab samples. Meat and ready-to-eat food samples were placed in sterile zip-lock bags. Each sample was assigned a unique code, and consent sheets were recorded for every sample. The samples were transported aseptically in a cool box containing ice packs with temperatures maintained at +2 to +6°C to the Laboratory of Emerging Infectious Diseases (LEID) at the University of Buea within one hour after collection. The samples were cultured and analysed to obtain MRSA isolates using previously described methods by Esemu et al [33]. Sample collection took place between 8:00 am and noon on designated days. Each location was visited on different days throughout the week for sample collection.

2.4 Bacterial isolates

A total of 76 MRSA isolates comprising 34 HA-MRSA recovered from hospital samples [32], 27 LA-MRSA recovered from meat retail shops [33], 11 LA-MRSA from the abattoir wastes [34] and four CA-MRSA from ready-to-eat-foods [35] were investigated in this study.

2.5 Amplification of *mecA* gene.

Genomic DNA was extracted from each isolate using the simple boiling method, followed by PCR for the amplification of the *mecA* gene using previously described methods by Esemu et al. [33]; oligonucleotide primers synthesised by Inqaba Biotech (Inqaba Biotechnical Industry, (Pty) Ltd., South Africa) (Table 1) were used. PCR was performed in Multigene Optimax thermocycler (Labnet International, Inc. USA) with 25 µL PCR mix (2x BioMix™ Red, BIOLINE) containing 5 µL of *S. aureus* DNA. The PCR products were separated electrophoretically in 1.5% agarose gel, visualised under ultraviolet light, and photographed in a Molecular Imager Gel Doc XR system (BIO-RAD, Hercules, CA, USA) followed by the identification of the target size (Table 1).

2.6 Sequencing and sequence analysis

Twenty-seven representative *mecA* PCR products were packaged and sent for purification and sequencing at Inqaba Biotech (Inqaba Biotechnical Industry, (Pty) Ltd., South Africa). For maximum data accuracy, the Sanger sequencing was done for both the forward and reversed strands.

Sequence quality assessment and similarity matches were conducted against sequences deposited in the GenBank using the nucleotide Basic Local Alignment Search Tool (BLASTN) search tool (http://www.ncbi.nlm.nih.gov/BLAST) of the NCBI database. All sequences were submitted to GenBank.

The *mecA* sequences of this study were queried through a blast search and alignment for similarity among the local strains. Query sequences were evaluated and compared with the GenBank database sequences.

The partial nucleotide and amino acid sequences in FASTA format were imported into BioEdit version 7.2.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), and multiple sequence alignments were done using the ClustalW algorithm and per cent identities determined by sequence identity matrix [1]. These sequences were comparatively analysed and aligned with seven reference sequences from different nations retrieved from GenBank.

2.7 PCR assay for typing of SCCmec elements.

SCCmec typing was performed for all the MRSA isolates using multiplex PCR (for detection of types I to V) following previously described conditions reported by Zhang and colleagues [10]. The conventional PCR method for the *ccr* and *mec* gene complexes (for detection of types VI to XIV) following previously described conditions reported by Youssef and colleagues [8]. The PCR assays used sixteen pairs of primers (Table 1) obtained from Inqaba Biotech specific for SCCmec typing [3, 8, 10]. The PCR products were separated using electrophoresis on a 1.5% agarose gel for gene sizes less than 1000 bp and on 1

% agarose gel for gene sizes greater than 1000 bp and then visualised and captured through photography under ultraviolet light in a Molecular Imager Gel Doc XR system followed by the identification of the different target sizes (Table 1).

Table 1: Sequences of PCR primers used in this study

Target genes	Primer	Nucleotide sequence (5′→3′)	Amplicon size (bp)	Reference
MRSA identification	*MecA* F *MecA* R	aaaatcgatggtaaaggttggc agttctgcagtaccggatttgc	533	[37]
SCCmec I	Type I F Type I R	gctttaaagagtgtcgttacagg gttctctcatagtatgacgtcc	613	[10]
SCCmec II	Type II F Type II R	cgttgaagatgatgaagcg cgaaatcaatggttaatg gacc	398	[10]
SCCmec III	Type III F Type III R	ccatattgtgtacgatgcg ccttagttgtcgtaacagatcg	280	[10]
SCCmec IVa	Type IVa F Type IVa R	gccttattcgaagaaaccg ctactcttctgaaaagcgtcg	776	[10]
SCCmec IVb	Type IVb F Type IVb R	tctggaattacttcagctgc aaacaatattgctctccctc	493	[10]
SCCmec IVc	Type IVc F Type IVc R	acaatatttgtattatcggagagc ttggtatgaggtattgctgg	200	[10]
SCCmec type IVd	Type IVd F Type IVd R	ctcaaaatacggaccccaataca tgctccagtaattgctaaag	881	[10]
SCCmec type V	Type V F Type V R	gaacattgttacttaaatgagcg tgaaagttgtacccttgacacc	325	[10]
Class A *mec* (MecI-*mec*R1)	mI6mA7b	cataacttcccattctgcagatgatatacca aacccgacaactaca	1965 (type II) 1797 (type III)	[38]
Class B *mec* (IS1272)	IS5 mA6	aacgccactcataacatatggaa tataccaaacccgacaac	1996	[11]
Class C *mec* (IS431-*mec*A)	IS2 mA2	tgaggttattcagatattt cgatgt aacgttgtaaccaccc caaga	2072	[39]
*ccr*1 (*ccr*A1)	*α 1*	aacctatatcatcaatcagtacgt	695	[40]

	Bc	attgccttgataatagccitct		
*ccr*2 (*ccr*A2)	*α 2* *Bc*	taaaggcatcaatgcacaaacact attgccttgataatagccitct	937	[8]
ccr3 (ccrA3)	α 3 Bc	agctcaaaagcaagcaatagaat attgccttgataatagccttct	1791	[41]
ccr4 (ccrA4)	A4.2 B4.2	gtatcaatgcaccagaactt ttgcgactctcttggcgttt	1287	[42]
ccr5 (ccrA5)	ϒ-F ϒ- R	cgtctattacaagatgttaaggataat cctttatagactggattattcaaaatat	518	[8]

2.8 Data analysis

All research findings were transferred and preserved in Microsoft Excel spreadsheets (Microsoft Corporation, Redmond, WA, USA). The data were exported into a Statistical Package for Social Science (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA), and descriptive statistics were used to summarise the prevalence of SCCmec types.

3. RESULTS

3.1 Amplification of *mecA* gene and submission to GenBank

The MRSA strains were amplified for the *mecA* gene, and *mecA* PCR products of 27 MRSA isolates comprising ten clinical (HA-MRSA), 15 livestock (LA-MRSA), and two RTE (CA-MRSA) were sequenced. The sequences were deposited into the GenBank and accession numbers OP042343 to OP042352 for HA-MRSA, OP042356 to OP042361, OP042363, and OP716861 to OP716868 for LA-MRSA (OP716861 – OP716862, OP042356, OP042360 – OP042361 for sequences from abattoir wastes and OP716863 – OP716868, OP042357 – OP042359 and OP042363 for sequences from meat-retail shops strains), and OP716859 to OP716860 for CA-MRSA were assigned.

3.2 Sequence analysis of *mecA* gene.

The BLASTN search tool of the NCBI database revealed high sequence quality and similarity (query coverage of between 99.5 - 100%, e-value of 0.0, and percentage identity of 99.9 - 100%) between the sequences in this study and the sequences deposited in the GenBank.

The multiple sequence alignments of the *mecA* sequences showed 1 or 2 substitutions in some nucleotide sequences: for CA-MRSA (CA-CMR) sequences at positions 55 and 385 (for OP716859) and positions 85 and 436 (for OP716860; for LA-MRSA (LA-CMR) sequences at position 4 (for OP042358),

positions 43 and 190 for OP716861, 238 for OP716862, 310 and 349 for OP716863, 151 and 289 for OP716865, 43 and 400 for OP716866, 298 for OP716867 and 97 and 343 for OP716868 (Figure 1).

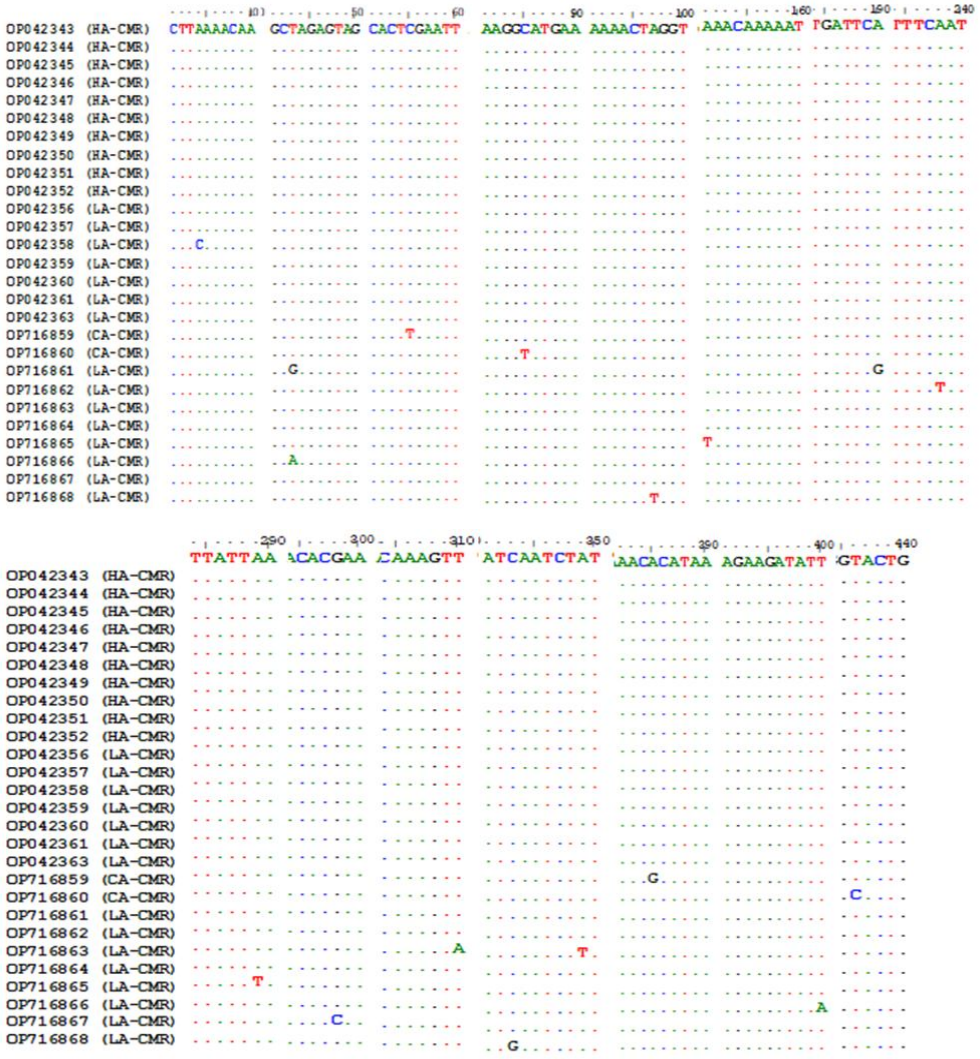

Figure 1: ClustalW alignment of the *mecA* gene nucleotide sequence of the MRSA strains in Fako.

The figure above shows the variable regions of the nucleotide sequences. Dots represent identical nucleotides. The labels in the bracket indicate the strain source (HA = Hospital-associated, CA = Community-associated, LA = Livestock-associated, CMR = Cameroon).

Alignment of the amino acid sequences revealed one amino acid substitution in some sequences due to nucleotide substitutions at the level of the nucleotide sequences. There was an amino acid substitution in some LA-CMR sequences, at position 1 for OP042358 as a result of nucleotide substitution at the nucleotide level at position 4, at position 96 for OP716865 and position 114 for OP716868 due to nucleotide substitution at the nucleotide level at positions 289 and 343 respectively. The nucleotide substitutions at the nucleotide level of CA-CMR sequences at positions 55 and 385 (for OP716859), and

positions 85 and 436 (for OP716860) were redundant. Similarly for some LA-CMR sequences, nucleotide substitutions at positions 43 and 190 for OP716861, 238 for OP716862, 310 and 349 for OP716863, 151 for OP716865, 43 and 400 for OP716866, 298 for OP716867 and 97 for OP716868 were all redundant thus led to no change in their respective coded amino acid (Figure 2).

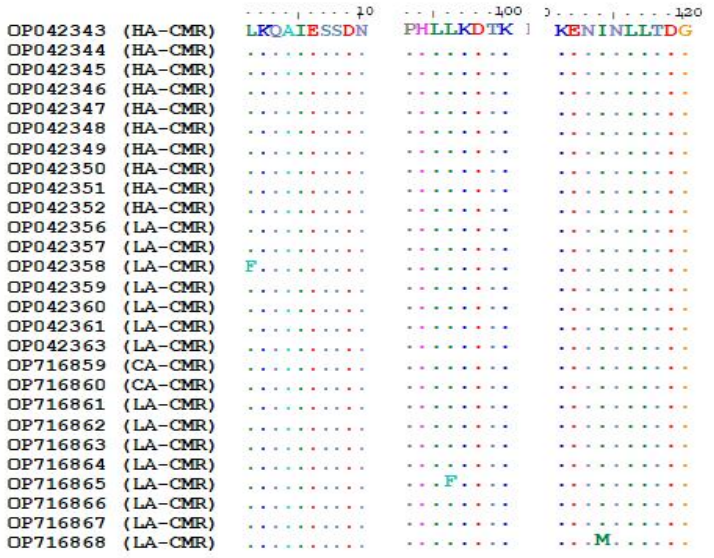

Figure 2: ClustalW alignment of the mecA gene amino acid sequences of the MRSA strains in Fako.

The figure above shows the variable regions of the amino acid sequences. Dots represent identical amino acids. The labels in the bracket are strain source (HA = Hospital-associated, CA = Community-associated, LA = Livestock-associated, CMR = Cameroon).

The *mecA* sequence identity analysis revealed between 99.1 to 100% similarity at the nucleotide level. All HA-MRSA (HA-CMR) sequences (OP042343 to OP042352) and seven LA-CMR sequences (OP42356 to OP042357, OP042360 to OP042363, and OP716864) showed 100% homology. The sequence identity analysis at the amino acid level showed a homology ranging from 98.6% to 100% among the sequences. All HA-CMR amino acid sequences and 14 LA-CMR (OP42356 to OP042357, OP042360 to OP042363, and OP716859 to OP71863, OP716866 to OP716867) showed 100% homology with each other.

Sequence analysis of the *mecA* gene compared with reference sequences from GenBank.

The *mecA* nucleotide and amino acid sequences were compared with those of the seven *mecA* GenBank reference sequences (MK659557 from Nigeria, AB221120 from Japan, JF778650 from India, MW052033 from Iran, and NG_047937 from the USA which were hospital-associated, NG_047939 from the Australian capital city Perth was community-associated and OK040767 from Nigeria was livestock-

associated). The reference sequences differed from the sequences in Fako by a few nucleotide substitutions at different positions (Figure 3). All HA-CMR sequences and seven LA-CMR sequences (OP42356 to OP042357, OP042360 to OP042363, and OP716864) showed 100% homology with the reference sequences of the HA-USA, HA-Japan, CA-Perth and HA-Iran sequences at the nucleotide level. These sequences were different from the other two reference sequences by four nucleotide substitutions:- at positions 22, 49, 199, and 310 for the LA-Nigeria and positions 431, 436, 439, and 447 for the HA-Indian and HA-Nigeria strains (Figure 3).

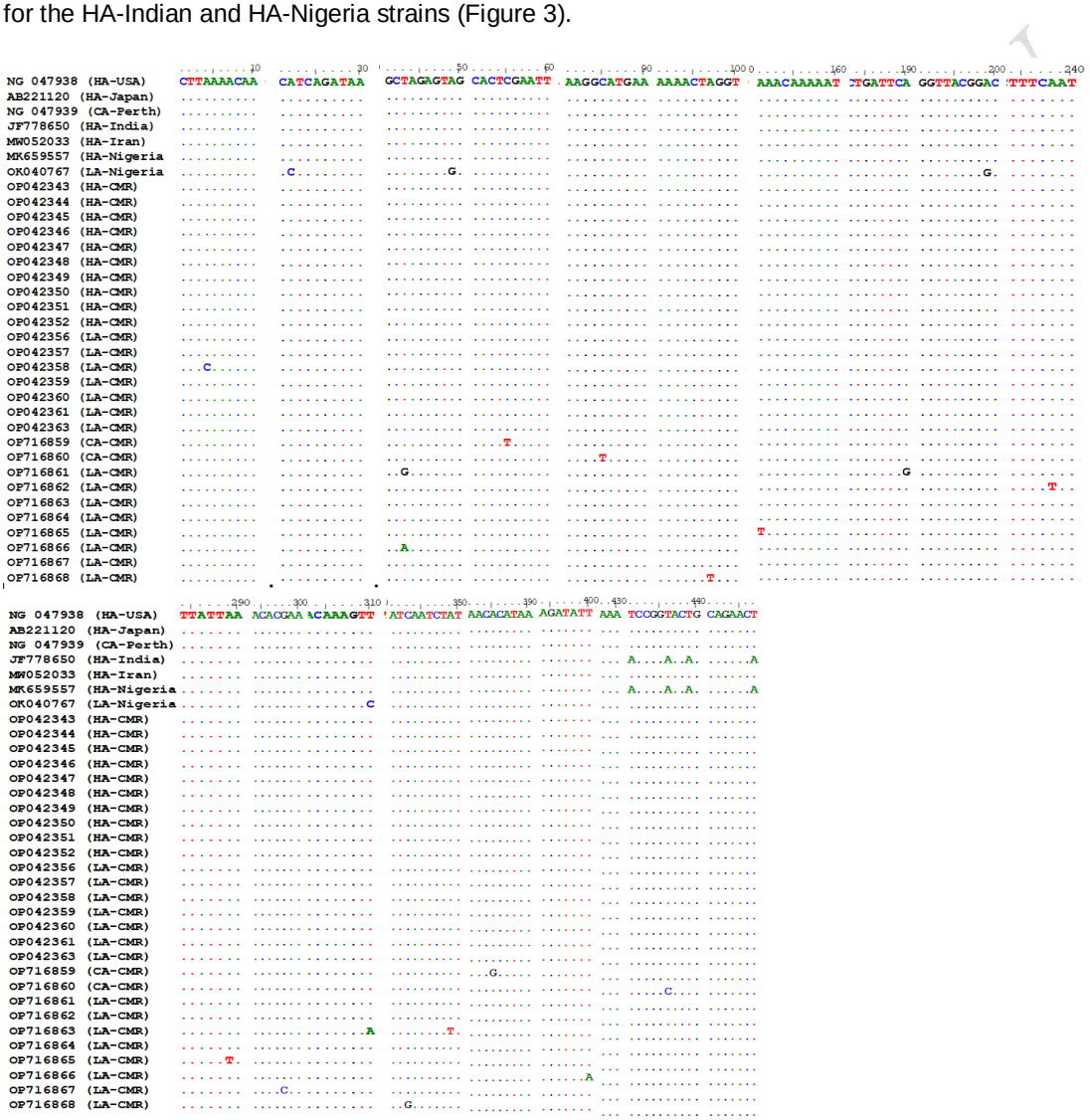

Figure 3: ClustalW alignment of mecA gene nucleotides sequences of the MRSA strains compared with GenBank reference sequences.

The figure above shows the variable regions of the nucleotide sequences. Dots represent identical nucleotides. The labels in the bracket indicate the strain source (HA = Hospital-associated, CA = Community-associated, LA = Livestock-associated, CMR = Cameroon).

The alignment of the amino acid sequences of the *mecA* strains in Fako compared with those of the seven *mecA* GenBank reference showed very few amino acid substitutions. For HA-India and HA-Nigeria strain with amino acid substitution at positions 144 and 149 due to the nucleotides substitution at the nucleotides level in positions 431, 436, 439 and 447 (Figure 4). The LA-Nigerian strain amino acid sequence was 100% similar to all of the HA-CMR strains in Fako regardless of its nucleotide substitutions at positions 22,49, 199, and 310. This implies that the nucleotide substitutions in the Nigerian livestock strain were redundant and did not affect its amino acid sequences.

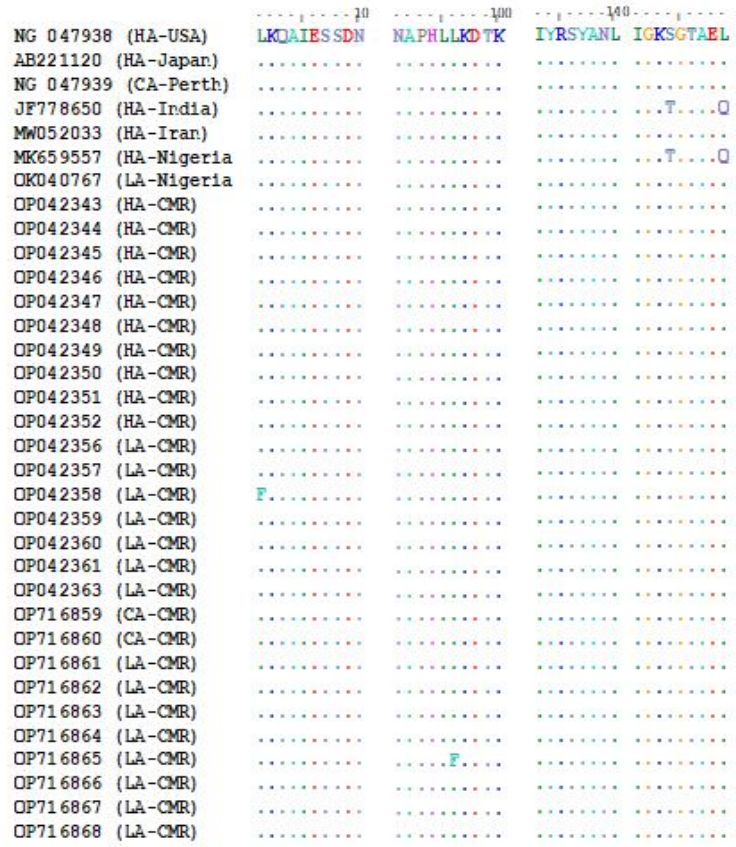

Figure 4: ClustalW alignment of the mecA gene amino acid sequences of MRSA strains compared with GenBank reference sequences.

The figure above shows the variable regions of the amino acid sequences. Dots represent identical amino acids. The labels in the bracket are strain source (HA = Hospital-associated, CA = Community-associated, LA = Livestock-associated, CMR = Cameroon).

The *mecA* sequence identity analysis of the *mecA* nucleotide sequences compared to the reference sequences revealed 98.2 to 100% similarity. When analysing the sequence identity at the amino acid level, it was found that the partial coding regions of the amino acid sequences of *mecA* strains in Fako

showed a homology ranging from 97.9% to 100% compared to seven reference amino acid sequences of mecA.

SCCmec typing of MRSA strains in Fako Division.

Amongst the 76 MRSA strains detected in Fako, five SCCmec types (types II, III, IVa, IVc, and V were identified (Figure 5).

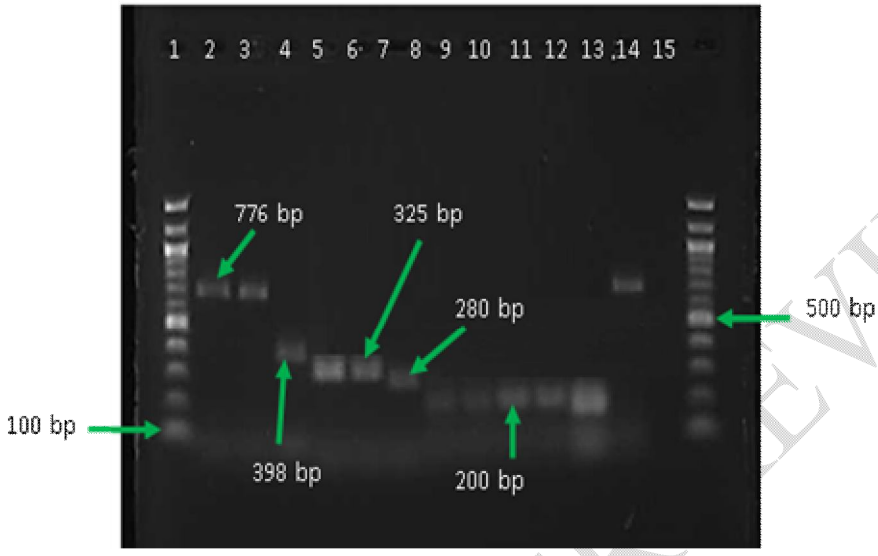

Figure 5. Visualisation of amplified PCR products of the SCCmec types on agarose gel.

Electrophoretic separation was done at 90 V for 1 hour. lane 1 and 15 (100 bp molecular weight marker), negative control (lane 14), SCCmec type IVa (lanes 2, 3 and 13), type II (lane 4), type V (lanes 5 and 6), type III (lane 7), and type IVc (lanes 8-12).

SCCmec type IV was highly prevalent, with 57.9%(44/76) prevalence comprising 30(39.5%) for sub-type IVa and 14(18.4%) for sub-type IVc. The second most prevalent type was SCCmec type V, with 21.1%(16/76) prevalence, followed by SCCmec type III and II, which had the same prevalence of 8(10.5%) each (Table 2). This study did not record the SCCmec types I, IVb, IVd, VI to XIV. The SCCmec types II and III noted to be specific for HA-MRSA were found in the MRSA from meat-retail shops at a prevalence of 3.7% (1/27) and 22.2%(6/27), respectively. The SCCmec types IV and V, noted to be specific for CA-MRSA, were found in the MRSA from hospital samples at a prevalence of 52.9%(18/34) and 20.6%(7/34), respectively. The MRSA strains from abattoir waste reported SCCmec types IV and V only, with a prevalence of 63.6%(7/11) and 36.4%(4/11), respectively. The MRSA strains from RTE reported SCCmec types IV and V only with a prevalence of 50.0% (2/4) each respectively.

Table 2: Staphylococcal chromosomal cassettes mec (SCCmec) types of MRSA strains circulating in Fako.

SCCmec types	HA-MRSA	LA-MRSA		CA-MRSA	Total N=76 (%)
	Clinical samples N=34 (%) [26]	Meat retail shop N=27 (%) [27]	Abattoir waste N=11 (%) [28]	Ready-to-eat food N=4 (%) [29]	
II	7(20.6)	1(3.7)	0(0.0)	0(0.0)	**8(10.5)**
III	2(5.9)	6(22.2)	0(0.0)	0(0.0)	**8(10.5)**
IV a	12(35.3)	12(44.4)	4(36.4)	2(50.0)	**30(39.5)**
IV c	6(17.6)	5(18.5)	3(27.2)	0(0.0)	**14(18.4)**
V	7(20.6)	3(11.1)	4(36.)	2(50.0)	**16(21.1)**
Total	**34 (100)**	**27(100)**	**11(100)**	**4(100)**	**76(100)**

- SCCmec types I, IVb, IVd, VI, VII, VIII, IX, X, XI, XII, XIII, and XIV were not detected in any MRSA strains circulating in Fako.

4. DISCUSSION

Methicillin-resistant *Staphylococcus aureus* is a pathogen that has become a significant burden on public health nationally and globally, with severe implications [43]. It is important to have a baseline understanding of the genetic diversity of the circulating strains to implement effective policies for controlling MRSA infections in hospitals, livestock and communities. This study was to establish the genetic variability of MRSA strains circulating within the Fako Division and identify any previously unacknowledged disparities that may enhance the effectiveness of infection control interventions.

The sequences in this study showed greater similarity to those in GenBank compared to previous research on MRSA strains from Sokoto, which reported query coverages ranging from 40-100%, an e-value of zero, and per cent identity sites between 13.65% and 100% [1]. The MRSA strains in this study, originating from various sources, exhibited one or two nucleotide substitutions and a high degree of sequence similarity. This indicates that MRSA can spread across different sources and settings, confirming that MRSA is exchanged between different sources and settings [44, 45]. This study found only a few nucleotide substitutions in the *mecA* sequences, unlike a similar study in Sokoto, where most of the *mecA* sequences from MRSA strains in clinical settings had more than three nucleotide substitutions [1].

A study in Iraq by Neamah and colleagues found a strong genetic link between MRSA strains in humans and cattle, indicating a significant connection between livestock and human isolates [46]. Similarly, this study observed related *mecA* sequences from both clinical and livestock sources. This study found that most of the nucleotide substitutions observed were redundant. Thus, the amino acid sequences of the *mecA* gene from both clinical and livestock sources displayed more remarkable similarity, with 14/15 sequences showing 100% homology with those of the clinical strains. Consequently, the amino acid sequences of the *mecA* gene from both clinical and livestock sources showed a high degree of similarity, with 14 out of 15 sequences being 100% identical to those of the clinical strains.

The alignment of the *mecA* sequences from this study with those retrieved from GenBank which included seven MRSA reference strains from various sources and countries (both developed and underdeveloped), showed 98.6%-100% similarity. This result was consistent with previous reports from Adelaide [47] and Egypt [48, 49], which found that identical homologs of the *mecA* gene of MRSA strains originated from diverse sources and locations. This similarity aligns with Velasco and colleagues' findings, which showed a genetic similarity between 108 clinical MRSA isolates and 133 *S. aureus* isolates from animals. Their study highlighted the high genetic similarity between the human and meat clones [50]. The high similarity between the sequences in this study and those from other countries suggests that the bacterial genome is conserved across geographical boundaries. Additionally, this similarity indicates that the *mecA* gene is unique to MRSA strains, with no equivalent locus in methicillin-susceptible bacteria, demonstrating that the *mecA* gene determinant was acquired by horizontal gene transfer [48].

In investigating MRSA's epidemiology and diversity, SCCmec typing is essential. The MRSA strains circulating in Fako predominantly exhibited SCCmec type IV, followed by type V. SCCmec type IV was most prevalent in both the HA-MRSA and LA-MRSA strains, with observed prevalences of 52.9%(18/34) and 63.2%(24/38), respectively. Similar findings of SCC*mec* type IV being most common in clinical settings in Switzerland [51] and Ethiopia [52], as well as in studies conducted in Uganda [53] and the Central and Northern parts of Cameroon [14, 22] of both hospital and community settings. In contrast, a study conducted on isolates from surgical site infections at Mulago Hospital in Kampala, Uganda [54], and in asymptomatic carriers in pastoral communities of rural South-Western Uganda [55], found that SCCmec type V was the most prevalent. In this study, SCCmec types IV and V had the same prevalence in CA-MRSA. Review studies on African MRSA strains aimed to provide updates on the clonal

characteristics and epidemiology of MRSA in Africa, including countries such as Madagascar, Morocco, Niger, Senegal, Algeria, Egypt, South Africa, Tunisia, Angola, Cameroon, Gabon, Ghana, Madagascar, Nigeria, Democratic Republic of Congo (DRC), Cape Verde, Kenya, and São Tomé and Príncipe; reported SCC*mec* type IV as the most common type across various settings [56, 57].

This study found SCC*mec* types II and III to be the least common, each accounting for 10.5% of cases. This is consistent with findings in Switzerland and Cameroon, where the SCC*mec* types II and III MRSA strains were also in the minority [14, 22, 51]. However, this study's findings contrast with those in South Africa, where SCCmec type III was the most predominant type found in MRSA blood culture isolates from a national laboratory-based and enhanced surveillance program [58]. Their study, conducted from 2010 to 2017 isolates, reported that SCCmec type III predominated every year, followed by type IV, except in 2011 when the opposite was observed [58].

SCCmec type IV is reported as the most virulent type [3], as confirmed in this study. It possesses a highly functional recombinase and the *mecA* gene and its superior mobility is due to its relatively small size. Consequently, the SCCmec type IV gene segment can be efficiently transmitted to methicillin-sensitive *S. aureus* within communities [3, 59, 60].

Of the 44 SCCmec type IV MRSA strains observed in this study, 30(39.5%) were of the SCCmec subtype IVa, and 14(18.4%) were of the SCCmec subtype IVc, with no instances of.SCCmec types IVb and IVd. This finding is slightly similar to the high prevalence of SCCmec subtype IVa among SCCmec type IV strains reported by Okuma and colleagues in the USA and Australia [11], as well as in a study from Uganda, where 27 out of 41 SCCmec type IV MRSA strains were of subtype IVa [53].

In this study, SCCmec type IV was the most dominant type among HA-MRSA strains, with a prevalence of 52.9% (18/34). This finding contrasts with similar studies in Iran [9, 15, 21, 61], three different geographical areas of India and Pakistan [62], Malaysia [19], Pakistan [63], and South Africa [25], where SCCmec type III was reported as the most predominant type among hospital MRSA strains. In South Africa, SCCmec type III was followed by types IV and II [25]. In Egypt, some studies found SCCmec type II to be the most predominant [8, 64], while another study reported SCCmec type V as the most dominant in HA-MRSA strains [65]. A survey in 11 Asian countries revealed SCCmec type II as the most prevalent in Japan and Korea, and SCCmec type III as the most prevalent in Saudi Arabia, Singapore, Indonesia, Vietnam, Sri Lanka, Thailand, India, and the Philippines, with type I being the least prevalent in all these

countries [16, 66]. Contrary to this study, a study in Japan found SCCmec type I to be the most prevalent [67], marking the first time SCCmec type I was predominant in hospital MRSA strains in some Asian countries.

Similar to our findings, studies in Iran [61], Ethiopia [52], and Egypt [8] did not report isolates for SCCmec types I, VI, VII, VIII, X to XIII. In contrast, while our study did not find any SCCmec types from VI to XIV, a study in Egypt identified SCCmec types VI, IX, and the recently discovered type XIV [8]. Additionally, studies in Lahore, Pakistan observed the SCCmec subtypes IIIa, IVd, IVe, and type XI [63], and in Germany and Japan SCC*mec* type XI was also reported [68, 69]. A six-year systematic review survey in African countries revealed two cases of SCCmec type I in South Africa, observed in 2014 and 2015 [57]. The distribution of the different SCCmec types has not been linked to any specific factor.

MRSA spreads among humans, animals, and their environments. SCCmec types I, II, and III are commonly associated with hospitals, while SCCmec types IV and V are typically found in community settings [7, 44, 70]. The distinction between HA-MRSA and CA-MRSA has become more complex due to the intermingling of CA-SCCmec types in hospitals and vice versa, leading to changes in disease patterns [57, 69 - 72]. In this study, 3.7% (1/27) and 22.2% (6/27) of MRSA strains from meat retail shop samples were of the HA-SCCmec types (type II and III, respectively). A similar scenario was observed in a study where two MRSA isolates of bovine origin carried the SCCmec type III, a HA-SCCmec type [72]. Since the firm establishment of MRSA in the clinical setting, it has emerged in the community, followed by livestock and foodstuff, revealing new reservoirs for MRSA [32, 45]. Adopting a One Health approach is essential to address the emergence of novel diseases and new reservoirs for infection [70 - 72].

The discovery of MRSA strains crossing between hospital, livestock, and community environments in this study conducted in an underdeveloped country, highlights the interconnectedness of human, animal, and environmental health. This emphasises the importance of the ‘One Health’ approach, which recognises that the health of people is closely connected to the health of animals and our shared environment. It suggests that infections and antimicrobial-resistant bacteria can spread more easily between humans, animals, and the environment; leading to outbreaks that are harder to control and treat. Thus, increasing the burden on healthcare systems, especially in resource-limited settings like Cameroon. As such strengthening healthcare infrastructure and establishing effective integrated surveillance systems to monitor these bacterial strains across different environments is crucial for early detection and response to potential outbreaks. Engaging communities through education on hygiene and safe agricultural practices,

and encouraging collaboration between healthcare providers, veterinarians, environmental scientists, and policymakers can lead to more effective strategies for managing infections. By adopting a One Health approach and implementing these public health strategies, resource-limited settings can better manage the risks associated with bacterial strain crossing and improve overall health outcomes.

In this study, the MRSA isolates from RTE foods (CA-MRSA) were exclusive of SCCmec types IV and V. This is similar to a cohort study on RTE foods in China, where SCC*mec* types IV and V were the most prevalent [13]. Our findings align with previous research indicating that CA-MRSA commonly carries SCC*mec*types IV and V [7, 44].

5. CONCLUSION

The study represents one of the few reports of a genetic relationship between MRSA strains circulating in Cameroon and the first in the Fako division. SCCmec type IV was the most prevalent (57.9%) type in the MRSA strains, followed by type V (21.1%). The study identified a genetic relationship between MRSA strains within the different settings in Cameroon's Fako division, suggesting the complexity of MRSA epidemiology in the region. Thus, the potential for increased risk of transmission of severe infections which highlights the need for adopting a One Health approach in effective disease prevention and control. The genetic analysis showed similarities between the circulating strains in Fako and those found globally, suggesting there is some degree of conservation of bacterial genome across geographical boundaries. All the sequences revealed high sequence identity when compared to those available on NCBI using the BLASTN. The sequences showed significant genetic similarity, ranging from 99.1% to 100% for the nucleotides and 98.6% to 100% for the amino acids. Compared with reference sequences retrieved from GenBank, similarity ranged from 98.2% to 100% and 97.9% to 100%, respectively.

Abbreviation

Methicillin-resistant *Staphylococcus aureus,* (MRSA); Basic Local Alignment Search Tool, (BLAST); Hospital-associated, (HA); Community-associated, (CA); Livestock- associated, (LA); South West Region (SWR); National Center for Biotechnology Information (NCBI); Staphylococcal chromosomal cassettes mec, (SCCmec); Statistical Package for Social Science, (SPSS); Cassette chromosome recombinase, (*ccr);* Multilocus sequence typing, (MLST); Pulsed-field gel electrophoresis, (PFGE); Cameroon, (CMR); Ready-to-eat, (RTE).

Ethical approval and consent to participate

Ethical approval to carry out this study was obtained from the Institutional Review Board of the Faculty of Health Sciences, University of Buea (Ref. No. 2020/1232-07/UB/SG/IRB/FHS of 04 August 2020). Samples used in the study were bacterial isolates. No patient specimens were used. Therefore, informed consent was not required.

Consent for publication

Not applicable

Availability of data and materials

The authors declare that all the supporting data and materials used in this study are presented sufficiently in the tables and figures.

Declaration

This article or work has not been published previously, nor is it under consideration for publication elsewhere. All authors approve the publication, and if accepted, it will not be published elsewhere, in English or any other language, without the copyright holder's written consent.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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