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Genetic resistance factors and antimicrobial resistance phenotypes in methicillin-resistant *Staphylococcus aureus* isolates of animals and humans

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ABSTRACT

Objective: To investigate the prevalence, genetic resistance factors, and antimicrobial resistance phenotypes of methicillin-resistant *Staphylococcus (S.) aureus* (MRSA) from clinically healthy animals (horses, dogs, and cats) and their human handlers, providing a baseline for broader genes sequencing based on One Health studies to the emergence of resistance as well as guides regarding specific therapies, hospital antibiotic usage/stewardship, and target-specific infection control measures.

Methods: This cross-sectional study involved both human and animals. *S. aureus* isolates derived were characterised for their antimicrobial phenotypes through antimicrobial susceptibility testing with PCR detection of *mecA*, *mecC*, and DNA sequencing of *mepR*, *mepA*, *mepB*, and *sapep* genes for correlation with their antimicrobial resistance phenotypes.

Results: Seventy *S. aureus* isolates from 149 human handlers, 240 horses, and 206 companion animals, including dogs and cats were studied. The prevalence of resistance was highest for penicillins (100.0%) and amoxicillin (94.3%), followed by erythromycin (87.7%), trimethoprim (78.6%), azithromycin (77.1%), imipenem (61.4%), and tetracycline (40.0%). Lower resistance prevalences were observed for ciprofloxacin (27.1%), chloramphenicol (20.0%), trimethoprim-sulfamethoxazole (12.9%), and gentamicin (2.9%). Twenty-six isolates had their DNA sequenced for *mepR*, *mepA*, *mepB*, and *sapep* genes for correlation with their antimicrobial phenotypes. Transcriptional profiling revealed that both animal and human MRSA isolates exhibited a gene cluster *mepRAB* (multidrug export protein gene), encoding a MarR-like transcriptional regulator (*mepR*), a M20/M25/M40 metallo-hydrolase protein gene (*sapep*) encoding resistance to biocides and carbapenems, and a hypothetical protein gene of unknown function (*mepB*).

Conclusions: This study demonstrated extensive multidrug resistance in MRSA, revealing similarities in the resistance patterns and multiple antibiotic resistance indices among the isolates. These findings suggest the potential presence of non-*mec* resistance mechanisms in MRSA, in addition to the *mec* gene mechanism.

KEYWORDS: MRSA; Community-acquired; Resistance; One Health

1. Introduction

Prior to the development of antibiotics, infection with *Staphylococcus (S.) aureus* was often fatal; *S. aureus* usually responds to lactam antibiotics as well as other antimicrobial agents[1]. However, with the rising prevalence of methicillin-resistant *S. aureus* (MRSA), a multidrug resistant strain, treatment has become a challenge. In 2017, the Centre for Disease Control of the United States estimated that MRSA was responsible for 119 247 cases of bloodstream infections with 19 832 associated deaths in the United States[2]. The infection frequently involves the skin and soft

Significance

The known antibiotic resistance determinants, *mecA* and *mecC*, detected in MRSA isolates exhibit a broad spectrum of cross-antibiotic resistance. This study identified three functional protein families, through sequencing, that confer extensive antibiotic resistance independent of *mecA* and *mecC*. Understanding the antimicrobial resistance patterns for MRSA is crucial for making informed decisions regarding antibiotic stewardship and infection control policies in a One Health context.

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tissue, but it can also cause other life-threatening systemic illnesses including endocarditis, meningitis, and toxic shock syndrome. Treatment of early infections usually involves incising and draining the lesion supplemented by beta-lactam antibiotic drugs[3].

Resistance to MRSA is mainly due to the carriage of *mecA* gene on a mobile genetic element (staphylococcal chromosome *mec*, SCCmec) encoding for the altered penicillin binding protein (PBP 2a), this results in low affinity for beta-lactam antibiotics[4]. In addition to *mecA*, another mechanism of resistance to penicillin in staphylococci is production of β-lactamase (encoded by *blaZ* gene)[4].

MRSA is an invasive pathogen that can cause disease in most tissues or organs of the body, especially in immune-compromised individuals[5]. The evolution of MRSA has been further advanced over the past decade when the emergence of community-associated (CA)-MRSA strains occurred[6]. The emerging community clones of MRSA were endemic in specific regions and spread between countries and continents. Evidence further indicated the entry of the CA-MRSA strains into healthcare facilities as a prevalent nosocomial pathogen[6]. MRSA has been isolated from several animals, including cattle, buffaloes, horses, pigs, poultry, dogs, cats, rabbits, and exotic species causing infection as well as in healthy animals as nasal carriage[7]. Companion animals can serve as a reservoir of multidrug-resistant staphylococci, which may be transferred to owners and others who handle companion animals[8,9]. Pet owners, breeders and veterinarians encompass the highest risk group that may become colonized from MRSA of companion animal origin[10].

Antimicrobial resistance takes place when bacteria change or adapt in a way that allows them to survive in the presence of antibiotics designed to kill them. Once strains of bacteria surpass the deadly effects of antibiotics, they are likely to pass the genes for resistance to other bacteria[11].

The high frequency of multiple drug resistant- (MDR-) *S. aureus* isolates in Africa has been attributed to irrational use of antibiotics in animal health and husbandry, self-medication, and substandard infection control and prevention practices[12,13]. Conversely, higher sensitivity has been reported for gentamicin compared to vancomycin by several authors working in Sub-Saharan Africa[14–17], while at the same time, other authors have reported considerably high frequency of gentamicin and erythromycin resistance[18]. The recovery of *S. aureus* isolates that are resistant to both vancomycin and oxacillin also parallels reports from recent studies in the region[15,18].

This study provides further evidence to the antimicrobial susceptibility pattern of MRSA and their genetic determinants of resistance among companion animals and human in-contacts in a state of the northern Nigeria.

2. Subjects and methods

2.1. Study area and period

The study was conducted in Kaduna State, located in northwestern Nigeria on latitude 7° 26’ 25” E and longitude 10° 31’ 23” N, with an altitude of 704 M (Google Earth, 2020). The study was conducted from November 1st, 2019, to August 30th, 2021.

2.2. Study subjects

Horses, dogs, cats, and their handlers whose sociodemographic information and informed consent were provided by the handlers or owners had their nasal swab samples collected. The animals and their handlers were only included in the sampling when affirmed to be free from antibiotic therapy at the time of data collection.

Each subject was anonymized on the information sheet, consent form, sterile cotton tipped swab and a receptacle for the swab containing sterile enrichment broth. For the duration of the study, samples were identified using only designated numbers rather than names or any other personal data, while respondents who tested positive for MRSA and indicated that they wanted to be informed of their culture results were confidentially notified.

2.3. Sample collection and microbiology processing

Nasal swab specimens were collected as previously described[19]. Each nasal swab sample was inoculated onto mannitol salt agar (Oxoid Basingstoke, UK) and then on Columbia agar with 5% sheep blood (Oxoid Basingstoke, UK). The 70 isolates obtained were identified using standard microbiological methods of colony morphology for *S. aureus*, Gram’s reaction, and biochemical tests (catalase and tube coagulase tests). The specific screening process of isolates for sequence analysis is presented in Figure 1. Isolates were confirmed as *S. aureus* after being subjected to testing using Microbact 12S (Oxoid Basingstoke, UK) following the manufacturers guide.

2.4. Antimicrobial susceptibility testing

A suspension of pure colony from each confirmed culture isolate was prepared in sterile normal saline and incubated at 37 ℃ for 15 min. The suspension was adjusted to 0.5 MacFarland standard. The Modified Kirby-Bauer disk diffusion technique[20] was implemented for antibiotic susceptibility testing using a total of 14 antibiotics. Isolates were classified as resistant, intermediate, and sensitive based on CLSI interpretative criteria[21]. Those isolates with resistance to cefoxitin (⩽21 mm) were confirmed as MRSA. *S. aureus* ATCC 33591 was used as control. Isolates displaying resistance to three or more antimicrobial classes were considered MDR. Multiple antimicrobial resistance (MAR) index was calculated as the ratio between the number of antibiotics that an isolate is resistant to and the number of antibiotics an isolate is exposed to. A MAR index of greater than 0.2 was considered indicative of antibiotic pressure (frequent antibiotic use on the source of the isolates)[22].

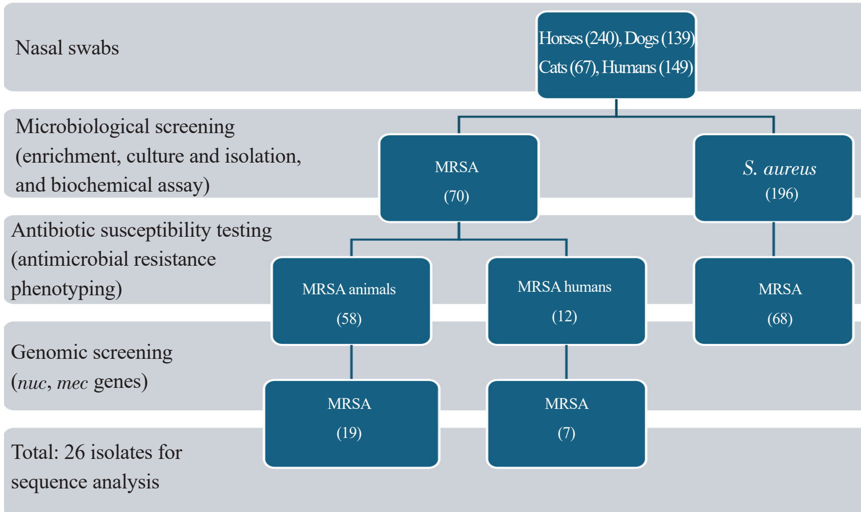

Figure 1. Screening flowchart of swab samples for sequence analysis.

2.5. Genomic analysis

DNA extraction protocol of the phenol-chloroform technique was employed for extraction of genomic DNA[23]. The tubes were allowed to air dry for 20 min to remove all traces of ethanol and finally eluted in 50 µL biological grade water. The genomic DNA was quantified using NanodropTM reading with 20 µg as the minimum acceptable concentration and stored afterwards at 2-4 ℃ until when required.

All cefoxitin-intermediate or cefoxitin-resistant isolates, that were *nuc* and *mec* genes positive were further screened by sequencing of *mepR*, *mepA*, *mepB*, and *sapep* genes. The primers used for PCR amplification The annealing temperature for the PCR reactions were set to 55 ℃ according to the temperature value of the primers, and the extension time was set to 1 min per 1 000 bp.

Amplification products of the 26 isolates extracted as described earlier were used as the template for sequencing and comparison of the *mepRAB* operon (1.410 kb) as well as the gene encoding sapep. The PCR products were sequenced (Psomagen, Rockville Maryland, USA; known formerly as Macrogen) using an ABI 3130 sequencer as described[24]. To detect mutations in relevant genes (*mepRAB* operon), comparisons were made between the sequences generated with the corresponding reference sequence strains. To exclude specific single-nucleotide polymorphisms in sequences that may also be present in susceptible strains, reference was made to the sequences of *S. aureus* M1279 (NCBI: txid1402767) and *S. aureus* 04-02981 (NCBI: txid703339). The entire genome sequence of these reference isolates has been completely studied and used as the reference genome of *S. aureus* in the genome database of NCBI (National Center for Biotechnology Information). BioNumerics v.8.0 (Applied Maths, Sint-Martens-Latem, Belgium) software, GeneiousTM software as well as the alignment tool (BLAST) of BioEdit 7.0.5.3 were used for analysis and annotation of the sequences generated from the isolates.

2.6. Ethical approval statement

The ethical approval of this study was released by the Ahmadu Bello University Ethics Committee on Use of Human or Animal Subjects for Research (ABUCUHSR/2019). This study has taken signed consent from the participants who agreed to take part in it. All subject information was maintained with strict confidentiality and used in compliance with ethical research

3. Results

All 70 *S. aureus* isolates from companion animals and human handlers were phenotypically characterized as MRSA exhibiting resistant to penicillin and cefoxitin. The isolates were susceptible to gentamicin, trimethoprim-sulfamethoxazole, chloramphenicol, and ciprofloxacin (97.2%, 87.1%, 80.0%, and 72.9% respectively). Additionalle, resistance was exhibited against trimethoprim (78.6%) and the macrolides: Erythromycin (85.7%) and azithromycin (77.1%), the aminopenicillin and beta-lactamase inhibitor: Amoxicillin (94.3%) and imipenem (61.4%). Resistance was also observed against tetracycline (40.0%) (Table 1).

Different MAR indices were observed, and thirty-three resistance patterns (antibiogram) were recorded. The lowest MAR index was 0.21 (Table 2). All isolates were observed to be multidrug resistant exhibiting resistance to an antibiotic in at least three classes of antimicrobials(Table 3).

Table 1. Number and percentage susceptibilities of Staphylococcus aureus isolates against fourteen antimicrobials (n=70).

Antimicrobial agents	Susceptibilites	Intermediate	Resistant
Oxacillin	0	0	70(100.0)
Methicillin	0	0	70(100.0)
Penicillin G	0	0	70(100.0)
Amoxicillin	4(5.7)	0	66(94.3)
Cefoxitin	0	0	70(100.0)
Azithromycin	5(7.1)	11(15.7)	54(77.1)
Erythromycin	1(1.4)	9(12.9)	60(85.7%)
Trimethoprim	6(8.6)	9(12.9)	55(78.6%)
trimethoprim-sulfamethoxazole	61(87.1)	5(7.1)	4(5.70%)
Gentamicin	68(97.2)	1(1.4)	1(1.4)
Tetracycline	28(40.0)	14(20.0)	28(40.0)
Ciprofloxacin	51(72.9)	15(21.4)	4(5.7)
Chloramphenicol	56(80.0)	13(18.6)	1(1.4)
Imipenem	14(20.0)	13(18.6)	43(61.4)

Data were expressed by n(%).

Four non-mec genes (*sapep*, *mepR*, *mepA*, and *mepB*) were amplified by PCR and sequenced. The sequences protein BLAST analysis results for these genes of 26 isolates showed similarity with typed isolates of MRSA with peculiar resistance to carbapenems and fluoroquinolone (Supplementary Table 1 and Supplementary Table 2). Transcriptional profiling revealed that both animal and human MRSA isolates exhibited a gene cluster *mepRAB* (multidrug export protein gene), encoding a *MarR*-like transcriptional regulator (*mepR*), a M20/M25/M40 metallo-hydrolase protein gene (*sapep*) encoding resistance to biocides and carbapenems, and a hypothetical protein gene of unknown function (*mepB*). All 26 isolates sequenced had one of these three genes encoded (Table 3). This study has also identified through sequencing three functional protein gene families known to confer resistance to a wide range of antibiotics that are non-mec dependent (Table 4).

Table 2. Antimicrobial resistance patterns of *Staphylococcus aureus* isolates and MAR indices (14 antibiotics, n=70).

Resistance patterns	MAR index (%)	Source			
		Horse	Dog	Cat	Human
E:FOX:IPM	0.21(1.4) (MDR)		1		
TIM:FOX:P:AMC		1			
TIM:E:FOX:AMC			1		
AZM:FOX:P:AMC			1		
TET:FOX:P:AMC	0.29(5.7) (MDR)		1		
CIP:E:FOX:P:AMC		1			
AZM:E:FOX:P:AMC		2			
TET:TIM:FOX:P:AMC				1	
TIM:AZM:E:FOX:P		1			
TIM:E:FOX:AMC:IPM	0.36(8.6) (MDR)		1		
TET:TIM:FOX:P:AMC:IPM			1		
AZM:E:FOX:P:AMC:IPM		1			1
TET:TIM:AZM:FOX:AMC:IPM		1	2		
TET:AZM:E:FOX:P:AMC		1			
TIM:AZM:E:FOX:AMC:IPM		1			
CIP:TIM:AZM;E:FOX:AMC		1			
TIM:AZM:E:FOX:P:AMC		1			
CIP:AZM:FOX:P:AMC:IPM		1			1
TIM:AZM:E:FOX:P:AMC		2	3		
TIM:E:FOX:P:AMC:IPM		1	2	1	
TET:AZM:E:FOX:P:AMC		1	1		
TET:AZM:E:FOX:AMC:IPM	0.43(17.1) (MDR)				1
TIM:AZM;E:FOX:P:AMC:IPM		5	3	2	4
TET:TIM:E;FOX:P:AMC:IPM		1		2	
TET:TIM:AZM:E:FOX:P:AMC		2			2
TET:TIM:AZM:E:FOX:AMC:IPM		1			1
TET:TIM:AZM:FOX:P:AMC:IPM		1			
SXT:TET:TIM:E:FOX:P:AMC					
SXT:TET:TIM:AZM:FOX:P:AMC	0.50(37.1) (MDR)	1	1		
TET:TIM:AZM:E:FOX:P:AMC:IPM		2	1		2
SXT:TET:TIM:AZM:E:FOX:P:AMC		1		1	
C:TIM:AZM:E:FOX:P:AMC:IPM				1	
CIP:SXT:TET:TIM:AZM;FOX:P:AMC	0.57(12.9) (XDR)	1			
Subtotal		31	19	8	12

Note: MAR index $\geqslant$0.2 indicates antibiotic pressure; AMC: Amoxicillin; TET: Tetracycline; C: Chloramphenicol; CIP: Ciprofloxacin; E: Erythromycin; FOX: Cefoxitin; CN: Gentamicin; SXT: Sulphamethoxazole/Trimethoprim; TEC: Teicoplanin; TIM: Trimethoprim; IPM: Imipenem; P: Peniciliin G; AZM: Azithromycin.

4. Discussion

Extensive multidrug resistance for MRSA has been demonstrated in this study with similarity between the isolates in the pattern of resistance and MAR Index. The resistance levels observed were at two extremes: Extensive resistance to macrolides, β-lactams, tetracycline, and trimethoprim, indicating significant antibiotic pressure (with a MAR index of $\geqslant$0.2), and low resistance to aminoglycosides, sulfamethoxazole/trimethoprim, fluoroquinolones, and phenicols.

These findings agree with the results of Grema[25] where *S. aureus* showed low level resistance to ciprofloxacin and sulphamethoxazole/trimethoprim. It also aligns with the findings of Garoy *et al.*[26] where very low resistance was observed against gentamicin. The result also agrees with that of Kaur and Chate[27], where resistance was reported against tetracycline, macrolides, penicillin's and cephalosporins. The low resistance of the isolates to sulfamethoxazole-trimethoprim and high resistance to trimethoprim alone is, however, not new, as MRSA from Australia and sub-Saharan Africa have been demonstrated to harbor the putative *dfrG* mobile genetic element that confers resistance against trimethoprim, without harboring any acquired genes or mutations that potentially confer resistance against sulfamethoxazole such as sulI, sulII, and sulIII, or mutations to *folP*[28,29].

The high prevalence of multidrug resistance may not be unconnected to the empirical use of antibiotics in animal husbandry, self-medication in humans (especially given the lowly and poor laboratory diagnostic facilities and manpower), as well as poor infection prevention and control practices in the community as reported by Falagas *et al.*[13] and Argaw-Derboba *et al.*[16]. The resistance indices and prevalence across the study populations make it nearly impossible to identify origin and cycle of transmission between these animals and human handlers. However, none of the isolates tested from this study was a pan-drug resistant strain.

The M20/M25/M40 metallo hydrolases super family gene (*sapep*) known to be the family of protein that produces the metallo beta-lactamases enzymes identified in the isolates perhaps explains the very high resistance rates observed against the lactam (penicillins, cephalosporins *etc*.) antibiotics including the extended spectrum beta lactamases (carbapenems, imipenem *etc*.). In addition, the multidrug resistance exhibited by these isolates may be because of the accumulation of multiple genes coding for a specific drug resistance[29] or by the increased expression of genes coding for multidrug efflux pumps known with the multidrug transporter protein (multidrug active transporter efflux) which was also identified in this study. This is in addition to the *MepB* family protein, a protein of unknown function but part of the *MepRAB* protein cluster which is known to play a role in conferring resistance against fluoroquinolones and disinfectants. These findings agree with the findings of Mohamed *et al.*[30] based on whole genome sequencing of the MRSA252 Sudanese isolate (SO-1977 strain). This may also explain the finding of this same protein from a horse isolate in this study because horses are frequently imported from

Table 3. Phenotypic and genotypic profiles of MRSA isolates (*n*=26).

Isolate ID	Antimicrobial resistance phenotype	Genetic resistance factors	MAR index	Source
LSHD 39	TEC:TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*	0.5	Human
LSHD 03	TET:TEC:TIM:E:AZM:AMC:FOX:IPM	*mecA*, *sapep*, *mepR*, *mepA*	0.5	Human
LSHD 40	TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*	0.4	Human
LSHD 50	TET:TEC:E:AZM:AMC:FOX:IPM	*mecC*, *sapep*	0.4	Human
HD 131	TEC:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*	0.4	Human
LSD 50	TIM:P:AMC:FOX	*mecA*, *sapep*	0.3	Dog
FGD 227	TEC:TIM:E:AZM:P:AMC:FOX	*mecA*, *sapep*	0.4	Dog
FGD 131	TEC:TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*	0.5	Dog
FGD 50	TEC:E:FOX:IPM	*mecA*, *sapep*	0.3	Dog
LSD 27	TET:TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*, *mepB*	0.5	Dog
LSHC 11	TET:TIM:E:AZM:P:AMC:FOX	*mecA*, *sapep*	0.4	Human
FGC 241	TET:TIM:E:P:AMC:FOX:IPM	*mecA*, *sapep*	0.4	Cat
FGC 4	TEC:C:TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*, *mepR*, *mepA*, *mepB*	0.6	Cat
HMIA 12	TIM:E:AZM:P:AMC:FOX	*mecA*, *sapep*	0.4	Human
HRS 85	TET:TEC:TIM:E:P:AMC:FOX:IPM	*mecA*, *sapep*	0.5	Horse
HRS 24	TIM:E:AZM:P:AMC:FOX	*mecA*, *sapep*	0.4	Horse
HRS 01	TEC:TIM:E:AZM:P:FOX	*mecA*, *sapep*	0.4	Horse
HRS 55	TEC:TIM:E:AZM:P:AMC:FOX	*mecA*, *sapep*	0.4	Horse
HRS 48	TET:TEC:TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*, *mepR*, *mepA*, *mepB*	0.6	Horse
HRS 87	TEC:TIM:E:AZM:P:AMC:FOX	*mecA*, *sapep*	0.4	Horse
HRS 41	TEC:TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*	0.5	Horse
HRT 57	E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*	0.4	Horse
HRT 72	TET:TIM:AZM:AMC:FOX:IPM	*mecA*, *sapep*	0.4	Horse
HRIA 22	TEC:TIM:E:AZM:P:AMC:FOX:IPM	*mecA*, *sapep*	0.5	Horse
HRIP 18	TET:SXT:CIP:TIM:AZM:P:AMC:FOX	*mecA*, *sapep*, *mepR*, *mepA*, *mepB*	0.5	Horse
HRIP 01	E:AZM:P:AMC:FOX	*mecA*, *mepB*	0.3	Horse

Note: MAR: multiple antibiotic resistance; AMC: Amoxicillin; TET: Tetracycline; C: Chloramphenicol; CIP: Ciprofloxacin; E: Erythromycin; FOX: Cefoxitin; SXT: Sulphamethoxazole/Trimethoprim; TEC; Teicoplanin; TIM: Trimethoprim; IPM: Imipenem; P: Peniciliin G; AZM: Azithromycin.

Sudan into Nigeria particularly our study area (Dongalows)[30].

Although speculative, there is possible conferment of non-mec resistance mechanisms to MRSA in addition to the mec gene mechanism that allow the isolates have low affinity for all beta-lactam antibiotics and use the efflux pumps against other antibiotics. The MRSA characteristic phenotype is, however, due to the presence of the mec gene which encodes the altered penicillin binding protein 2a with reduced affinity for beta-lactams[31,32]. These results suggest that the existence of yet unidentified or unknown function determinants involved in transcriptional enhancement of *mecA* gene exists and may point to a revision of the *mecA* resistance regulatory mechanism in MRSA isolates.

Ongoing investigations into the virulence and resistance mechanisms of MRSA are focused on addressing the morbidity and mortality associated with this evolving pathogen. Using high throughput gene sequencing and molecular diagnostic assays, several genes coding for functional proteins have been annotated and shown to confer resistance to several classes of antibiotics making the organism MDR[33].

Table 4. Distribution of non-*mec* antibiotic resistance genes from MRSA isolates (*n*=26).

Genes	Horses	Dogs	Cats	Humans	Occurrence (%)
sapep	11	5	2	7	25(96.2)
mepB	3	1	1	0	5(19.2)
mepR and *mepA*	2	0	1	1	4(15.4)

5. Conclusions

The resistance epidemiology of MRSA in Nigeria, Sub-Saharan Africa appears to exhibit important regional variation and the substantial proportion of MDR MRSA phenotypes reported in this study indicates the need for locally appropriate prescription recommendations. Such a result should be closely considered in planning effective therapeutic protocols.

Conflict of interest statement

The authors claim there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Abdulkadir A and Kabir J conceptualized the study, designed the experimental framework; Abdulkadir A performed the laboratory experiments and contributed to the data collection. Abdulkadir A, Kabir J, Bello M, and Olayinka B were involved in data analysis, preliminary draft and critical review of the manuscript. All authors read, revised and approved the final manuscript.

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Supplementary materials

Supplementary Table 1. Sequence match score of the non-mec antibiotic resistance genes detected from MRSA isolates of the study area against reference MRSA isolates from NCBI.

Gene description	Reference organism	Similarity score	Accession number	Sequence match score
M20/M25/M40 family metallo-hydrolase	*Staphylococcus aureus*	97.1	NGV92051.1	1.4
MepB family protein	*Staphylococcus aureus*	89.0	WP_031910817.1	1
TPA: multidrug transporter	*Staphylococcus aureus*	83.2	HAR4588006.1	1

Sequence match score: the number of (unique) 7-base oligomers shared between a sequence and a given RDP sequence divided by the lowest number of unique oligonucleotides in either of the two sequences.

Supplementary Table 2. Primer sequences used in amplification of spa, mepR, mepA, mepB, sapep genes sequenced and their expected amplicon sizes.

Target gene	Primer	Oligo Sequence (5'-3')	Amplicon size (bp)	Reference
mepR	F*mepR*	ACTTGGATTGCGTTGTATCCAT	≥1000	Renchi *et al*., 2020
	R*mepR*	GTTCAATACTCCTTGTCTTTTCCA		
mepA	F*mepA*	ACTGGTAGAAATCAGTCACTTTGT	≥1000	Renchi *et al*., 2020
	R*mepA*	TTTACACATATGCCAATCTCACTTT		
mepB	F*mepB*	CGTCCACTTCTGGCTGAGTA	≥1000	Renchi *et al*., 2020
	R*mepB*	AGTAGGACAAGAAACCGTTCA		
Sapep	*sapep*F	ACGCTCGAGTTATTCCTCCACGCATAATG	1410	Girish and Gopal, 2010
	*sapep*R	GTTCGCTAGCATGTGGAAAGAAAAAGTTCA		