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Genomic Patterns of Methicillin-Resistant *Staphylococcus Aureus* Isolates obtained from Patients seeking Treatment in Nakuru County Referral and Teaching Hospital, Kenya

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Abstract

Background: *Staphylococcus aureus is a bacterial pathogen and the global cause of both community and hospital acquired infections, posing a serious threat of antimicrobial resistance. Methicillin-Resistant Staphylococcus aureus is a global health concern that is associated with significant morbidity and mortality. This study was done to find out the molecular characterization of staphylococcus aureus. MRSA lineages and strains was identified using various typing methods, such as), multilocus sequence typing (MLST), SCCmec typing, Centre of Genomic Epidemiology (CGE), PUBMLTS and spa typing.*

Objective: *To determine the genotypic patterns of MRSA isolates obtained from patients seeking treatment in Nakuru County Referral and Teaching Hospital.*

Study design: *A descriptive cross-section study with a laboratory experimental component.*

Setting: *It was carried out in Nakuru County Referral and Teaching Hospital which is based in Nakuru County. This Hospital serves residents of Nakuru County and its neighboring Counties.*

Subject: *A total of 25 staphylococcus aureus isolates underwent Whole genome sequencing for molecular characterization.*

Results: *A significant proportion of MRSA isolates showed high resistance to multiple antibiotics, including penicillin G, erythromycin, clindamycin, ciprofloxacin, and gentamycin. Molecular analysis revealed a diverse range of clonal complexes and strain types, with CC8 and ST152 being predominant. A variety of resistance genes were detected, with mecA being the most common methicillin resistance gene. High prevalence of virulence genes, including aur, hlgA, hlgB, and icaC, was also observed. Multiple plasmid replicons were identified, indicating their role in spreading resistance and virulence factors among MRSA strains.*

Conclusion: *This study provided comprehensive insights into the resistance patterns, and molecular characteristics of staphylococcus aureus isolates at Nakuru County Referral and Teaching Hospital. The high antibiotic resistance observed underscores the urgent need for effective antimicrobial stewardship. The molecular diversity and high prevalence of resistance and virulence genes highlight the complex nature of MRSA infections, necessitating continuous surveillance, stringent infection control measures, and the development of new antimicrobial agents to manage the rising threat of MRSA and improve patient outcomes*

Keywords: *Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Antimicrobial resistance, SCCmec, Hospital-acquired MRSA (HA-MRSA), Community-associated MRSA (CA-MRSA), Molecular characterization.*

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I. Introduction

Staphylococcus aureus is a bacterial pathogen and the global cause of both community and hospital acquired infections, posing a serious threat of antimicrobial resistance (Nyasinga et al., 2020). Staphylococcus aureus is considered a "superbug" because of constant emergence of new clones and has a collection of virulence factors and the ability to acquire resistance to most antibiotics (Chambers & DeLeo, 2009). Methicillin-resistant S. aureus (MRSA) strains remain the hallmark of S. aureus multidrug resistance (MDR). Importantly, MRSA is a leading cause of most hospital-acquired infections, bacteremia, pneumonia, sepsis, endovascular infection and skin and soft tissues infections (SSTIs) making drug-resistant bacterial pathogens to pose a major challenge in global health ((Foster, 2017), (Hiramatsu et al., 2013), (Liu et al., 2011), (Turner et al., 2019). At first, between 1961 and 1990, MRSA was associated with healthcare institutions. New cases of MRSA infection were later reported in individuals with no previous history of hospitalization in the 1990s, resulting to the separate definitions for hospital- acquired MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) (DeLeo et al., 2010),

(Turner et al., 2019). The various risk factors for getting MRSA infections within hospital settings include surgery, hemodialysis and prolonged residence in a healthcare facility (David & Daum, 2010).

We first describe the origin of MRSA, with emphasis on the diverse nature of staphylococcal cassette chromosome *mec* (SCC*mec*). *mecA* and its new homologues (*mecB*, *mecC*, and *mecD*), SCC*mec* types (13 SCC*mec* types have been discovered to date), and their classification criteria are discussed as well as their antimicrobial resistance has been linked to evolution of MRSA in environments such as hospitals, among livestock and in the community ((Diep et al., 2006), (Hiramatsu et al., 2013)). Some SSC*mec* contain mobile genetic elements (MGE), a term referring to components that enhance intracellular DNA mobility. These cassettes include SCC*mec* II and SSC*mec* III (53kb and 67kb respectively) and contain transpons, integrated plasmids, commonly associated with HA-MRSA. SCC*mec* types IV and V are shorter (24kb and 27kb respectively) hence more prone to non-beta-lactam antibiotics and are associated with CA-MRSA (Miragaia, 2018), (Partridge et al., 2018)).

MRSA lineages and strains was identified using various typing methods, such as), multilocus sequence typing (MLST), SCC*mec* typing, Centre of Genomic Epidemiology (CGE), PUBMLTS and *spa* typing. The information hence obtained can be epidemiologically useful for tracing outbreaks, identifying the likely source of colonization (such as livestock or human associated), and distinguishing between community and hospital strains. Strains sharing alleles in all loci belong to the same sequence type (ST), while STs sharing at least five alleles belong to larger groupings called clonal complexes (CCs). PVL is a bicomponent leucocidin that has been linked to severe skin and soft tissue infections and necrotizing pneumonia (Diep et al., 2006).

Genetic variability has been attributed to the transmissibility and persistence of certain strains in distinct geographical settings.

Clonal complexes (CCs) CC5, CC8, CC22, CC30, CC80, and CC88, with CC5 being prevalent were depicted as African MRSA strains according to a 2015 systematic analysis (Miragaia, 2018). In Kenya, reported MRSA rates have varied between 3.7% in two private hospitals (Song et al., 2011), 27.8% in Kenya's largest national referral hospital (Stefani et al., 2012), and 53.4% from the same facility (Turner et al., 2019). A number of studies have reported on the molecular characterization of MRSA in Kenya, where the predominance of HA-MRSA strain ST239/241-t037-SCCmec III(3A), especially among inpatients, has been observed, with other strains being infrequently encountered ((WHO, 2017), (Chambers & DeLeo, 2009), (David & Daum, 2010)).

We then describe various typing methods applied to study the molecular epidemiology and evolutionary nature of MRSA, starting with the historical methods and we applied whole-genome sequencing to gain insights into the population structure, antibiotic resistance, and virulence gene carriage among 23 *staphylococcus aureus* isolates from different clinical, demographic, and temporal backgrounds.

Molecular characterization of MRSA is essential for several reasons. It informs healthcare providers about prevalent MRSA strains and their resistance profiles, aiding in the selection of appropriate therapies (Turner et al., 2019). Moreover, it helps track the spread of MRSA within healthcare facilities and the community, facilitating targeted infection control measures. Understanding MRSA epidemiology in resource-limited settings like Nakuru County contributes to the global understanding of MRSA and guides efforts to combat its spread.

II. Methods

2.1 Study site

The study was conducted in Nakuru County Referral and Teaching Hospital which serves patients in Nakuru County and Neighboring Counties. This study site was selected because it attends to a large population of 3.6 million patients from the South Rift region, North Rift, Nyanza, Western and Central parts of Kenya.

2.2 Study design

A descriptive cross-section study with a laboratory experimental component was conducted.

2.3 Study samples

Laboratory samples that were randomly collected from different laboratory samples/sites: blood, urine, wound, nasal swabs, pus, tissue, abscess, sputum, ear swabs, as well as cerebrospinal fluid (CSF) culture, skin swabs and synovial fluid culture in Nakuru County Referral and Teaching Hospital for Microbiology Culture and Sensitivity Test.

2.4 Sample size

The sample size of 25 samples used to study the genotypic patterns of MRSA was derived from Staphylococcus aureus isolates from a related study that determined prevalence of Staphylococcus aureus and MRSA in laboratory samples.

2.5 Sampling method and processing

All laboratory samples that were collected from different laboratory samples/sites: blood, urine, wound, nasal swabs, pus, tissue, abscess, sputum, ear swabs, as well as cerebrospinal fluid (CSF) culture, skin swabs and synovial fluid culture. The isolated cases were obtained from routinely collected samples from patients in Nakuru

County Referral and Teaching Hospital. All collected samples meeting the inclusion criteria were considered for testing.

2.6 Laboratory testing

2.6.1 Bacterial Culture and identification of Staphylococcus aureus

-The collected samples were inoculated in blood agar, Chocolate Blood Agar and MacConkey Agar plates obtained commercially and incubated at 37^0C for 24 hours.

-*S. aureus* isolate was identified using catalase and coagulase tests and also gram stain.

-To get pure colonies, *Staphylococcus aureus* colonies were sub cultured into Mueller-Hinton Agar and incubated for 24 hours at 37^0C. The pure *S. aureus* colonies were stored in the freezer at -20^0C and used whenever it's required.

-1ml of Tryptic Soy Broth(TSB) was placed in 2 micro vials (1 for AST and 1 for WGS) and then colonies of *S. aureus* were applied into the vials, mixed and then stored at -80^0C until the desired number of isolates were achieved for further testing.

-The frozen staphylococcus aureus isolates stored at -80^0C whenever we required it was defrosted and sub cultured on blood agar overnight at 37^0C for later use on AST and PCR.

2.6.2 Antimicrobial susceptibility testing

To test antimicrobial susceptibility, disk diffusion method was applied as advised by Clinical and Laboratory Standards Institute (CLSI) (2017). The testing of antimicrobial agents was as follows: penicillin G (10 units), Cefoxitin (30 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (25 µg), gentamicin (10 µg), ciprofloxacin (5 µg), clindamycin (10 µg) on the *S. aureus* blood agar plates.

Methicillin-resistant *Staphylococcus aureus* strains was determined by agar diffusion test method where cefoxitin disk is 30 micrograms.

2.6.3 DNA Extraction, Purity Check and Quantification

Bacterial genomic DNA was extracted using Qiagen DNA extraction kit according to the manufacturer's instructions. Colonies obtained after activation of *Staphylococcus aureus* on the MH agar plate was used. The extracted DNA was stored at 4°C awaiting further analysis. The DNA quantity and purity was assessed fluorometrically using the Qubit HS dsDNA quantification kit along with Qubit™ 3 fluorometer. First, the bacterial colony was emulsified in Phosphate Buffer Saline (PBS). Lysis buffer was then added and heated at 95°C for 5 minutes. This assisted in lysing the bacterial cell wall. Proteinase K was then added and heated at 70°C for 10 minutes to digest any contaminant proteins that may be present. 100% ethanol was added to precipitate the DNA. The solution was passed through a spin column which traps the DNA. Two washes were done after which the DNA was eluted into a clean tube and used for further processes immediately or stored at -20°C awaiting processing.

2.6.4 Library Preparation and Sequencing on Illumina Sequencing Technology

Next-generation sequencing technique was used for whole genome sequencing of *Staphylococcus aureus* to determine the variant genotypes using Illumina MiSeq instrument. Extracted DNA was used for library preparation. First, the bacterial DNA genome was tagmented. This is the process by which the DNA is tagged and fragmented. The tagmented libraries was cleaned up and indexing adaptors was added to them, which labeled each sample uniquely. These tagmented libraries was then amplified by PCR to increase the amplicons. After PCR, quantification for each sample was done using Qubit HS dsDNA quantification. The DNA quantities was used to calculate the equimolar amounts of each sample required for library pooling. After pooling samples into one tube, the concentration of the library pool was checked again by Qubit HS dsDNA quantification. Normalization was done, and the final library pool was denatured into a single stranded DNA library then loaded into the Illumina MiSeq sequencer as per manufacturer's instructions. PhiX was used as an internal control for Illumina sequencing of the samples.

2.7 Bioinformatics Analysis

2.7.1 De novo Genome Assembly and Quality check

De novo genome assembly of the raw reads was performed using Flye (Version 2.8.3) high-quality sequencing reads was assembled into contigs, scaffolds, or complete genomes. The algorithm iteratively joins overlapping reads, ultimately reconstructing the genome. The resulting assemblies was visualized using Bandage (Version 2.0). Quality check on the assemblies was performed using BUSCO (Version 5.2.2) to assess completeness and QUAST (Version 5.0.2) to obtain genomic features such as genome length and GC content.

2.7.2 Genome Annotation

Annotation involved assigning biological context to the assembled genome by identifying features like coding sequences, genes, and providing putative functions based on similarity to known sequences. The *S. aureus* genomes was annotated using prokka (Version 1.14.6) and visualized using BRIG visualizer (Version 0.95)

2.7.3 Database Querying for AMR, Virulence Genes, Plasmids

AMR genes, virulence genes and plasmid replicons were screened using ABRicate (Version 1.0.1) against the NCBI AMR Plus finder database, VFDB and PlasmidFInder database respectively

2.7.4 Strain Type, Spa Type, SCCMec Type and Clonal Complex

Determination of the strains type, clonal complex, spa type and SCCmec type for the *S. aureus* isolates was performed using PubMLST (https://pubmlst.org/).

2.8 Data analysis

Data was analyzed using IBM SPSS 21.0 software. The genotypic patterns were presented as percentages as per the resistance patterns, population structure, resistance genotypes, virulence structure and plasmid replicons.

2.9 Ethical consideration

Ethical approval to conduct the study was sought from the Kenya Medical Research Institute (KEMRI) Scientific Ethics Review Unit (SERU), National Commission for Science, Technology and Innovation (NACOSTI) and Nakuru County Teaching and Referral Hospital. Anonymity of participants and confidentiality of their information was maintained.

III. Results

3.1 Resistance pattern to commonly prescribed antibiotics on MRSA

In total, 9 (36%) of *S. aureus* were resistant to doripenem, 16 (64%) were resistant to Penicillin G, 8 (32%) to cefoxitin and 16 (64%) to erythromycin. Similarly, 16 (64%) were resistant to clindamycin, 14 (56%) to ciprofloxacin, and 14 (56%) to gentamycin while ‘6 (24%) was resistant to STX (Table 4.1).

Table 4.1 Resistance pattern to commonly prescribed antibiotics on MRSA

Variable	**n=25** **Frequency (%)**
Doripenem	
Resistant	9 (36.0)
Intermediate	3 (12.0)
Susceptible	13 (52.0)
PenicillinG	
Resistant	16 (64.0)
Intermediate	1 (4.0)
Susceptible	8 (32.0)
Cefoxitin	
Resistant	8 (32.0)
Intermediate	5 (20.0)
Susceptible	12 (48.0)
Erythromycin	
Resistant	16 (64.0)
Intermediate	3 (12.0)(
Susceptible	6 (24.0)
Clindamycin	
Resistant	16 (64.0)
Intermediate	2 (8.0)
Susceptible	7 (28.0)
Ciprofloxacin	
Resistant	14 (56.0)
Intermediate	3 (12.0)
Susceptible	8 (32.0)
Gentamycin	
Resistant	14 (56.0)
Intermediate	4 (16.0)
Susceptible	7 (28.0)
SXT	
Resistant	6 (24.0)
Intermediate	2 (8.0)
Susceptible	17 (68.0)

3.2 Population structure

Of the 25 specimens that were done molecular analysis, 23 (92%) were identified as Staphylococcus aureus while 1 was Proteus maribilis and 1 Acinetobacter baumannii. The clonal complex was commonly CC8 (20%) and CC15 (12%) while 56% (14 specimens) were unassigned. Strain type was mainly ST152 (52%) and t355 Spa type was

the most common at 32%. The SCC mec type were mostly type_III(3A) (12%) and type_IVa(2B) (12%) while 64% was unassigned (Table 4.2).

Table 4.2: Population structure

Variable	Frequency (%)
Species ID	
Acinetobacter baumannii	1 (4.0)
Proteus mirabilis	1 (4.0)
Staphylococcus aureus	23 (92.0)
Clonal complex	
CC1	1 (4.0)
CC8	5 (20.0)
CC15	3 (12.0)
CC22	1 (4.0)
CC30	1 (4.0)
Unassigned	14 (56.0)
ST type	
ST8	2 (8.0)
ST22	1 (4.0)
ST152	13 (52.0)
ST188	1 (4.0)
ST241	1 (8.0)
ST243	1 (4.0)
ST2019	1 (4.0)
ST2126	1 (4.0)
ST7635	2 (8.0)
Unassigned	2 (8.0)
Spa type	
t21	1 (4.0)
t37	3 (12.0)
t84	2 (8.0)
t189	1 (4.0)
t355	8 (32.0)
t1172	1 (4.0)
t1476	2 (8.0)
t1941	1 (4.0)
t2119	1 (4.0)
t5047	1 (4.0)
t10499	1 (4.0)
t17184	1 (4.0)
Unassigned	2 (8.0)
SCC mec	
SCC mec_type_III(3A)	3 (12.0)
SCC mec_type_IV(2B&5)	2 (8.0)
SCC mec_type_IVa(2B)	3 (12.0)
SCC mec_type_VII(5C1)	1 (4.0)
Unassigned	16 (64.0)

Table 4.3: Population Structure Type

Genome ID	Clonal complex	ST Type	Spa Type	SCC mec Type	Virulence genes
STP 01	CC1	ST188	t189		
STP 02					
STP 03	CC8	ST241	t37	type iii (3A)	
STP 04	CC8	ST635	t37	type iii (3A)	
STP 05	CC22	ST22	t1784		
STP 06	CC8	ST7635	t37	type iii (3A)	
STP 07		ST152	t355		√
STP 08	CC15	ST15	t84		√
STP 09	CC30	ST243	t21		√
STP 10		ST152	t355		√
STP 11		ST152	t355		√
STP 12	CC8	ST8	t1476	meca type iv (2B and s)	
STP 13		ST152	t1941		√

STP 14		ST2019	t10499		
STP 15	CC8	ST8	t1476	meca type iv (2B and s)	
STP 16		ST152	t355	meca type iv (2B and s)	√
STP 17		ST152	t355	type iva (2B)	√
STP 18		ST152	t355	type iva (2B)	√
STP 19					
STP 20		ST152	t355		√
STP 21		ST152	t355	meca type iva (2B)	
STP 22	CC15	ST15	t84		√
STP 23		ST152	t5047		√
STP 24	CC15	ST2126	t2119		
STP 25		ST152	t1172		√

-As indicated in table 4.2, Isolate typing indicated great diversity among the 23 *staphylococcus aureus* with the identification of 5 distinct clonal complexes which is CC1,CC8,CC15,CC30 and CC22.The majority were CC8 at 20%.CC8 was assigned different ST types but but for the spa types it was greatly associated with t37 and t1476.
-A majority of ST type was ST 152 and it was associated with spa type t355 as well as SCCmec type IV and type VII (both CA MRSA).ST7635 was associated with spa type t37 and was the main contributing factor to HA-MRSA (SCCmec type III)
-Spa typing revealed 13 different spa types with the predominant types t355 (32%) and t37 (12%).Spa types t355 and t37 were associated with CA-MRSA and HA-MRSA respectively.
-Out of the 23 staphylococcus aureus isolates, 9 (39.13%) isolates showed the presence of SCCmec cassette ranging from type III (3A), type IV (2B & 5), type IIVa (2B), and type VII (5C1).The presence of mec a gene was identified in both ST 8 and ST 152.The genes that were associated with MRSA included t37,t355 and t1476.

3.3 Resistance genotypes

This study determined 28 unique antimicrobial resistance genes. mec a gene was predominantly resistant to cefoxitin at 24% as well as mecR1 and mecl at 12%.

Antibiotics resistance genes against SXT and penicillin G were more frequent at 80%. The most common gene against SXT was dfrG (64%). blal(68%) and blalR1(60%) genes encoding B lactamase had widespread resistance to penicillin. Resistant genes against erythromycin were at 36%, 24% for ciprofloxacin and 16% for gentamycin. The lowest resistant genes identified were those against clindamycin (4%) and doripenem (4%) (see table 4.4).

Table 4.4: Resistance genotypes

Drug	**Total isolates with resistant genes (n=25)**	**Type of genes detected**	
	Frequency (%)	**Gene**	**Frequency (%)**
SXT	20 (80.0)	dfrA7	1 (4.0)
		dfrG	16 (64.0)
		dfrS1	5 (20.0)
Penicillin G	20 (80.0)	Blal	3 (12.0)
		blalPC1	3 (12.0)
		blalR1*	15 (60.0)
		blal*	17 (68.0)
		blaZ	2 (8.0)
		blaZ*	15 (60.0)
Methicillin	9 (36.0)	mecA	6 (24.0)
		mecR1^	3 (12.0)
		mecA*	3 (12.0)
		mecR1	3 (12.0)
		mecl^	3 (12.0)
Erythromycin	9 (36.0)	erm(A)	3 (12.0)
		erm(B)*	1 (4.0)
		mph(E)	1 (4.0)
		msr(E)	1 (4.0)
		erm(C)	2 (8.0)
		mph(C)*	3 (12.0)
		msr(A)	3 (12.0)
Ciprofloxacin	6 (24.0)	gyrA_S84L	6 (24.0)
		parC_S80F	4 (16.0)
		parC_S80Y	2 (8.0)

Clindamycin	1 (4.0)	Inu(A)'	1 (4.0)
Doripenem	1 (4.0)	blaOXA-98	1 (4.0)
Gentamycin	4 (16.0)	aac(6')-Ie/aph(2")-Ia	2 (8.0)
		aac(6')-Ie/aph(2")-Ia*	2 (8.0)

4.4 Virulence profile

(92%) virulence genes were detected among the 23 staphylococcus aureus strains. The most common genes were *aur, hlgA, hlgB* and *icaC* identified in 92% of the specimens. Others which appeared more frequently were *hld* (88%), *scn* (84%) and *sak* (68%). Notably, virulence genes that came about as a result of resistance were *lukF-PV* and *lukS-PV* which were identified in 52% of the specimens and are identified as major genes. Molecular characterization showed that PVL positive staphylococcus aureus belonged to different genotypes (see table 4.5).

Table 4.5: Virulence profile

Variable	**Frequency (%)**
Virulence genes detected	
Yes	23 (92.0)
No	2 (8.0)
Virulence profile	
aur	23 (92.0)
cna	13 (52.0)
ednB	10 (40.0)
hld	22 (88.0)
hlgA	23 (92.0)
hlgB	23 (92.0)
hlgC	11 (44.0)
icaC	23 (92.0)
lukD	9 (36.0)
lukE	9 (36.0)
lukF-PV	13 (52.0)
lukS-PV	13 (52.0)
sak	17 (68.0)
scn	21 (84.0)
sea	3 (12.0)
sec3	1 (4.0)
sei	2 (8.0)
sek	4 (16.0)
sel	1 (4.0)
sel26	6 (24.0)
selX	5 (20.0)
sem	2 (8.0)
sen	2 (8.0)
seo	1 (4.0)
seq	4 (16.0)
seu	2 (8.0)
splA	9 (36.0)
splB	9 (32.0)
splE	9 (36.0)
tst	4 (12.0)

4.5 Plasmid replicons

The highest number of replicons were 2 (36%) and 3 (32%). Replicons were 56% rep5a_1_repSAP001 (pN315), 52% rep16_3_rep (pSaa6159) and 32% rep7a_16_repC (Cassette). 57 plasmid replicons were present in 23 staphylococcus aureus isolates. A majority of the isolates had 2 plasmids in a genome at 36% while those with 4 plasmids in a genome were at 12% (see table 4.6).

Table 4.6: Plasmid replicons

Variable	**Frequency (%)**
Number of replicons	
1	5 (20.0)
2	9 (36.0)
3	8 (32.0)
4	3 (12.0)
Replicons	
rep5a_1_repSAP001(pN315)	14 (56.0)
rep5c_1_rep(pRJ9)	2 (8.0)

rep7a_12_repC(pKH17)	1 (4.0)
rep7a_16_repC(Cassette)	8 (32.0)
rep7c_1_rep(MSSA476)	2 (8.0)
rep9a_1_repA(pAD1)	1 (4.0)
rep9b_2_prgW(EF62pC)	1 (4.0)
rep10_3_pNE131p1(pNE131)	3 (12.0)
rep10b_2_rep(pSK6)	1 (4.0)
rep15_1_repA(pLW043)	2 (8.0)
rep16_2_CDS6(pSJH101)	1 (4.0)
rep16_3_rep(pSaa6159)	13 (52.0)
rep16_4_SAP056A024(SAP056A)	1 (4.0)
rep19_7_repA(SAP019A)	1 (4.0)
rep21_18_rep(pLNU3)	1 (4.0)
rep21_22_CDS1(pUSA01)	1 (4.0)
rep21_9_rep(pKH12)	1 (4.0)
repUS21_1_rep(pWBG764)	1 (4.0)
repUS5_1_CDS20(pETB)	1 (4.0)
ColRNAI_1	1 (4.0)

IV. Discussion

5.1 Resistance Pattern to Commonly Prescribed Antibiotics on MRSA

The resistance pattern observed among MRSA isolates from Nakuru County Referral and Teaching Hospital shows significant resistance to several commonly prescribed antibiotics. Of the 25 MRSA isolates analyzed, the resistance rates were as follows: doripenem (36%), penicillin G (64%), cefoxitin (32%), erythromycin (64%), clindamycin (64%), ciprofloxacin (56%), gentamycin (56%), and sulfamethoxazole-trimethoprim (SXT) (24%).

These findings align with global patterns where MRSA exhibits substantial resistance to beta-lactam antibiotics, particularly penicillin G and cefoxitin, due to the mecA gene encoding penicillin-binding protein 2a (PBP2a) which confers resistance to these antibiotics (Chambers & DeLeo, 2009). The high resistance rates to erythromycin and clindamycin are also consistent with previous studies indicating that MRSA strains frequently acquire resistance to macrolides and lincosamides (Turner et al., 2019).

The resistance to doripenem observed (36%) is significant but lower than resistance to penicillin G (64%). This aligns with the findings of (Ekwanzala et al., 2017) in a similar study in Kenya, which reported high resistance rates to beta-lactam antibiotics in MRSA isolates. The 64% resistance to erythromycin and clindamycin is consistent with the patterns reported (Khamis et al., 2015), who noted high resistance rates to these antibiotics in MRSA strains isolated from hospital settings. The 56% resistance to ciprofloxacin is slightly higher than the global average, which ranges between 40-50% as reported (WHO, 2017)

5.2 Population Structure

The molecular analysis of the 25 specimens identified 92% as Staphylococcus aureus, with the remaining 8% identified as Proteus mirabilis and Acinetobacter baumannii. The predominant clonal complexes were CC8 (20%) and CC15 (12%), with 56% of isolates being unassigned. The strain type ST152 (52%) and spa type t355 (32%) were most common. The SCCmec types identified were type III (3A) (12%) and type IVa (2B) (12%), with 64% unassigned. The dominance of CC8 and ST152 strains is consistent with findings (David & Daum, 2010), who reported that CC8 is a common clonal complex associated with both hospital and community-acquired MRSA globally. The presence of SCCmec type III and IVa corresponds with global trends, where type III is commonly associated with hospital-acquired MRSA (HA-MRSA), and type IVa with community-acquired MRSA (CA-MRSA) (Hiramatsu et al., 2013).

5.3 Resistance Genotypes

The methicillin-resistant genes were identified in 36% of isolates, with the mecA gene being the most prevalent (24%). Resistance genes against SXT and penicillin G were found in 80% of isolates. Notably, the dfrG gene was most common for SXT resistance (64%), and bla genes were prevalent for penicillin G resistance. The prevalence of the mecA gene at 24% is lower than some studies, (Miragaia, 2018), which reported higher mecA prevalence in MRSA isolates. The high frequency of dfrG and bla genes aligns with the study (Miragaia, 2018), which highlighted the role of these genes in conferring resistance to SXT and penicillin G respectively.

5.4 Virulence Profile

Virulence genes were detected in 92% of the specimens. Commonly identified genes included aur, hlgA, hlgB, and icaC. The presence of lukF-PV and lukS-PV, which are linked to virulence and resistance, was notable in 52% of the specimens. The high prevalence of virulence genes, such as aur and hlgA, is consistent with findings (Diep et al., 2006), who reported these genes as common in virulent MRSA strains. The presence of PVL genes

(lukF-PV and lukS-PV) in 52% of isolates aligns with the increasing trend of PVL-positive MRSA reported (DeLeo et al, 2009 and 2010)

5.5 Plasmid Replicons

The most common replicons identified were rep5a_1_repSAP001 (56%) and rep16_3_rep (52%), with the highest number of replicons found in 36% of isolates. The distribution of plasmid replicons in MRSA isolates, particularly rep5a_1_repSAP001 and rep16_3_rep, is similar to findings (DeLeo et al, 2010), indicating these replicons play a significant role in antibiotic resistance and virulence.

V. Conclusion

This study provides comprehensive insights into the prevalence, resistance patterns, and molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) isolates obtained from patients at Nakuru County Referral and Teaching Hospital, Kenya. The findings reveal significant resistance of MRSA to several commonly prescribed antibiotics, underscoring the urgent need for effective antimicrobial stewardship.

A substantial proportion of MRSA isolates exhibited resistance to multiple antibiotics, including penicillin G, erythromycin, clindamycin, ciprofloxacin, and gentamycin. This high level of resistance poses a significant challenge to treatment options, highlighting the necessity for continuous monitoring and development of new therapeutic strategies.

The molecular characterization identified a diverse range of clonal complexes and strain types, with CC8 and ST152 being predominant. The presence of various SCCmec types, including type III (3A) and type IVa (2B), further indicates the genetic diversity and adaptability of MRSA strains in this region.

The study detected a variety of resistance genes, with mecA being the most common methicillin resistance gene. Additionally, a high prevalence of virulence genes, including aur, hlgA, hlgB, and icaC, was observed. The presence of PVL genes (lukF-PV and lukS-PV) in a significant proportion of the isolates suggests a considerable potential for severe infections.

The identification of multiple plasmid replicons, particularly rep5a_1_repSAP001 and rep16_3_rep, indicates the role of plasmids in the spread of resistance and virulence factors among MRSA strains.

The findings underscore the critical need for stringent infection control measures in healthcare settings. There is a necessity for continuous surveillance of MRSA and prudent use of antibiotics to mitigate the spread of resistant strains.

The study highlights the complex and multifaceted nature of MRSA infections in Nakuru County, emphasizing the importance of ongoing research, effective infection control policies, and the development of new antimicrobial agents to combat the rising threat of MRSA. These efforts are essential to improve patient outcomes and manage the burden of antibiotic-resistant infections in the region.

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