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Genomic characterization of methicillin-resistant *Staphylococcus aureus* isolated from patients attending regional referral hospitals in Tanzania

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Abstract

Background Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization increases the risk of subsequent infection by MRSA strain complex interlinking between hospital and community-acquired MRSA which increases the chance of drug resistance and severity of the disease.

Objective Genomic characterization of *Staphylococcus aures* strains isolated from patients attending regional referral hospitals in Tanzania.

Methodology A laboratory-based cross-sectional study using short read-based sequencing technology, (Nextseq550,Illumina, Inc. San diego, California, USA). The samples used were collected from patients attending selected regional referral hospitals in Tanzania under the SeqAfrica project. Sequences were analyzed using tools available in the center for genomic and epidemiology server, and visualization of the phylogenetic tree was performed in ITOL 6.0. SPSS 28.0 was used for statistical analysis.

Results Among 103 sequences of *S. aureus*, 48.5% (50/103) carry the *mecA* gene for MRSA. High proportions of MRSA were observed among participants aged between 18 and 34 years (52.4%), in females (54.3%), and among outpatients (60.5%). The majority of observed MRSA carried plasmids rep5a (92.0%), rep16 (90.0%), rep7c (90.0%), rep15 (82.0%), rep19 (80.0%) and rep10 (72.0%). Among all plasmids observed rep5a, rep16, rep20, and repUS70 carried the *blaZ* gene, rep10 carried the *erm(C)* gene and rep7a carried the *tet(K)* gene. MLST and phylogeny analysis reveal high diversity among MRSA. Six different clones were observed circulating at selected regional hospitals and MRSA with ST8 was dominant.

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Conclusion The study reveals a significant presence of MRSA in *Staphylococcus aureus* strains from Tanzanian regional hospitals, with nearly half carrying the mecA gene. MRSA is notably prevalent among young adults, females, and outpatients, showing high genetic diversity and dominance of ST8. Various plasmids carrying resistance genes indicate a complex resistance profile, highlighting the need for targeted interventions to manage MRSA infections in Tanzania.

Keywords Genomic characterization, Methicillin resistance, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is a Gram-positive spherical-shaped bacterium from the family *Staphylococcaceae* appears in a grape-like cluster upon Gram staining [1, 2]. *S. aureus* is one of the most widespread infectious agents in hospital settings and community environments resulting in high morbidity and mortality rates [2]. It is estimated that *S. aureus* is associated with an over 30% mortality rate worldwide whereby, in developed countries, the incidence of *S. aureus* infections ranges from 9.3 to 65 cases per 100,000-person years [3]. *S. aureus* is known to cause a variety of diseases ranging from skin infection, bloodstream (septicemia) infection, infection in tissue, lower respiratory infection, catheter-associated infection, and toxic shock syndrome [4–7].

Horizontal gene transfer of resistance genes and rapid mutation rates among *S. aureus* have led to an increase in drug resistance worldwide and the development of multidrug resistance in *S. aureus* [8]. Methicillin-resistant *Staphylococcus aureus*(MRSA) is a strain of *S. aureus* known for its ability to resist methicillin group antibiotics [9]. Resistance to methicillin antibiotics is mediated by the *mecA* gene acquired by horizontal gene transfer of a mobile genetic element *Staphylococca*l cassette chromosome mec *(SCCmec)* [9, 10]. The *mecA* gene encodes an enzyme penicillin-binding protein 2a (PBP2a) which is responsible for crosslinking the peptidoglycan layer in the bacteria cell wall resulting in resistance to methicillin and all β-lactams antibiotics [11, 12]. Rapid mutation and the ability to acquire resistance genes among MRSA make them capable of possessing antibiotic resistance to one or more classes of antibiotics [12].

In the East African region prevalence of MRSA infections varies from 7.0 to 63.0% whereby, Tanzania is reported to have less burden of MRSA compared to other East African regions [13–17]. MRSA colonization increases the risk of subsequent infection by MRSA strains of which complex interlinking between hospital and community-acquired MRSA increases the chance of drug resistance and severity of the disease [2]. The development of vancomycin resistance among MRSA is the most feared genetic adaptation to *S. aureus* since it is the drug of choice for MRSA infections [18]. Antimicrobial resistance is among the top 10 global public health threats facing humanity whereby, resistance bacterial infections are associated with over 4.95 million deaths per year [19].

The main challenge of *S. aureus* in a clinical setting is that it can quickly acquire resistance genes against multiple antibiotics affecting patient care, and it can cause a variety of infections including Blood, skin, and other organs, and can lead to toxic shock syndrome [5, 7, 12]. MRSA increases healthcare costs due to more expensive therapy, prolonged hospitalization, and high morbidity rates [20]. It is estimated that by 2050, drug resistance alone will cause more deaths than all cancers combined [21].

Tanzania like many other developing countries, faces challenges in managing and controlling the spread of infectious bacteria particularly MRSA within the healthcare facilities. This also is attributed to antibiotic misuse in our population which increases the risk of drug resistance and clonal changes among the bacterial isolates [22, 23]. A report from Northern Tanzania showed an MRSA prevalence of 33.3% among archived *S. aureus* samples [17]. There is a discrepancy in the prevalence of MRSA globally in which low- and middle-income countries are mostly affected. The increased prevalence of MRSA infections in low- and middle-income countries has raised concerns about patient safety, treatment options, and effectiveness of infectious control measures. The use of next-generation sequencing technology in analyzing pathogenic bacteria is rare in low- and middle-income countries, there is little information regarding diversity among the MRSA circulating in Tanzania.

The genomic characterization of MRSA in this study involves detailed analysis of the bacterial genome to identify resistance gene, plasmid abundance, and associated drug resistance gene and finally, strain relatedness among all *S. aureus* isolates from patients attending regional referral hospitals of Tanzania. By the use of advanced genomic techniques such as whole genome sequencing, this study aims to reveal the genetic diversity of MRSA strains circulating at regional referral hospitals in Tanzania. The following drugs classes are used to treat *Staphylococcus* infections in Tanzania: Beta-lactam Antibiotics (Penicillin), Macrolides, Trimethoprim Sulfamethoxazole, Fluoroquinolones, Tetracycline and Glycopeptides.

Methodology

Study design, study participants, and study site

A cross-sectional laboratory-based study using *S. aureus* isolates collected from patients attending four regional referral hospitals in Tanzania mainland, which included Tabora Regional Referral Hospital, Dodoma Regional Referral Hospital Songea Regional Referral Hospital, Morogoro Regional Referral Hospital and Mnazi Mmoja hospital in Zanzibar island.

S. Aureus recovery

Various *S. aureus* isolates were thawed at ambient temperature. A loop of isolates was then subcultured on a Blood agar (BA) plate and incubated at 37 degrees Celsius for 24 h. Cultured catalase and coagulase-positive *S. aureus* strains were prepared for DNA extraction and sequencing as detailed below.

DNA extraction and whole genomic sequencing

Genomic DNA (gDNA) from collected *S. aureus* strains was extracted using Quick-DNA™ Fungal/Bacteria Miniprep Kit. Quantification of the gDNA were performed using a Qubit® version 4.0 fluorometer. Library preparation of the extracted DNA involves fragmentation, adaptor ligation, size selection, and indexing or barcoding as per NEBNext® Ultra™ II FS DNA Library Prep Kit manual 2020. Before loading in the Illumina Nextseq550 sequencer platform for sequencing, the prepared library was normalized and combined with Phix control.

Table 1 Social demographic and clinical characteristics of the study participants ($N=103$)

Variable	Frequency (*n*)	(*n/N*) %
Age		
<18	21	20.4
18–34	42	40.8
>34	40	38.8
Median ([a]IQR)	29.0 (45.0, 20.0)	
Sex		
Male	57	55.3
Female	46	44.7
Patient category		
Outpatients	38	36.9
Inpatients	65	63.1
Nature of the sample		
Stool	1	1.0
Blood	17	16.5
Peritoneal fluid	1	1.0
[b]HVS	5	4.9
Urine	13	12.6
Pus wound swab	65	63.1
Sputum	1	1.0
Health facility		
Dodoma Regional Referral Hospital	22	21.4
Morogoro regional referral hospital	32	31.1
Mnazi mmoja Hospital, Zanzibar	35	34.0
Tabora Regional Referral Hospital	13	12.6
Songea Regional Referral Hospital	1	1.0
Total	103	100

[a]IQR- Interquartile range, [b]HVS- High vaginal swab, DRRH- Dodoma regional referral hospital, MRHH- Morogoro regional referral hospital, ZRRH- Zanzibar regional referral hospital, TRRH- Tabora regional referral and SRRH-Songea regional referral hospital

Bioinformatics and statistical analysis

FastQC 0.12.0 was used for quality control of the sequenced raw data and assembly (de novo assembly) was performed in SPAdes 3.15.5 [24, 25]. The final output files were in fasta format containing several contigs. Bacterial Analysis Pipeline (BAP 3.3.2) which is based on the services available at the Center for Genomic Epidemiology (CGE) (https://www.genomicepidemiology.org/services/) was used. Identification of species was determined using Kmerfinder 3.2 [26–28], resistance genes were identified using Resfinder 4.1 [29–31] and the presence of plasmid was determined by using PlasmidFinder 2.1 [29, 32], Multilocus sequence typing was determined by using MLST 2.0 and Resistance in plasmids were determined by using MGE finder 1.0.3 [33]. Phylogenetic tree construction was done by using CSIPhylogeny 1.4 [34] with reference strain USA500 2395 (accession number CP007499, chromosomal length 2,955,646). Visualization of the tree was done by using ITOL V6 [35].

mecA gene was used to define Methicillin Resistance *Staphylococcus aureus* (MRSA). All these services are found in the CGE tool.

Statistical analysis was performed using SPSS version 28. Descriptive statistics was done where categorical variables were summarized using frequency and proportion. The Chi-square test was used to check if there is a significant difference in the distribution of MRSA and resistance genes. A p-value of <0.05 was considered statistically significant.

Results

Social demographic and clinical characteristics of the study participants

There were 103 participants, from which *Staphylococcus aureus* was isolated and sequenced. The median age (IQR) of all participants was 29.0 (45.0, 20.0), whereby the majority (40.8%) of all participants were aged between 18 and 34 years old. Most of the participants (55.3%) were male and 63.1% of all participants were inpatients. Among all samples, most collected samples were pus wound swab (63.1%) and blood (16.5%). Most of the samples collected were from Mnazi mmoja Hospital, Zanzibar (34.0%) and Morogoro regional referral hospital (31.1%) (Table 1).

Prevalence of MRSA gene (mecA gene)

Among 103 sequenced *S. aureus*, 48.5%,50/103) carry the *mecA* gene MRSA and the remaining (51.5% ,53/100) were methicillin-sensitive *Staphylococcus aureus* (MSSA). High proportions of MRSA gene (*mecA*) were observed among; participants aged between 18 and 34 years old (52.4%), among female participants MRSA carriage was (54.3%), and among outpatients (60.5%). Participants from Morogoro Regional Referral Hospital MRSA carriage was (71.9%) and Dodoma Regional Referral Hospital was (40.9%) (Table 2.). There were no significant differences in the proportions of *mecA* gene carriage among different demographic and clinical characteristics except for the proportion of *mecA* among the health facilities (p-value=0.029).

Plasmid abundance with their respective drug resistance genes among MRSA and MSSA

From 50 MRSA and 53 MSSA, different numbers of plasmids were observed.

Among MRSA the most abundant plasmids were rep5a (92.0% ,46/50) rep16 (90.0%,45/50) rep7c (90.0%,45/50) rep15 (82.0%,41/50) rep 19 (80%,40/50) and rep10. (72.0% ,36/50)

For MSSA the most abundant plasmids were rep5a (75.5%,40/53) and rep16 (69.8%,37/53).

Among all rep family observed rep5a, rep16, rep20, and repUS70 carry the *blaZ* resistance gene for β-lactams, rep10 carries the *erm(C)* resistance gene for macrolides and rep7a carries *tet(K)* resistance gene for tetracyclines (Table 3).

Resistance gene carriage among MRSA and MSSA

Among all 103 *S. aureus* isolates, the most prevalent resistance genes were 100/103 (97.1%) *blaZ* gene which codes for β-lactamase(79/103 ,76.7%) *dfrG* gene which codes enzyme for Folate synthesis inhibitors, (48/103 ,46.6%) *aac(6')-aph(2")* gene which codes enzyme for aminoglycosides resistance, (46/103 ,44.7%) *erm(C)* gene which codes enzyme for macrolides antibiotics resistance, and (36/103 ,35.0%) *tet(K)* gene which code enzyme for tetracycline resistance. Other observed resistance genes were below 3.0%. There were statistically significant differences (p value<0.05) in the distribution of resistance gene *dfrG*, *erm (C)*, *aac(6')-aph(2")* and *tet(K)* among MRSA and MSSA isolates of which, high proportional were observed among MRSA (Table 4).

Table 2 Proportional of mecA gene (MRSA gene) among different participants and clinical characteristics ($N=103$)

Variable	*n* (%)	mecA gene		[a]*p*-value
		Yes	No	
Age				
<18	21 (20.4)	8 (38.1%)	13 (61.9%)	0.549
18–34	42 (40.8)	22 (52.4%)	20 (47.6%)	
>34	40 (38.8)	20 (50.0%)	20 (50.0%)	
Sex				
Male	57 (55.3)	25 (43.9%)	32 (56.1%)	0.29
Female	46 (44.7)	25 (54.3%)	21 (45.7%)	
Patient category				
Outpatients	38 (36.9)	23 (60.5%)	15 (39.5%)	0.063
Inpatients	65 (63.1)	27 (41.5%)	38 (58.5%)	
Nature of the sample				
Stool	1 (1.0)	1 (100%)	0 (0.0%)	0.154
Blood	17 (16.5)	11(64.7%)	6 (35.3%)	
Peritoneal fluid	1 (1.0)	0 (0.0%)	1 (100%)	
[b]HVS	5 (4.9)	1 (20.0%)	4 (80.0%)	
Urine	13 (12.6)	9 (69.2%)	4 (30.8%)	
Pus wound swab	65 (63.1)	28 (43.1%)	37 (56.9%)	
Sputum	1 (1.0)	0 (0.0%)	1 (100%)	
Health facility				
Dodoma Regional Referral Hospital	22 (21.4)	9 (40.9%)	13 (59.1%)	0.029
Morogoro Regional Referral Hospital	32 (31.1)	23 (71.9%)	9 (28.1%)	
Mnazi mmoja Hospital, Zanzibar	35 (34.0)	13 (37.1%)	22 (62.9%)	
Tabora Regional Referral Hospital	13 (12.6)	5 (38.5%)	8 (61.5%)	
Songea Regional Referral Hospital	1 (1.0)	0 (0.0%)	1 (100%)	
Total		50 (48.5%)	53 (51.5%)	

a- *p*-value for Pearson chi-square, [b]HVS- High vaginal swab

Table 3 Plasmid abundance and resistance gene carriage among MRSA and MSSA

Isolates	Plasmid	*n* (%)	Resistance gene in plasmid
MRSA (50)	**rep family**		
	rep5a	46 (92.0)	*blaZ*,
	rep5e	1 (2.0)	-
	rep7a	24 (48.0)	*tet(K)*
	rep7c	45 (90.0)	-
	rep10	36 (72.0)	**erm(C)**
	rep13	3 (6.0)	-
	rep15	41 (82.0)	-
	rep16	45 (90.0)	*blaZ*
	rep19	40 (80.0)	-
	rep20	2 (4.0)	*blaZ*
	repUS21	6 (12.0)	-
	Other plasmids		
	Col156	3 (6.0)	-
	Col440I	2 (4.0)	-
	FIA(pBK30683)	1 (2.0)	-
	IncFII(29)	1 (2.0)	-
	IncFIB(K)(pCAV1099-114)	1 (2.0)	-
MSSA (53)	**rep family**		
	rep5a	40 (75.5)	*blaZ*
	rep7a	11 (20.8)	*tet(K)*
	rep7c	5 (9.4)	-
	rep10	11 (20.8)	*erm(C)*
	rep16	37 (69.8)	*blaZ*
	rep20	4 (7.5)	*blaZ*
	repUS21	7 (13.2)	-
	repUS70	1 (1.9)	*blaZ*
	Other plasmids		
	Col156	1 (1.9)	-

n- number of *S. aureus* isolates with plasmid, MRSA-Methicillin Resistance *Staphylococcus aureus*, MSSA-Methicillin Sensitive *Staphylococcus aureus*

Table 4 Resistance gene carriage among MRSA and MSSA (*N* = 103)

Antibiotic class	AMR gene	*n* (%)	MRSA *n* (%)	MSSA *n* (%)	[a]*p*-value
Folate synthesis-inhibitor	*dfrG*	79 (76.7)	48 (60.8)	31 (39.2)	0.001
	sul2	3 (2.9)	1 (33.3)	2 (66.7)	0.593
Macrolides	*mph(A)*	1 (1.0)	0 (0.0)	1 (100)	0.329
	mph(E)	1 (1.0)	1 (100)	0 (0.0)	0.301
	msr(E)	1 (1.0)	1 (100)	0 (0.0)	0.301
	erm(C)	46 (44.7)	36 (78.3)	10 (21.7)	0.001
Aminoglycosides	*aac(6')-aph(2")*	48 (46.6)	47 (97.9)	1 (2.1)	0.001
	aph(3')-III	2 (1.9)	2 (100)	0 (0.0)	0.141
Tetracyclines	*tet(K)*	36 (35)	26 (72.2)	10 (27.8)	0.001
	tet(39)	2 (1.9)	1 (50.0)	1 (50.0)	0.967
Amphenicol	*cat(pC233)*	1 (1.0)	0 (0.0)	1 (100)	0.329
β-lactam	*blaZ*	100 (97.1)	49 (49.0)	51 (51.0)	0.593
	blaTEM-1B	1 (1.0)	0 (0.0)	1 (100)	0.329
	blaADC-25	2 (1.9)	1 (50.0)	1 (50.0)	0.967

a- *p*-value for Pearson chi-square

Multi-locus sequence typing (MLST), *SCCmec* typing and spa typing

Multi-locus sequence typing of all 103 *S. aureus* sequenced isolates shows that majority belong to ST8 (38.8%,40/103), ST152 (20.4%,21/103), ST 88 (11.7%,12/103), and ST 121 (7.8%,8/103). Other sequence types observed were below (5.0%,5/103)(Fig. 1).

"ST8 (36.9%,18/50) was exclusively found in MRSA whereas ST152 (20.4%,11/53) was exclusively found in MSSA" (Fig. 2).

SCCmec typing of all 50 MRSA isolates revealed that (86.0%,43/50)were *SCCmec* type IV based on homology to whole cassette, (10.0%,5/50) *SCCmec* type V based on *SCCmec* gene, (2.0%,1/50) *SCCmec* type IVa based on *SCCmec* gene and (2.0%,1/50) *SCCmec* type V based on homology to whole cassette (Fig. 3).

Spa typing was done for all *S. aureus* isolates in which the majority were t1476 (31.1%,32/103), t355 (18.4%,19/103) and t4333 (8.7%,9/103). The rest were below (6.0%,6/103), (7.8%,8/103) of isolates had unknown spa types from the database (Fig. 4).

All t1476 (31.1%,16/50) were found in MRSA and all t355 (18.4%,10/53) were found in MSSA (Fig. 5).

Phylogeny relatedness among MRSA and MSSA

The observed maximum single nucleotide polymorphism (SNP) differences for MRSA were 14,188 while for MSSA were 23,609 nucleotides and the minimum was zero(0) SNP for both. Six different clones in MRSA and MSSA with SNP differences of less than 25 nucleotides were observed. The clones observed for MRSA had a combination of ST88 and spa type t4333, ST8 and spa type t1476, and ST8 and spa type t498 (Fig. 6). For MSSA observed clone had a combination of ST152 and spa type t355, ST122 and spa type t4499, and finally ST88 and spa type t4333 (Fig. 7).

Discussion

The present study aimed at genomic characterization of Methicillin resistance *Staphylococcus aureus* isolated from patients attending regional referral hospitals in Tanzania by the use of whole genome sequencing technology. Both MRSA and MSSA were sequenced for mec A gene, Plasmid abundance, and resistance gene carriage, Multi-locus sequence typing (MLST), SCCmec typing, and spa typing *MRSA* is an important infectious agent in hospital and community settings, genomic characterization is important for disease diagnosis, treatment, infectious

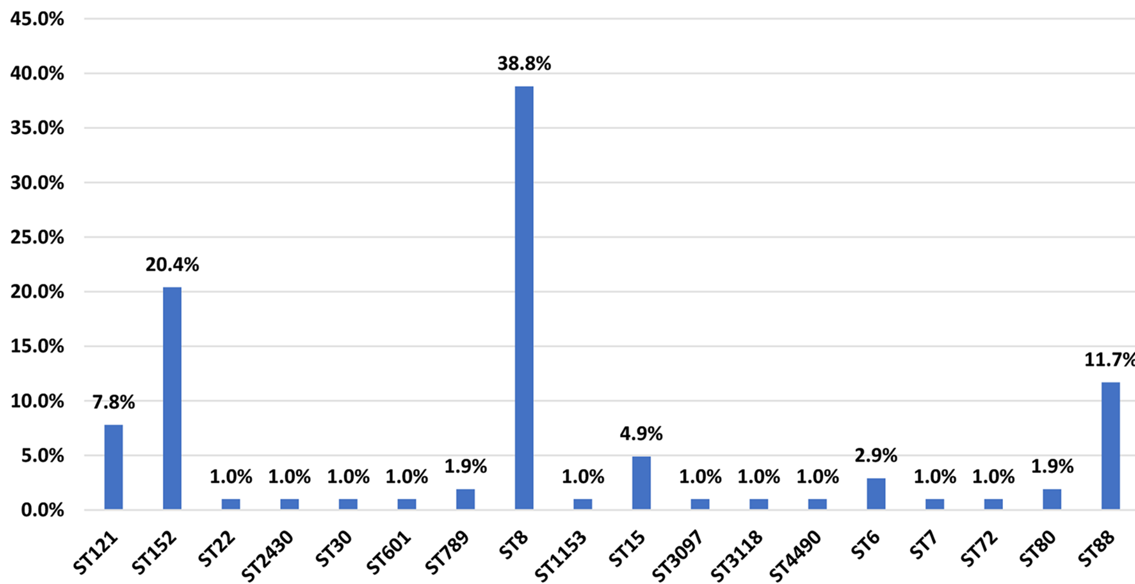

Fig. 1 MLST of all sequenced *S. aureus* isolated ($N=103$)

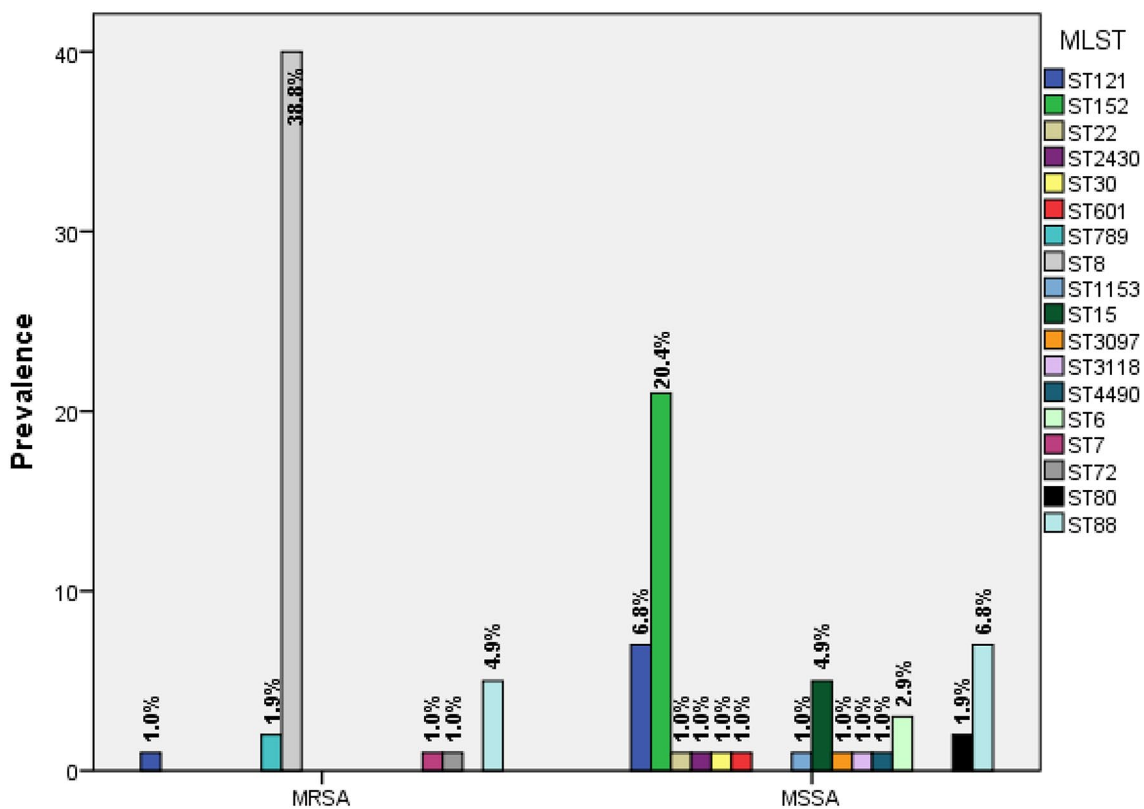

Fig. 2 Sequence type distribution among MRSA and MSSA

control prevention of nosocomial outbreaks, and surveillance systems.

Whole genome sequencing of the collected *S. aureus* isolates showed that about (48.5%,50/103) of the isolates carry resistance gene *mecA* for MRSA (Table 2). There were no significant differences in the proportion of *mecA* gene (MRSA gene) among different demographic and clinical characteristics; However, the proportion of MRSA observed from different health facilities varied significantly (p-value=0.029), portraying the role of environmental factors. These results deviate from a previous study conducted in Tanzania [16, 17, 36], but the results

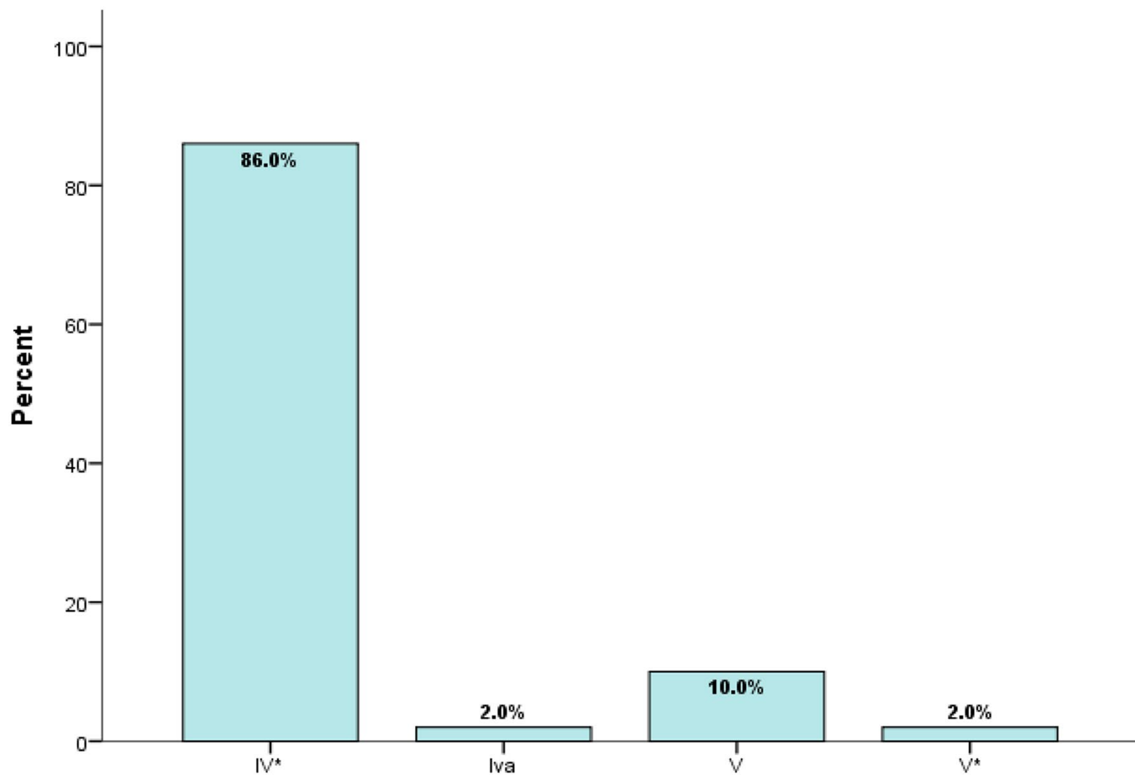

Fig. 3 SCCmec type of the sequenced MRSA ($N=50$). * No *SCCmec* element was detected, prediction was based on homology to the whole cassette

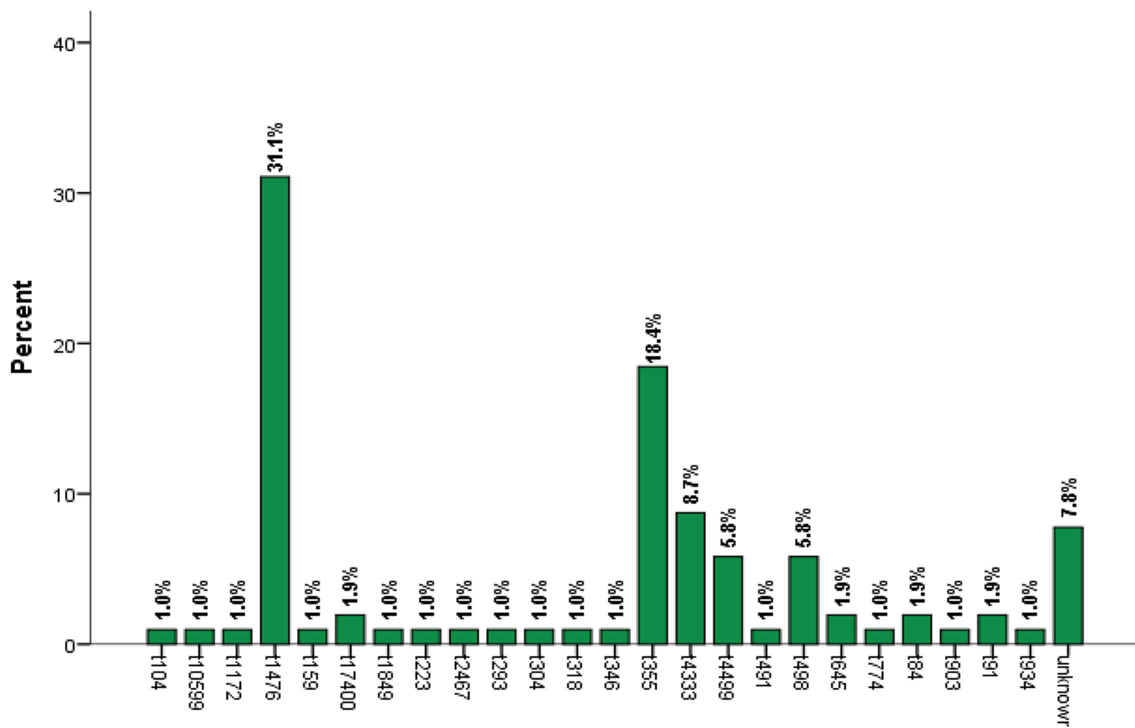

Fig. 4 Spa typing of all S. aureus isolates ($N=103$)

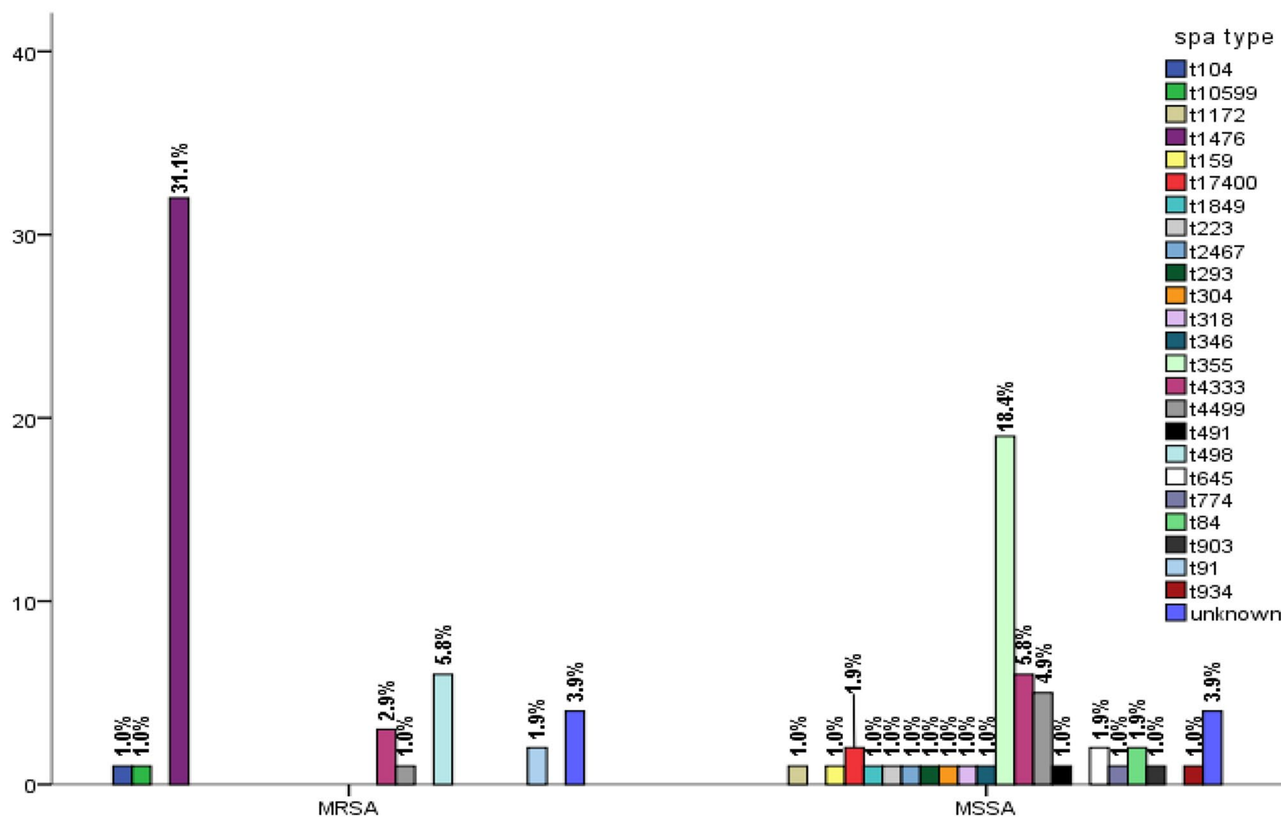

Fig. 5 Spa type distribution among MRSA and MSSA

are in line with a study from Kenyatta National Hospital Kenya [15]. Variations in the prevalence of MRSA in Africa have also been reported [37]. The observed differences could be attributed to different environmental and genetic factors in which hospital-related MRSA and community-related MRSA share genes by horizontal gene transfer creating diversity [38, 39]. Also, a high proportion of MRSA in this study could be due to a large study area when compared to other studies focusing on a single hospital. The observed high proportion of MRSA isolates is associated with increasing disease complications, high treatment costs, and more morbidity and mortality rates in the studied sites.

The present study also reported different plasmid-mediated drug carriage among fifty(50) MRSA isolates and ffty three(53) MSSA isolates. Plasmids rep5a, rep16, rep7c, rep15, rep 19, and rep 10 were most abundant among MRSA while for MSSA the most abundant plasmids were rep5a and rep16. Among all rep families observed rep5a, rep16, rep20, and repUS70 carried the *blaZ* gene, rep10 carried the *erm(C)* gene, and rep7a carried the *tet(K)* gene (Table 3). These resistance genes confer resistance to antibiotics such asβ-lactams, macrolides, and tetracyclines antibiotics respectively. A study conducted in South Africa also reported that all MRSA isolated were plasmid positive in which 74.1% of MRSA isolates from clinical samples carried plasmid with multidrug resistance [40]. Factors that favor plasmid-carried resistance genes have not been fully established. However, the interval between antibiotic treatment and antibiotic dosage can accelerate either the plasmid-carrying resistance gene or the chromosome-carrying resistance gene [41].

Among all 103 sequenced *S. aureus* isolates, the most prevalent chromosomal carried resistance genes were 97.1% *blaZ* gene which codes for β-lactamase enzyme which confers resistance to beta lactam antibiotics 76.7% *dfrG* gene which codes for enzymes that confer resistance against Folate synthesis-inhibitors 46.6% *aac(6')-aph(2")* gene which codes for enzymes resistant to aminoglycosides, *erm(C)* gene which code for enyme conferring resistance to macrolides antibiotics, and 35.0% *tet(K)* gene which code for enzymes resistant to tetracycline antibiotics (Table 4). Another report from northern Tanzania also showed that among all sequenced MRSA, almost all had the same resistance gene as reported in this study [17]. There was a significant difference in the distribution of resistance genes among MRSA and MSSA. A high proportion was observed among MRSA isolates. Other resistance genes observed were below 3.0% *(sul2, mph(A), mph(E), msr(E), aph(3')-III, tet(39), cat(pC233), blaTEM-1B and blaADC-25)* this may be

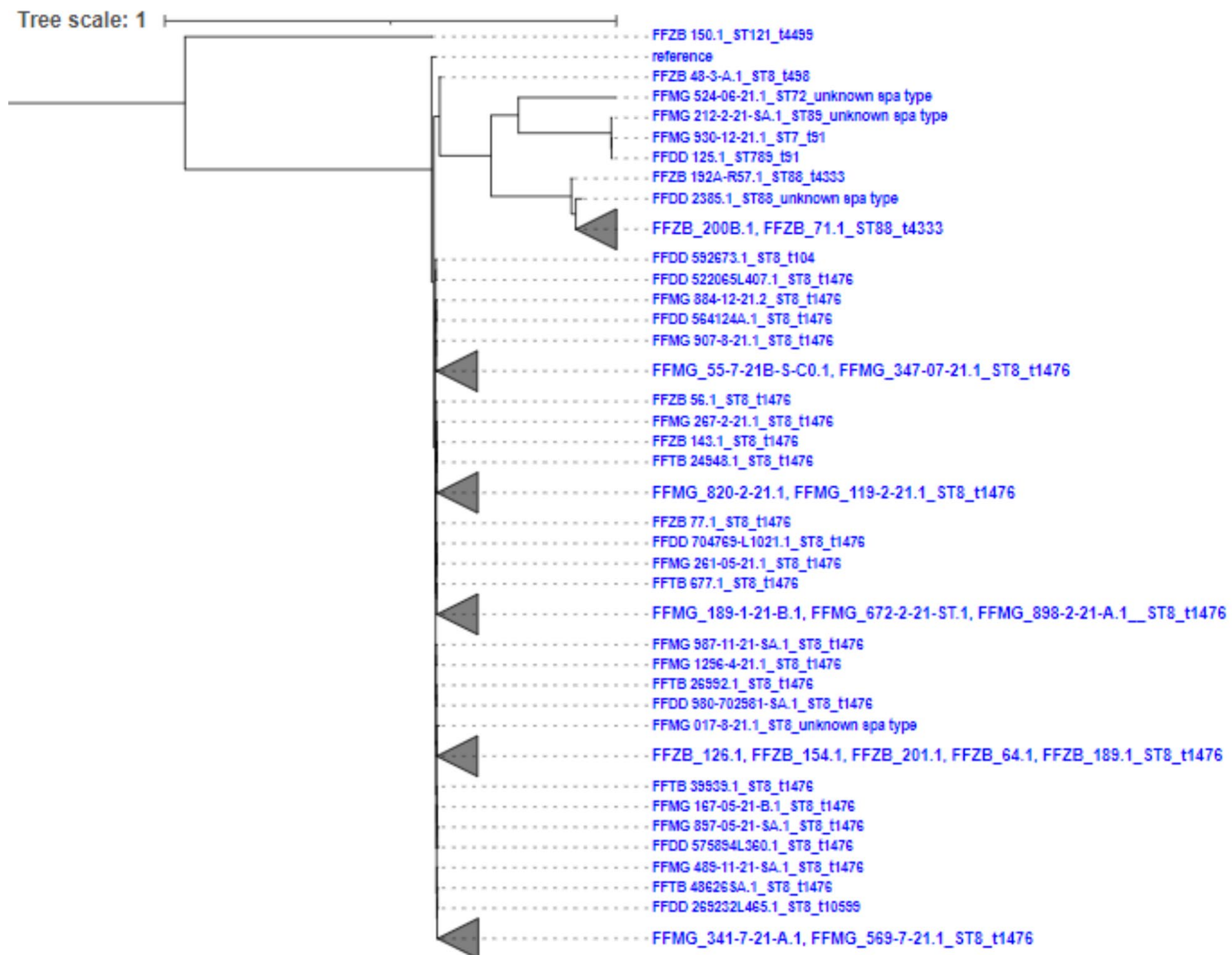

Fig. 6 Phylogeny analysis of 50 MRSA isolates with their sequence type and spa type

due to low consumption of antibiotics targeting these genes for treatment of *S. aureus* infections [42]. There was no resistance gene to vancomycin antibiotics which is the reserved drug for MRSA treatment in Tanzania. This brings hope in handling MRSA infection although, drug misuse and cross-contamination of microorganisms in hospital settings can lead to the development of resistance.

The present study also reports high variations among *S. aureus* in which 18 different sequence types were observed (ST) with the prevalent being 36.9% ST8, 20.4% ST152, 8.7% ST 88, and 6.8% ST 121. (Fig. 1). The results are in line with a study conducted in Kilimanjaro Tanzania, which also reports ST8 as the most predominant sequence type among MRSA [17]. The presence of novel ST indicates genetic changes in *S. aureus* isolates by mutation and HGT. High diversity among *S. aureus* gives them the ability to sustain different environmental conditions [43, 44]. The predominant ST8 observed has been reported in other studies to be associated with community-acquired MRSA (CA-MRSA) [45, 46]. This has been confirmed by *SCCmec* typing of MRSA isolates which also reveal that all MRSA belong to mec type IV and V (Fig. 3), indicating that they are CA-MRSA. The results deviate from the report of Iran which reports mec type III and II as the most predominant [47]. However, the results are in line with the study from Pakistan [48].

Spa typing also revealed the majority of *S. aureus* isolates were t1476, t355, and t4333 (Fig. 4). A study from Tanzania among HIV patients also reports spa type t1476, ST8, and mec type IV as the most predominant of which all isolates were negative for Panton-Valentine Leucocidin (PVL) [49].

Phylogeny analysis among MRSA and MSSA also reveals variations among the *S. aureus* isolates in which the observed maximum SNP differences for MRSA were 14,188 while for MSSA were 23,609 nucleotides. Six different clones for MRSA and MSSA were observed, clones observed for MRSA had a combination of ST88 and spa type t4333, ST8 and spa type t1476, and ST8 and spa type

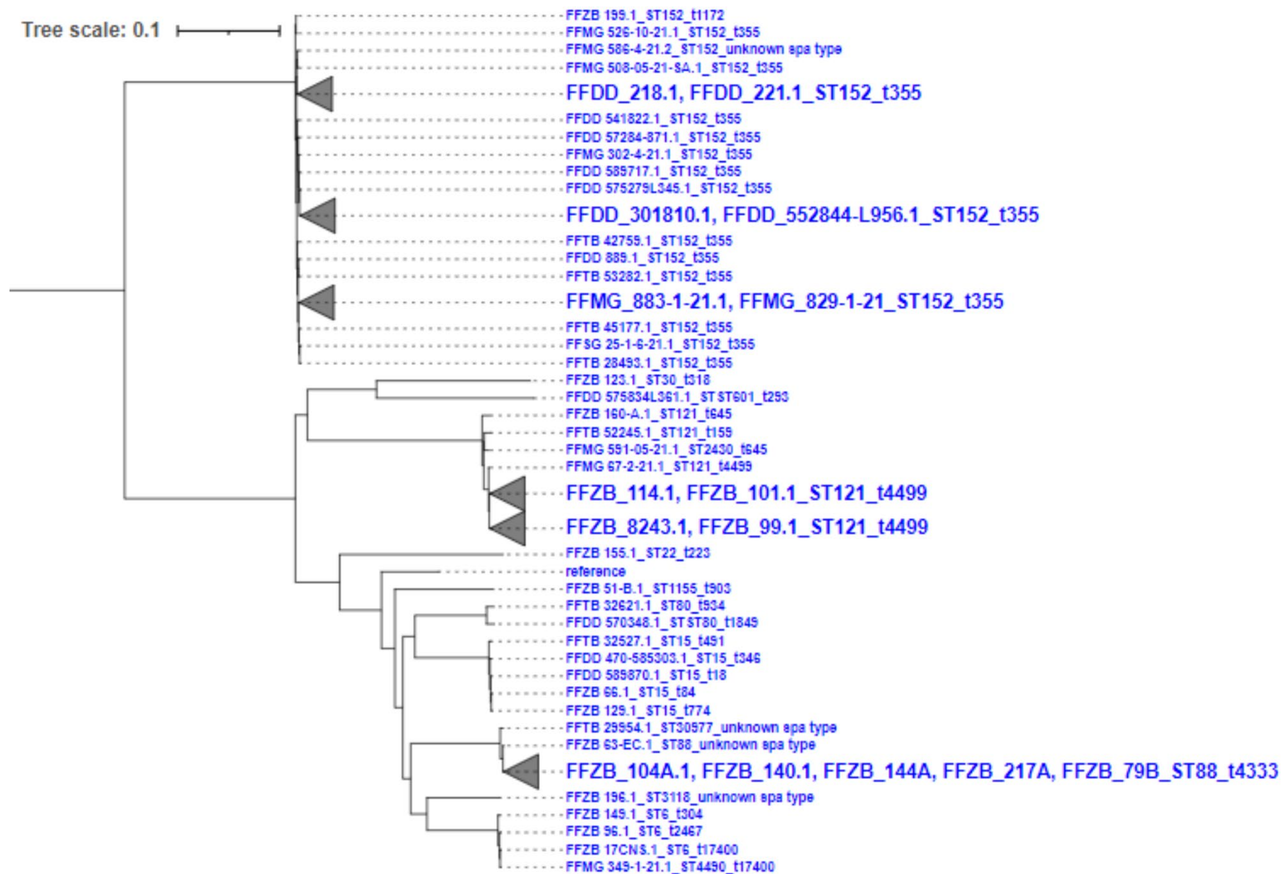

Fig. 7 Phylogeny analysis of 53 MSSA isolates with their sequence type and spa type

t498. For MSSA observed clone had a combination of ST152 and spa type t355, ST122 and spa type t4499, and finally ST88 and spa type t4333 (Figs. 6 and 7). Clones with ST8 and spa type t1476 were also reported in Kilimanjaro and Dar es Salaam Tanzania [17, 49]. The existence of the same clone in other regions indicates clonal transmission due to the movement of people since results also showed that these clones are community-acquired.

The clinical implications for the observed results are that treatment options and management for the *S. aureus* infection should be reviewed mostly in low- and middle-income countries including Tanzania, whereby, depending on the site and severity of infection, there are different antibiotics used to treat MRSA infections. These include but are not limited to Linezolid, Clindamycin, Trimethoprim-sulfamethoxazole, Tetracycline, Rifampin, etc. Additionally, observed findings suggest proper use of antibiotics to reduce the development of resistance. Infected individuals should be handled well in hospital settings to prevent the spread of highly diverse microorganisms in particular *S. aureus.* Nevertheless, this study also had limitations, such as, it only focused on Plasmids analysis, multi-locus sequence typing (MLST), SCCmec typing, and spa typing without looking at virulence factors analysis which could reveal more information regarding the virulence status.

Conclusion

The study reveals a significant presence of MRSA in Staphylococcus aureus strains from Tanzanian regional hospitals, with nearly half carrying the mecA gene. MRSA is notably prevalent among young adults, females, and outpatients, showing high genetic diversity and dominance of ST8. Various plasmids carrying resistance genes indicate a complex resistance profile, highlighting the need for targeted interventions to manage MRSA infections in Tanzania.

Community health education has to be given to society since all observed MRSA isolates were community-acquired. Even though observed MRSA had an abundance of plasmid and resistance genes, there were no resistance genes for vancomycin antibiotics, suggesting that Vancomycin remains the first-line drug for the management of MRSA infections in Tanzania.

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Author contributions
MG, ES, TS, HK, and develop an idea and present it to all others. All authors prepare and read the concept note. LK, SM, and BW conduct DNA sequencing together with MB, MZ, MJD, DK and BM for data analysis and interpretation. MG was the major contributor in writing the manuscript together with discussion from all authors. All authors read and approve the final manuscript draft.

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Data availability
The assembled S. aureus genome from this study has been submitted to the European Nucleotide Archive with project accession number PRJEB71932.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of National Institute for Medical Research (NIMR) in Tanzania, and assigned number NIMR/HQ/R.8a/Vol.IX/3273. Informed consent was obtained from all subjects or their legal guardian before using the sample for research purposes under SeqAfrica project.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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