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Research Article

Multiplex PCR detection of antibiotic resistance and virulence genes in multidrug-resistant *Staphylococcus aureus* isolated from chickens, humans, rodents, and soil in Northern Tanzania

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Abstract

Staphylococcus aureus (*S. aureus*) is a zoonotic pathogen with public health and veterinary importance. We investigated the presence of antibiotic resistance genes (ARGs) and virulence genes (VGs) in 57 multidrug-resistant (MDR) *S. aureus* isolated from humans (n=17), chickens (n=14), rodents (n=13), and soil (n=13) using multiplex PCR. Overall, the distribution of ARGs revealed that the *tetK* was found in 18/57 (31.6%), *mecA* in 16/57 (28.1%), *tetL* in 5/57 (8.9%), and *ermC* in 1/57 (1.8%), while *ermA* and *tetM* were not detected. For VGs, the *clfB* was found in 6/57 (10.5 %), *coa* in 8/57 (14.0%), *clfA* in 3/57 (5.3%), *hlg* in 1/57 (1.8%), *ebpS* in 2/57 (3.5%), *fnbB* in 2/57 (3.5%), *luk-PV* in 6/57 (10.5%) and *tst* in 1/57 (1.8%). Resistance genes (*tetK* and *mecA*) and virulence determinants (*clfB*, *coa*, and *luk-PV*) were common in all sample sources, while *tst*, *hlg*, and *fnbB* were specific to human, chicken, and rodent isolates, respectively. Erythromycin phenotypic resistance results correlated with the presence of *ermC* (r=0.42), *tetL* (r=0.98), and *mecA* (r=0.51), while tetracycline resistance correlated with *tetL* (r=1.00) and *mecA* (r=0.57) genes and methicillin resistance correlated with *mecA* (r=0.55) and *tetL* (r=0.98) genes. Positive correlations were noted between ARG (*ermC*) and VGs; *clfA* (r=0.57), *hlg* (r=1.00), and *clfB* (r=0.43), and between *tetK* and *clfB* (r=0.39); *tetK* and *coa* (r=0.36) genes. Principal component analysis (PCA) shows that *tetL*, *ermC*, and *mecA* contributed to tetracycline, erythromycin, and methicillin resistance, respectively. The widespread presence of resistance and virulence genes, often in combination, among MDR *S. aureus* in isolates from humans, chicken, rodents, and soil samples require comprehensive One-Health interventions.

Keywords: AMR genes, Chicken, Humans, Multidrug-resistant, Rodents, *Staphylococcus aureus*

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Introduction

Staphylococcus aureus (*S. aureus*) is a globally recognized opportunistic bacteria that colonizes humans, and animals and is found in the environment (Katakweba et al., 2016; Wang et al., 2017). In humans, *S. aureus* causes a wide spectrum of infections ranging from skin and soft tissue to life-threatening infections such as pneumonia, osteomyelitis, endocarditis, and bacteremia (Kayili and Sanlibaba, 2020; Cheung et al., 2021). *S. aureus* is the leading cause of mastitis in dairy animals (Kashoma et al., 2015) and causes several diseases in chickens, including omphalitis, dermatitis, and arthritis (Abou-Zahr et al., 2018; Gornatti-Churria et al., 2018; Benrabia et al., 2020).

Drug-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) strains, cause infections that are difficult to treat and have been associated with higher mortality and morbidity rates (Watkins et al., 2012), and are on the World Health Organization (WHO) list of high priority antibiotic-resistant bacteria (Savoldi et al., 2019). The carriage of virulence factors confers some evolutionary benefit to bacteria, which favors resistant strains (Derakhshan et al., 2021). *S. aureus* infections have been associated with several

virulence factors, including; hemolysins (*hla*, *hlb*, *hld* genes), staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see* genes), toxic shock syndrome toxin-1 (TSST-1) and Panton-Valentine Leucocidin (PVL) (*lukF* and *lukS* genes) (Liu et al., 2014; Zhao et al., 2016; Li et al., 2021).

Molecular techniques like Multiplex PCR are important in the detection of resistance genes, including *ermA*, *ermB*, *tetA*, and *tetD* (Adwan, 2013), as well as virulence factors such as *hla*, *hlb*, *hld*, *sea*, *seb*, *sec*, *lukF* and *lukS* (Ote et al., 2011; Zhao et al., 2016; Hait et al., 2021). Antibiotic resistance is recognized as a quintessentially One-Health issue, involving the cross-transmission of resistant bacteria or their resistance genes between humans, animals, aquaculture, and the environment through their interactions (Ampaire et al., 2016; Mouiche et al., 2019).

Genetic determinants of antimicrobial resistance, often located on mobile genetic elements, can be easily transmitted among different hosts, including humans, animals, and the environment (Baquero et al., 2019). Indeed, antimicrobial resistance studies using a One-Health approach have found similarities in multidrug-resistance genes in many important and common pathogens such as *E. coli*, *K. pneumoniae*, and *S. aureus*, suggesting their potential transmission between humans, animals, aquaculture, and the environment (van Duin and Paterson, 2016). The interaction between these compartments, governed largely by human anthropogenic activities, is key in spreading of antimicrobial resistance (AMR) genes that can be depicted by molecular studies (Nathan and Cars, 2014).

In Tanzania, several studies conducted in Karatu in the Northern part of the country have revealed intense interactions between rodents, humans, and livestock, with the occurrence of outbreaks of zoonoses, including plague (Kilonzo et al., 2006; Makundi et al., 2008; Ziwa et al., 2013b; Makundi et al., 2015). Molecular studies specifically assessing the occurrence and distribution of genes encoding for antimicrobial resistance and virulence factors have not been done. Knowledge regarding genetic diversity, antimicrobial resistance, and virulence factors is needed to effectively control the spread of antimicrobial resistance, given the ability of *S. aureus* to acquire antimicrobial resistance determinants and extensive virulence factors (Sonola et al., 2021).

We therefore undertook this study to determine and compare profiles of antimicrobial resistance and virulence genes among multidrug resistance (MDR) *S. aureus* strains isolated from chickens, humans, rodents, and soil in Karatu, Northern Tanzania, where such interactions are intense.

Materials and methods

Bacterial isolates

A total of 57 MDR *S. aureus* isolates from chicken cloaca swabs (n=14), human nasal swabs (n=17), rodents deep pharyngeal swabs (n=13), and household soil (n=13) samples were preserved in tryptic soy broth (TSB) with 50% glycerol (v/v) at -80°C, pending DNA extraction. These isolates exhibited phenotypic resistance to at least three different classes of antibiotics.

Genomic DNA extraction

Isolates were subcultured on nutrient broth media (NB, Merck, Germany) and incubated at 37°C for 24h. Genomic DNA was extracted using Zymo Research Fungal and Bacterial Genomic DNA MiniPrepTM kit (Zymo Research, Irvine, USA), according to the manufacturer's recommendations. The extracted DNA's purity, quality, and quantity were determined using a Nanodrop device (NanoDrop, Thermo Scientific, USA), gel electrophoresis, and spectrophotometer. The extracted genomic DNA was stored at -80°C pending PCR analyses.

Detection of antibiotic resistance and virulence genes

Multiplex PCR was used to amplify the resistance (*tetK*, *tetL*, *tetM*, *ermA*, *ermC*, and *mecA*) and virulence (*clfB*, *cna*, *coa*, *clfA*, *hlg*, *ebpS*, *fnbB*, *luk-PV*, and *tst*) genes according to the previously described protocols (Ote et al., 2011; Zhao et al., 2016).

The primers used for PCR amplification of different genes are listed in Table 1. Lyophilized primers (Macrogen, Amsterdam, The Netherlands) for targeted genes were reconstituted using DNase/RNase-free sterile water to obtain 100 μM stock solutions stored at -20°C and diluted to a working concentration of 10 μM. The cycling conditions for all reactions are shown in Table 2. PCR products were run on 1.5% (w/v) agarose gel using electrophoresis, stained with gel red (Merck, Darmstadt, Germany) at 120 Volts for one hour, and visualized under UV light using a BioDoc-it™ imaging system (Ultra-Violet Products, Cambridge, UK) by using GeneRuler 100 bp Plus DNA Ladder (Bioneer, Republic of Korea).

Statistical analysis

The data obtained were arranged and entered into an Excel spreadsheet (Microsoft® Office Excels 2010) and analyzed. The differences in prevalence of the genes (%) between categories were compared by Chi-square test while distributions and relationships among the genes in their respective sources of isolates were managed by principal component analysis (PCA). All statistical analyses were performed using R software, and any *p-value* less than 0.05 was considered significant.

Results

Distribution of resistance genes among MDR *S. aureus* isolates from different sample sources

As shown in Table 3, the overall distribution of resistance genes was as follows: *tetK* (31.6%), *mecA* (28.1%), *tetL* (8.9%), and *ermC* (1.8%). These genes were detected in isolates from human 12/17 (70.6%), chicken 14/14 (100.0%), rodent 6/13 (61.5%), and soil 6/13 (46.2%) samples (Figure 1).

Table 1: List of primers that were used for the detection of resistance and virulence genes among the MDR *S. aureus* isolates.

Targeted gene	Primer name	Primer sequence (5'-3')	Amplicon size (bp	Annealing temperature (°C)	Reference
tetK	*tetK*-1	TTAGGTGAAGGGTTAGGTCC	360	55	Abdolmaleki et al. (2019)
	tetK-2	GCAAACTCATTCCAGAAGCA			
tetL	*tetL*2-2	ATAAATTGTTTCGGGTCGGTAAT	1077	55	Trzcinski et al. (2000)
	*tetL*2-1	AACCAGCCAACTAATGACAAGAT			
tetM	*tetM*-1	GTCCGTCTGAACTTTGCGGA	158		
	tetM-2	GCGGCACTTCGATGTGAATG			
ermA	Tn554-1(*ermA*)	AAGCGGTAAACCCCTCTGA	139	55	Sidhu et al. (2002)
	Tn554-2 (*ermA*)	TTCGCAAATCCCTTCTCAAC			
ermC	*ermC*-2	AATCGGCTCAGGAAAAGG	562	55	Pérez-Serrano et al. (2020)
	ermC-1	ATCGTCAATTCCTGCATG			
mecA	*mecA*-1F	AACTCTGTTATTAGGGAAGAACA	293	55	Abdolmaleki et al. (2019)
	mecA-2R	CCACCTTCCTCCGGTTTGTCACC			
ebpS	*ebpS*-1	CATCCAGAACCAATCGAAGAC	186	55	Peacock et al. (2002)
	ebpS-2	CTTAACAGTTACATCATCATGTTTATCTTTG			
cna	*cna*-1	AGTGGTTACTAATACTG	560		
	cna-2	CAGGATAGATTGGTTTA			
clfA	*clfA*-1	ATTGGCGTGGCTTCAGTGCT	292	55	Tristan et al. (2003)
	clfA-2	CGTTTCTTCCGTAGTTGCATTTG			
clfB	*clfB*-1	ACATCAGTAATAGTAGGGGGCAAC	203		
	clfB-2	TTCGCACTGTTTGTGTTTGCAC			
coag	*coag*-1	ACCACAAGGTACTGAATCAACG	812	55	Mullarky et al. (2001)
	coag-2	TGCTTTCGATTGTTCGATGC			
luk-PV	*luk-PV*-1	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	55	Jarraud et al. (2002)
	luk-PV-2	GCATCAASTGTATTGGATAGCAAAAGC			
hlg	*hlg*-1	GCCAATCCGTTATTAGAAAATGC	938		
	(*hlg*-2	CCATAGACGTAGCAACGGAT			
tst	*tst*-1	CATCTACAAACGATAATATAAAGG	476	55	Vannuffel et al. (1995)
	tst-2	CATTGTTATTTTCCAATAACCACCCG			
fnbB	*fnbB*-1	GTAACAGCTAATGGTCGAATTGATACT	523	55	Tristan et al. (2003)
	tst-2	CAAGTTCGATAGGAGTACTATGTTC			

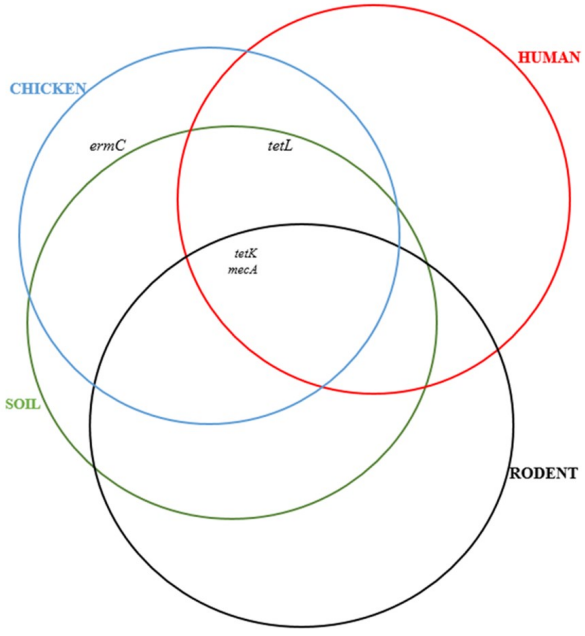

Figure 1: Comparative occurrence of resistance genes among MDR *S. aureus* isolates from all sources.

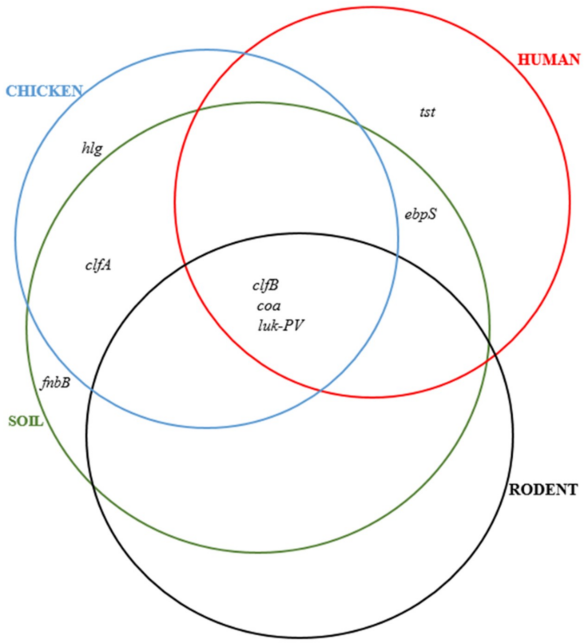

Figure 2: Comparative occurrence of virulence genes among MDR *S. aureus* from all samples.

Table 2: The protocol used during multiplex PCR amplification of resistance and virulence genes of MDR *S. aureus* isolates.

Program	Amplified genes	Initial denaturation	Denaturation	Annealing	Primer extension	Final extension	Amplification cycles
PCR 1	*ermA*	94°C, 1 min	94°C, 1 min	55°C, 1 min	72°C, 1 min	72°C, 10 min	25
	ermC						
	tetK						
PCR 2	*mecA*	94°C, 3 min	94°C, 30 sec	55°C, 30 sec	72°C, 30 sec	72°C, 3 min	30
	tetL						
	tetM						
PCR 3	*fnbB*	94°C, 5 min	94°C, 1 min	55°C, 1min	72°C, 1 min	72°C, 10 min	25
	clfA						
	clfB						
	cna						
	ebpS						
	coa						
PCR 4	*tst*	94°C, 5 min	94°C, 30 sec	55°C, 1 min	72°C, 1 min	72°C, 10 min	30
	luk-PV						
	hlg						

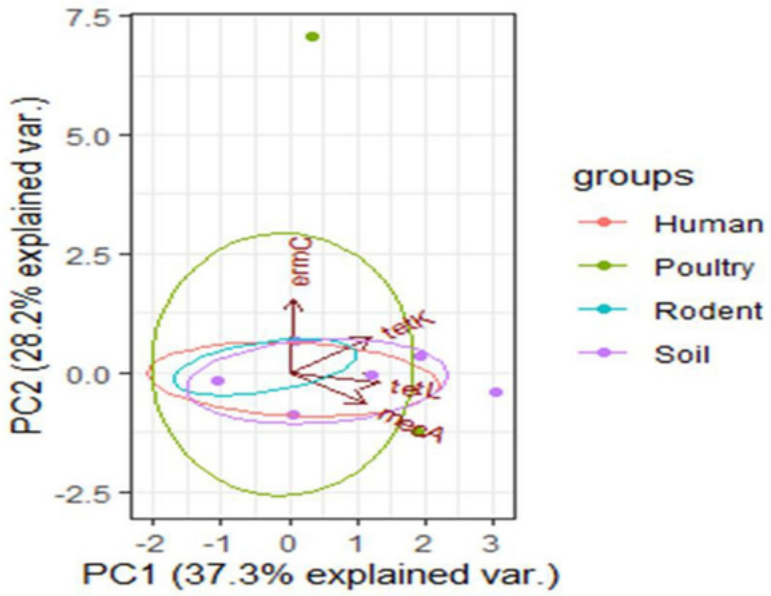

Figure 3: Principal component analysis of resistance genes of MDR *S. aureus*. The dots represent isolates from different sample sources, arrows indicate vectors for resistance genes, and ellipses show a 95% confidence interval for all samples of a particular source.

Table 3: Prevalence of antibiotic resistance genes in MDR *S. aureus* isolates from different samples .

Genes	Different types of sample sources no. (%)				
	Human (n=17)	Chicken (n=14)	Rodent (n=13)	Soil (n=13)	Total isolates (n=57)
ermC	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	1 (1.8)
tetK	5 (29.4)	5 (35.7)	6 (46.2)	2 (15.4)	18 (31.6)
tetL	3 (17.6)	2 (14.3)	0 (0.0)	0 (0.0)	5 (8.9)
mecA	4 (23.5)	6 (42.9)	2 (15.4)	4 (30.8)	16 (28.1)
Total	12 (70.6)	14 (100.0)	8 (61.5)	6 (46.2)	40 (70.2)
Chi-square	32.6	37.5	29.5	20.67	
p-value	0.001	0.001	0.001	0.002	

PCR Amplification of virulence genes

The PCR shows positive bands for *clfB* (10.5%), *coa* (14.0%), *clfA* (5.3%), *hlg* (1.8%), *ebpS* (3.5%), *fnbB* (3.5%), *luk-PV* (10.5%) and *tst* (1.8%) genes (Table 4). These genes were distributed in isolates from human 7/17 (41.2%), chicken 6/14 (42.9%), rodent 5/13 (38.5%), and soil 11/13 (84.6%) samples, as shown in Figure 2.

We observed multiple occurrences of up to three genes among isolates from human, chicken, and soil samples. Isolates from rodents had a maximum of two genes. For virulence determinants, combinations of up to four genes were common in chicken isolates, while co-occurrence of three genes was common in human and soil isolates (Table 5).

As shown in figures 1 and 2, resistance genes (*tetK* and *mecA*) and virulence genes (*clfB*, *coa*, and *luk-PV*) were common in isolates from all sample sources. However, virulence genes *tst*, *hlg*, and *fnbB* were specific to human, chicken, and rodent isolates respectively.

Correlation between phenotypes and genotypes AMR results

We found positive correlations between erythromycin resistance and *ermC* (r=0.42), *tetL* (r=0.98) and *mecA* (r=0.51), tetracycline with *tetL* (r=1.00) and *mecA* (r=0.57) and methicillin with *mecA* (r=0.55) and *tetL* (r=0.98) (Table 6).

As shown in Table 7, we found positive correlations between resistance (*ermC*) and virulence genes; *clfA* (r=0.57), *hlg* (r=1.00) and *clfB* (r=0.43), *tetK*, and *clfB* (r=0.39); *tetK* and *coa* (r=0.36), while other correlations were weak and negative (Table 7).

Principal component analysis (PCA) for resistance genes

Along PC1 (37.3% explained variance), the vectors for *tetL* and *mecA* genes each form a small angle with the x-axis, indicating that the genes had a higher contribution to tetracycline and methicillin resistance of MDR *S. aureus* isolates. The small angle between these vectors indicates greater and positive correlations between

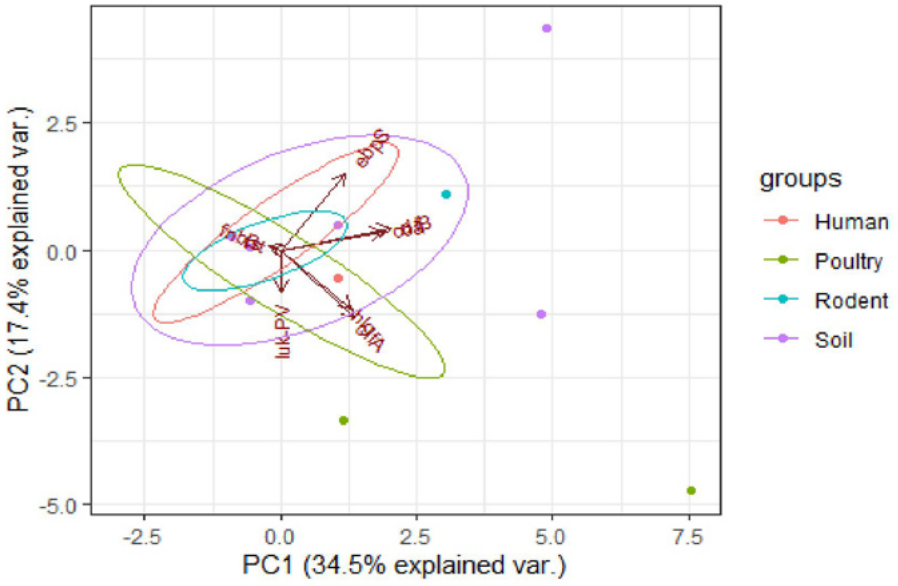

Figure 4: Principal component analysis of virulence genes of MDR *S. aureus* isolates. The dots represent isolates from different sample sources, arrows indicate vectors for virulence genes, and ellipses show a 95% confidence interval for all samples of a particular source.

Table 4: Distribution of virulence genes in MDR *S. aureus* isolates from different samples.

Genes	Samples number (%)				
	Human (n=17)	Chicken (n=14)	Rodent (n=13)	Soil (n=13)	Total isolates (n=57)
clfB	2 (11.8)	1 (7.1)	1 (7.1)	2 (15.4)	6 (10.5)
coa	2 (11.8)	1 (7.1)	2 (15.4)	3 (23.1)	8 (14.0)
clfA	0 (0.0)	2 (14.3)	0 (0.0)	1 (7.7)	3 (5.3)
hlg	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	1 (1.8)
ebpS	1 (5.9)	0 (0.0)	0 (0.0)	1 (7.7)	2 (3.5)
fnbB	0 (0.0)	0 (0.0)	0 (0.0)	2 (15.4)	2 (3.5)
luk-PV	1 (5.9)	1 (7.1)	2 (15.4)	2 (15.4)	6 (10.5)
tst	1 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)
Total	7 (41.2)	6 (42.9)	5 (38.5)	11 (84.6)	29 (50.9)
Chi-square	4.8	6.0	11.2	7.82	
p-value	0.5697	0.6472	0.1906	0.4514	

the genes. Considering PC2 (28.2% explained variance), the vector for *ermC* shows a greater and positive correlation with PC2, implying a higher contribution of the *ermC* gene to the erythromycin resistance of the isolates. The ellipses indicate that most of the resistance genes were found in isolates from chicken samples. The overlapping ellipses for rodents, humans, and soil indicate that the prevalence of resistance genes did not vary much across these sample sources (Figure 3).

Principal component analysis (PCA) for virulence genes

According to Figure 4, *clfB*, *coa*, *hlg*, and *clfA* genes each form a small angle with PC1 (34.5% variance), indicating their higher influence on the virulence of the MDR *S. aureus* isolates. The genes displayed greater positive correlations, particularly between *clfB* and *coa* and *hlg* and *clfA*. Like wisely, the *fnbB* gene had a higher contribution to the virulence of isolates but was negatively correlated with *hlg* and *clfA* genes. Along PC2 (17.4% variance), *luk-PV* and *ebpS* were close to the y-axis, showing their higher contribution to the virulence of the isolates. However, the vectors of these genes tended to opposite directions, indicating that *luk-PV* and *ebpS* genes are negatively correlated. The biplot shows a larger size of soil-ellipse followed by humans and rodents, extending in the positive quadrant, indicating that most of the virulence genes were found in soil isolates, followed by those from humans and rodents. The shrunk chicken ellipse extends downward in the negative quadrant, showing that MDR isolates from chicken samples had a comparatively lower prevalence of virulence genes.

Table 5: Co-occurrence between resistance and virulence genes of MDR *S. aureus* from different sample sources.

Sample ID	Source	Resistance genes	Virulence genes
EDM 2H	Human	*tetK*, *tetL*, *mecA*	–
EEG 23H		*tetK*, *mecA*	*clfB*, *coa*,*ebpS*
EEG 16H		*tetL*, *mecA*	–
KS 3H		*tetK*, *tetL*	*coa*, *luk-PV*
BSA 2P	Chicken	*tetK*, *tetL*, *mecA*	–
BSG 6P		*ermC*, *tetK*	*clfB*, *clfA*, *coa*, *hlg*
SL 1P		*tetK*, *mecA*	–
Ert 13P		*tetL*, *mecA*	–
Ert 12P		*mecA*	*clfA*, *luk-PV*
BSG 14Ra	Rodents	*tetK*, *mecA*	–
KM 3R2		*tetK*	*luk-PV*
Ert 10R2b		*tetK*	*luk-PV*
Ert 10Ra		*tetK*	*clfB*, *coa*
KS 4S	Soil	*tetK*	*clfB*, *coa*, *clfA*
Ert 9S		*mecA*	*luk-PV*
KS 14S		*mecA*	*fnbB*
KS 3S		*tetK*, *mecA*	*fnbB*
Ert 12S		*tetK*, *tetL*	*luk-PV*
Ert 13S		*tetK*, *tetL*	*clfB*, *coa*, *ebpS*
BSG 2S		*tetK*, *tetL*, *mecA*	–

Table 6: Correlation between resistance phenotypes and genotypes of MDR *S. aureus*.

Phenotypes of isolates	Correlation coefficients (r) between phenotypes and genotypes			
	Resistance genes of isolates			
	ermC	*tetK*	*tetL*	*mecA*
Erythromycin	0.42	0.49	0.98	0.51
Tetracycline	0.39	0.36	1.00	0.57
Methicillin	0.45	0.46	0.98	0.55

Table 7: Correlation between virulence and resistance genes of MDR *S. aureus*.

Virulence genes	Correlation coefficients (r) between virulence and resistance genes			
	Antibiotic resistance genes			
	ermC	*tetK*	*tetL*	*mecA*
clfB	0.43	0.39	0.05	-0.08
coa	0.36	0.36	0.16	-0.14
clfA	0.57	0.14	-0.10	0.01
hlg	1.00	0.17	-0.05	-0.09
ebpS	-0.03	0.24	0.20	0.08
fnbB	-0.03	0.04	-0.08	0.28
luk-PV	-0.05	0.20	0.19	0.01
tst	-0.02	-0.11	-0.05	-0.09

Discussion

According to the literature review, there is limited data regarding the genetic profile of AMR and virulence genes involving MDR *S. aureus* isolated from humans, poultry, rodents, and household soil in Tanzania or surrounding countries. This study was conducted in Karatu in Northern Tanzania, where interactions between them are very intense, with a likelihood for the spread of resistomes and virulence genes (Haule, 2013; Ziwa et al., 2013a,b; Makundi et al., 2015; Sonola et al., 2021). A phenotypic study conducted in Karatu indicated high levels of resistance to tetracycline, erythromycin, and clindamycin in samples collected from humans, poultry, rodents, and household soil (Sonola

et al., 2021).

Therefore, this study focused on determining the genetic basis of the observed phenotypic resistance and went further to find the type of virulence factors in the isolates. The resistance genes detected in our study were *ermC* (1.8%), *tetK* (31.6%), *tetL* (8.9%), and *mecA* (28.1%). These genes were found in all isolates from chicken (100%) followed by human (70.6%), rodent (61.5%), and soil (46.2%) samples. This finding is plausible with reports of extensive clindamycin, tetracycline, erythromycin, and amoxicillin-clavulanate resistance in poultry and humans in the Karatu district and other areas in northern Tanzania (Hassell et al., 2019; Gwenzi et al., 2021).

Tetracyclines remain the first-line treatment for many human and veterinary infections in many parts of the world, including Tanzania (Sangeda et al., 2021). Our findings show that tetracycline resistance was predominated by *tetK* genes (31.6%) compared with *tetL* genes (8.9%), which is in agreement with previous studies (Jamali et al., 2015; Safarpoor Dehkordi et al., 2017). The *tetK* and *tetL* encode an efflux pumping mechanism during *S. aureus* resistance to tetracycline (Lim et al., 2012). Overall, we found that 40.0% of MDR *S. aureus* isolates had the *mecA* gene, which is greater than the 29% reported by Silva et al. (2021) in their study on slaughtered quails.

In our study, the *ermC* gene was less frequently detected (1.8%), which is unlike other studies reporting prevalence rates ranging from 27.02% to 90.1% (Jamali et al., 2015; Safarpoor Dehkordi et al., 2017; Li et al., 2019; Silva et al., 2021). The lower prevalence of *ermC* genes in our study indicates that other mechanisms of resistance, such as drug efflux (mediated by *msrA* gene), rather than methylation of ribosomal sites of *S. aureus*, which is usually mediated by *ermC*, might have influenced the resistance of isolates to erythromycin and clindamycin (Vandendriessche et al., 2011). We noted a co-existence of resistance genes, where the combination of *tetK*, *tetL*, and *mecA* genes was the most common in most isolates. The *mecA* gene, which is carried on Staphylococcal Cassette Chromosome mec (SCCmec), is associated with genes encoding resistance to several non-β-lactam antibiotic classes, such as tetracyclines and aminoglycosides (Fatholahzadeh et al., 2008).

With regard to virulence genes, the most frequent genes were *clfB* (10.5%), *coa* (14.0%), *luk-PV* (10.5%), and *clfA* (1.8%). We found a lower prevalence of *hlg* (1.8%), *ebpS* (3.5%), *fnbB* (3.5%), and *tst* (1.8%). Most of the genes were found in isolates from soil (84.6%), followed by those from chicken (42.9%), human (41.2%), and rodent (38.5%) samples. Our findings have two implications: i) most of these isolates displayed an ability to cause infections that are difficult to treat (Mullarky et al., 2001; Peacock et al., 2002; Tristan et al., 2007); and ii) both rodents and soil environment are potential reservoirs (Lupindu et al., 2015; Hassell et al., 2019; Gwenzi et al., 2021).

The virulence genes; *ebpS*, *clfA*, *clfB*, and *fnbB* encode for binding proteins that facilitate bacterial adherence to host epithelial cells during invasive infections (Ionescu et al., 2015; Wang et al., 2018). The *luk-PV* and *hlg* genes encode the production of toxins that disrupt host immunity, resulting in skin lesions and severe pneumonia, while the coagulase gene (*coa*) is responsible for the protection of *S. aureus* cells against phagocytosis and host immunity (Cotar et al., 2010). The *tst* gene codes for producing toxic shock syndrome toxin-1 (TSST-1) protein associated with skin rashes and kidney failure (Bertelloni et al., 2015).

According to PCA results, *clfB*, *coa*, *ebpS*, *fnbB*, *luk-PV*, *fnbA*, and *tst* genes had a higher influence on the virulence of the MDR *S. aureus* isolates, and there were positive correlations, particularly between *clfB* and *coa*, as well as between *hlg* and *clfA*, which comprehends with the results reported by other related studies (Ionescu et al., 2015; Preda et al., 2021). We noted a co-occurrence between resistance and virulence genes in MDR *S. aureus* isolates from humans (*tetK*, *mecA*, *clfB*, *coa* and *ebpS*), chicken (*ermC*, *tetK*, *clfB*, *clfA*, *coa* and *hlg*), rodents (*tetK*, *clfB* and *coa*) and soil (*tetK*, *tetL*, *clfB*, *coa* and *ebpS*).

Indeed, PCA confirmed positive correlations between resistance and virulence genes of MDR *S. aureus*, highlighting the possibility of co-transmission of plasmid-mediated genes through horizontal gene transfer (Sidhu et al., 2002). Notably, resistance genes (*tetK* and *mecA*) and virulence determinants (*clfB*, *coa*, and *luk-PV*) were common in all sample sources, while *tst*, *hlg*, and *fnbB* were only specific to human, chicken, and rodent isolates, respectively. Our results agree with the findings from different studies on the prevalence of virulence and antibiotic-resistance genes in *S. aureus* (Tristan et al., 2007; Preda et al., 2021; Silva et al., 2021).

In summary, our results show the occurrence and co-occurrence of AMR and virulence genes in most of the isolates implying that the circulating MDR *S. aureus* strains are capable of causing infections that are difficult to treat (Ionescu et al., 2015; Wang et al., 2018). Certainly, the predominance of *tetK*, *tetL*, and *mecA* in all sample sources reflects the reported pattern of antibiotic usage in the area (Sonola et al., 2021). The carriage of virulence genes was high, even in isolates from soil samples. We are recommending the following: 1) progressive stewardship of antibiotics usage in human and veterinary medicine; 2) improving One-Health interventions to minimize the risks of spreading infections among humans, chickens, and soil environment; and 3) rodent control practices in households. This will require a multisectoral and multidisciplinary collaborative effort of human, animal, and environmental sectors to attain optimal health for people and animals and protect the environment.

Lastly, we acknowledge that although our study provides important insight regarding the profile of AMR and virulence genes among MDR *S. aureus* strains circulating in Karatu, sequence typing is needed to explore genetic diversity and relatedness, which have consequences in managing the spread and control of these strains between reservoirs.

Conclusions

This study has shown that the *S. aureus* isolates recovered from humans, poultry, rodents, and household soil contain a variety of resistance genes, mainly *tetK*, *tetL*, and *mecA*, and virulence genes mostly; clumping factor B (*clfB*), coagulase protein (*coa*), leukocidal toxins (*luk-PV*) and clumping factor A protein (*clfA*). PCA revealed that *clfB*, *coa*, *hlg*, and *clfA* genes had a higher influence on the virulence of the MDR *S. aureus* isolates. Resistance genes (*tetK* and *mecA*) and virulence determinants (*clfB*, *coa*, and *luk-PV*) were common in all sample sources, while *tst*, *hlg*, and *fnbB* were only specific to human, chicken, and rodent isolates, respectively. AMR and virulence genes were found in rodents and soil environments, implying that both are potential reservoirs. The large battery of AMR and virulence genes among MDR *S. aureus* strains circulating in the area indicate their ability to cause infections that are difficult to treat, endangering public and animal health.

Article Information

Ethics approval and consent to participate. Institutional Review Board Statement; The ethical clearance for the study was issued by the National Institute for Medical Research (NIMR) of Tanzania (NIMR/HQ/R.8a/Vol.IX/3386). NIMR is the national health research coordinating body that ensures all health research follows the national health ethics requirements. Sokoine University of Agriculture (SUA) and the Institutional Animal Care and Use Committee (IACUC) approved the use of animals in this study. The permission to work in the study area was sought from the Regional Administrative Office (Arusha). Informed Consent Statement: Informed verbal consent was obtained from all subjects involved in the study.

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