Infection and Drug Resistance

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ORIGINAL RESEARCH

Multidrug-Resistant *Staphylococcus aureus* in Diabetic Foot Infections (DFI) from Beira, Mozambique: Prevalence and Virulence Profile

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Introduction: Diabetic foot infection (DFI) represents a growing public health problem in Africa, caused by several microorganisms, with *Staphylococcus aureus* being one of the most prevalent pathogens associated with subsequent complications. This study aimed to characterize *S. aureus* isolated from the wounds of patients with type 2 diabetes, treated at a health center in Beira, Mozambique, in terms of antibiotic resistance and virulence genes.

Methods: Samples were collected by swab, after ulcer debridement, and cultivated onto mannitol salt Columbia agar supplemented with 5% sheep blood, for 24 to 48 h, at 37°C. The antibiotic resistance was assessed by disk diffusion on Mueller–Hinton agar, and Multiplex PCR was used to screen 32 virulence and seven antibiotic resistance genes.

Results: *S. aureus* isolates showed high phenotypic resistance to penicillin (100%), cefoxitin (53.3%), trimethoprim/sulfamethoxazole (40%) and vancomycin (22.2%), and a high percentage of multidrug resistance (68.9%). The most prevalent resistance genes were *blaZ* (penicillin, 100%), *mecA* (cefoxitin, 53.3%) and *vancA* (vancomycin, 28.9%). The most frequent virulence genes were *TSST (*toxic syndrome staphylococcal toxin, 57.8%), and the colonization factor *clfB* (37.8%), followed by the Panton-Valentine leukocidin (PLV) genes, *lukPV* (26.7%) and *lukED* (15.6%). The regulator factor coded by *arcA* (37.8%) and the adhesion factors coded by *cap5* (20%) and by *icaA* (17.8%) were also found.

Conclusion: A high presence of virulence genes encoding exotoxins and colonization and adhesion factors, associated with a high rate of multidrug resistance, was found in *S. aureus* isolates. This anticipates increasing difficulty in treating DFI. The greatest resistance was to commonly used antibiotics, particularly penicillin, cefoxitin and vancomycin, with resistance genes, *blaZ, mecA* and *vancA*, frequently detected. This emphasizes the urgent need for improved antimicrobial stewardship, routine molecular surveillance, and improved management strategies for DFI in resource-limited settings to mitigate disease complications and reduce the burden of antimicrobial resistance.

Keywords: diabetic foot infection, DFI, Beira-Mozambique, multidrug-resistance, methicillin resistant *Staphylococcus aureus*, MRSA, staphylococcal exotoxin genes

Introduction

Diabetic foot infection (DFI) is a common complication resulting from soft tissue or bone infection, often associated with neuropathy or peripheral arterial disease in patients with type 2 diabetic mellitus.[1] DFI is associated with considerable morbidity, an increased risk of lower limb amputation, and a high mortality.[2] DFI is mainly polymicrobial, caused by different bacterial communities, and the presence of this wide variety of species is a factor that complicates diagnosis and

Received: 10 February 2025
Accepted: 23 May 2025
Published: 31 May 2025

treatment.[3,4] *Staphylococcus aureus* is one of the most frequently isolated pathogens of DFI, often exhibiting worrying trends of high virulence and antibiotic resistance.[5]

Staphylococcus is one of the most common genera on the skin and nasal mucosa of humans and animals.[5,6] The presence of *Staphylococcus* in wounds can cause increased inflammation and delay the healing process.[6] This scenario is aggravated by the combination of the presence of different virulent genes and high antibiotic resistance, which may compromise the success of the treatment. The presence of virulence genes, such as those encoding the protein adhesin A (*SpA*), which facilitates the attachment of bacteria to host cells and tissues, fibronectin (*FnBP*), which binds to molecules on the surface of the host cells, and α-and-β-hemolysins (*hlA* and *hlB*) that can cause damage to host cells and induce cell death, contributes to the successful invasion and pathogenicity of *S. aureus*.[7,8] Exfoliative toxins, expressed by the *ET* genes, are exotoxins produced by several staphylococcal species, capable of causing blistering in human and animal infections.[9] Three of these toxins, encoded by the *etA, etB*, and *etD* genes, are directly linked to infections in humans.[10] Toxic shock syndrome-associated toxin (*TSST*, or *TSST-1*), which encodes a protein considered a superantigen, and enterotoxins, such as those of the SE family (*seA, seB, seD, seI* and *seH*), are other examples of *S. aureus* virulence genes.[11,12]

The occurrence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), is another factor that increases the severity of *S. aureus* infections in diabetic patients, making treatment of these infections challenging to say the least.[13,14] MRSA strains thus play a significant role in DFI and have become a public health concern due to their high virulence and resistance to an increasingly broad spectrum of antibiotics.[15] MRSA infections are associated with prolonged hospital stay, increased morbidity and mortality rates, and higher healthcare costs compared with infections caused by methicillin-sensitive strains.[16] Furthermore, MRSA strains pose a significant threat to public health due to their propensity for nosocomial transmission and their ability to form antibiotic-resistant biofilms on medical devices.[17] Biofilm formation involves several steps, such as adhesion, accumulation, maturation and biofilm dispersion, determined by several genes. In this process, the *ica*ABCD operon, which encodes Polysaccharide Intercellular Adhesion (PIA), the main component of biofilms, plays a very relevant role.[18,19] The regulation of the genes of this operon is complex and affected by several factors, including the presence of antimicrobials, the presence of cell wall-binding proteins and the presence of other bacterial virulence genes.[20]

S. aureus is a major opportunistic pathogen in DFIs and is frequently associated with persistent and severe infections in diabetic patients, often leading to poor healing outcomes, increased risk of amputation, and prolonged hospitalization.[5,21] The evaluation of surveillance of this opportunistic pathogen is critically important for guiding effective clinical management and controlling the spread of antimicrobial resistance.[21,22] Regular monitoring of its antibiotic resistance patterns and virulence profiles enables early detection of multidrug-resistant and highly virulent strains, informing targeted antimicrobial therapy and infection control measures.[22] Moreover, surveillance data are essential for developing local and regional treatment guidelines, optimizing antibiotic stewardship programs, and shaping public health interventions aimed at reducing the clinical and economic burden of DFIs, particularly in resource-limited settings.[23,24]

In Africa, the increasing incidence and inadequate management of type 2 diabetes represent crucial public health challenges, requiring active surveillance.[25] In Mozambique, there is a progressive increase in the prevalence of type 2 diabetes. The lack of awareness among the population about the disease and its control strategies is alarming.[26] In view of this, there is an urgent need for immediate adaptation in health service delivery, especially in the diagnosis and characterization of opportunistic pathogens associated with type 2 diabetes, with a view to improving diabetes management. In this context, this study aimed at the molecular characterization of *S. aureus* isolates associated with DFI from patients assisted at the Matacuane military health center in the city of Beira, Mozambique.

Material and Methods

Study Area

The Matacuane Military Health Center is a military health facility integrated into the national health system of Mozambique, dedicated to providing healthcare services to military and paramilitary forces. However, as part of the

national health system, this facility also provides primary healthcare services to civilians living in the vicinity of the health center. Located in the urban area of the municipality of Beira, on average, the center provides primary healthcare services to 1900 to 2500 patients per month, from various neighborhoods, with the majority of patients coming from the Matacuane neighborhood.[27]

The most important services offered include general medicine consultations, pediatric consultations, monitoring of patients on antiretroviral therapy and nursing services. Nursing services involve the administration of intramuscular or intravenous medications and the treatment of ulcers, wounds or surgical dressings, in patients referred by the Beira Central Hospital.

Inclusion and Exclusion Criteria

All civilian patients with a confirmed diagnosis of type 2 diabetes mellitus, who presented clinical signs of infected foot ulcers and provided informed consent, as stated under ethical considerations, were included in the study. On other way, military or paramilitary personnel, civilian patients who refused or were unable to provide informed consent, and those who had not initiated antibiotic treatment were excluded from the study.

Sampling and Characteristics of Patients and Lesions

Sampling was conducted between September 2023 and January 2024, in 45 civilian patients with type 2 diabetes and DFI ulcers. Ethical considerations were strictly observed. All patients were taking antibiotics to treat the infection. Demographic and lesion information, including age, sex, duration of DFI, occurrence of diabetes medication, lesion characteristics, and lesion location were collected.

Isolation and Identification of Staphylococcus aureus

A sample was collected from each patient by swab, after debridement of the ulcer. A total of 45 swabs were sampled and, respectively, inoculated into Brain Heart Infusion (BHI) broth (Oxoid Ltd., Basingstoke, UK) and incubated overnight at 37°C. From this culture broth, Mannitol Salt Agar (MSA) (Oxoid Ltd., Basingstoke, UK) and Columbia agar (supplemented with 5% sheep blood) plates were inoculated and incubated at 37°C for 24 to 48 h. The plates were inspected for characteristic staphylococcal morphology and presumptive *Staphylococcus aureus* colonies were identified using conventional methods (Gram staining, catalase and coagulase reactions) according to standard microbiological methods. Identification as *S. aureus* was performed by PCR targeting the 447-bp fragment of *nuc* gene, with the forward primer 5′-GCGATTGATGGTGATACGGTI-3′ and reverse primer 5′-AGCCAAGCCTTGACGAACTAAAGC-3′.[28,29] The primers were commercially synthesized by STAB VIDA (STAB VIDA, Caparica, Portugal). *S. aureus* (ATCC 25923) and *S. epidermidis* (ATCC 12228) were used as positive and negative controls, respectively.

DNA extraction was performed using the QIAamp DNA Mini® Kit (Qiagen, Hilden, North Rhine-Westphalia, Germany) according to the manufacturer's instructions. Briefly, bacterial pellets were resuspended in lysis buffer containing proteinase K and incubated at 56°C to ensure complete cell lysis. Ethanol was then added to the lysate, and the mixture was transferred to a QIAamp spin column. DNA was bound to the silica membrane, followed by successive washing steps to remove contaminants. Finally, purified DNA was eluted in the provided elution buffer and stored at –20°C until use.

The mix for PCR was prepared with 12.5 µL of Taq DNA Polymerase NZYTaq II2x Colourless Master Mix (MZYTech, Lisboa, Portugal), 1 µL of each forward and reverse primer (final concentration 0.3 µM), 2 µL of template DNA and sterile ultrapure water to fill 25 µL of total volume. The reaction mixtures were subjected to the following amplification conditions: initial denaturation, 94°C for 5 min, followed by 30 cycles of denaturation 94°C for 30 s, annealing 52°C for 30 s, elongation 72°C for 30 s, and a final elongation at 72°C for 5 min. PCR reactions were run in a thermocycler GeneAmp® PCR System 9700, Bio-Rad (Bio-Rad Laboratories, Segrate, Milan, Italy). The resulting PCR products were resolved on 2% (m/v) agarose gels in 1× TAE buffer, in an EC330 Thermo Fisher Scientific tank (Atlanta, GA, USA) at 8 V/cm for 60 min. The gels were stained with GelRed (Frilabo, Maia, Portugal) and analyzed using a Gel Doc™ EZ System (Bio-Rad Laboratories, Segrate, Milan, Italy). For calculating the size of the PCR products, the molecular marker 100 bp DNA Ladder (Invitrogen, CA, USA) was used.

Antibiotic Resistance Profile

Antimicrobial resistance profiling was performed by the disk diffusion method on Mueller–Hinton (MH) agar plates (Biokar Diagnostics, Beauvais, France) with antibiotic disks (Liofilchem, Roseto degli Abruzzi, Italy), according to the Clinical Laboratory Standards Institute (CLSI, 2021).[30] Colonies grown on Trypto-Casein-Soy agar (Biokar Diagnostics, Beauvais, France) for 22 ± 2 h at 37°C were suspended in sterile saline until the turbidity was equivalent to the McFarland 0.5 standard (ca. 10^6 CFU/mL). Of the resulting bacterial suspensions, 100 µL was used to inoculate MH plates under the conditions described by CLSI.[30] After deposition of the antibiotic disks, the plates were incubated for 18 ± 2 h at 37°C. Eight antibiotics were tested: cefoxitin (FOX) 30 µg; penicillin G 10U; vancomycin (VAN) 5 µg; chloramphenicol (CHL) 30 µg; tetracycline (TET) 30 µg; gentamicin (GEN) 10 µg; trimethoprim/sulfamethoxazole (SXT) 1.25/23.75 µg; erythromycin (ERY) 16 µg. On each 90 mm diameter plate, four different antibiotic disks were placed. For each isolate, two replicates were performed.

To assess the antimicrobial resistance profile of the *S. aureus* isolates, inhibition zones diameters were measured (millimeter) and compared to those described in the CLSI (2021).[30] Isolates were considered non-susceptible to a given antibiotic when they showed intermediate or full resistance to that antibiotic, according to the CLSI clinical breakpoints. Multidrug resistance was considered as non-susceptibility to at least one agent, in three or more antimicrobial categories and/or resistance to methicillin, cefoxitin or oxacillin.[31]

Identification of Virulence and Antibiotic Resistance Genes

Multiplex PCR (MPCR) investigated the presence of 32 virulence genes (Table 1). Namely: Genes encoding hemolysin (*hlA, hlB* and *hlgCB*); genes associated with colonization, such as fibronectins (*fnbA* and *fnbB*); genes encoding adhesins

Table 1 Primers Used for the Detection of Virulent Genes and Respective Sizes of Amplified Product

Target Gene	Primer Sequence (5′–3′)	Size (bp)	Annealing Temperature (°C)	References
fnbA	CACAACCAGCAAATATAG	1362	50	Peacock et al[32]
	CTGTGTGGTAATCAATGTC			
fnbB	GTAACAGCTAATGGTCGAATTGATACT	523	55	Tristan et al[33]
	CAAGTTCGATAGGAGTACTATGTTC			
clfA	ATTGGCGTGGCTTCAGTGCT	1584	56	Peacock et al[32]
	CGTTTCTTCCGTAGTTGCATTTG			
clfB	TGGCGGCAAATTTTACAGTGACAGA	404	61	Campbell et al[34]
	AGAAATGTTCGCGCCATTTGGTTT			
sasX	AGAATTAGAAGTACGTCTAAATGC	521	53	Li et al[26]
	GCTGATTATGTAAATGACTCAAATG			
hlA	ATGAAAACACGTATAGTCAGCTCAGTAACAAC	960	72	Lin et al[35]
	TTAATTTGTCATTTCTTCTTTTTCCCAATCGA			
hlB	GTGCACTTACTGACAATAGTGC	309	60	Jarraud et al[36]
	GTTGATGAGTAGCTACCTTCAGT			

(*Continued*)

Table 1 (Continued).

Target Gene	Primer Sequence (5′–3′)	Size (bp)	Annealing Temperature (°C)	References
hlgCB	GCCAATCCGTTATTAGAAAATGC	938	55	Kumar et al[37]
	CCATAGACGTAGCAACGGAT			
etA	CTATTTACTGTAGGAGCTAG	741	53	Ružicková et al[38]
	ATTTATTTGATGCTCTCTAT			
etB	CAGATAAAGAGCTTTATACACACATTAC	612	55.5	Li et al[39]
	AGTGAACTTATCTTTCTATTGAAAAACACTC			
etD	CCCGTTGATTAGTCATGCAG	606	55	Strommenger et al[40]
	TCCAGAATTTCCCGACTCAG			
TSST	AAGCCCTTTGTTGCTTGCG	447	55	Becker et al[41]
	ATCGAACTTTGGCCCATACTTT			
*luk*ED	TGAAAAAGGTTCAAAGTTGATACGAG	269	61	Jarraud et al[36]
	TGTATTCGATAGCAAAAGCAGTGCA			
*luk*S/F-PV	ATCATTAGGTAAAATGTCTGGACATGATCC	432	58	Strommenger et al[40]
	GCATCAAGTGTATTGGATAGCAAAAGC			
icaA	GATTATGTAATGTGCTTGGA	770	50	Peacock et al[32]
	ACTACTGCTGCGTTAATAAT			
icaB	CTGATCAAGAATTTAAATCACAAA	302	56	Solati et al[42]
	AAAGTCCCATAAGCCTGTTT			
icaC	TAACTTTAGGCGCATATGTTTT	400	56	
	TTCCAGTTAGGCTGGTATTG			
icaD	ATGGTCAAGCCCAGACAGAG	198	55.5	Arciola et al[18]
	AGTATTTTCAATGTTTAAAGCAA			
agr I	ATCGCAGCTTATAGTACTTGT	739	50	Peacock et al[32]
	CTTGATTACGTTTATATTTCATC			
agr II	AACGCTTGCAGCAGTTTATTT	691	50	
	CGACATTATAAGTATTACAACA			
agr III	TATATAAATTGTGATTTTTTATTG	712	50	
	TTCTTTAAGAGTAAATTGAGAA			
agr IV	TATATAAATTGTGATTTTTTATTG	683	50	
	TTCTTTAAGAGTAAATTGAGAA			
ACME-*arc*A	GCTCAAACTTTGAGAGATGAA	216	58	Strommenger et al[40]
	TTACGTACGCCAGCCATGAT			

(*Continued*)

Table 1 (Continued).

Target Gene	Primer Sequence (5′–3′)	Size (bp)	Annealing Temperature (°C)	References
seA	GAAAAAAGTCTGAATTGCAGGGAACA	560	52	Salamandane et al[43]
	CAAATAAATCGTAATTAACCGAAGGTTC			
seB	ATTCTATTAAGGACACTAAGTTAGGGA	404	52	
	ATCCCGTTTCATAAGGCGAGT			
seC	GTAAAGTTACAGGTGGCAAAACTTG	297	52	
	CATATCATACCAAAAAGTATTGCCGT			
seD	GTGGTGAAATAGATAGGACTGC	384	58.5	
	ATATGAAGGTGCTCTGTGG			
seE	CAAAGAAATGCTTTAAGCAATCTTAGGC	480	52	
	CACCTTACCGCCCAAAGCTG			
SeH	CAACTGCTGATTTAGCTCAG	358	55	Strommenger et al 2008[40]
	GTCGAATGAGTAATCTCTAGG			
seI	AGGCAGTCCATCTCCTGTATAA	568	60	Moura et al[44]
	TGCTCAAGGTGATATTGGTGTAG			
Cap5	CGAACCGATGATTGATGCTATTG	555	61	Acosta et al[45]
	TGCTATGACTGCACCAGTATTT			
cap8	GGAGGAAATGACGATGAGGATAG	608	61	
	TAGCTTCTGTAGCGGTGAATG			

(*clfA* and *clfB*) and gene *sasX* related to nasal colonization; accessory gene regulators (*agr I, Agr II, agr III, agr IV* and *arcA*); biofilm regulator genes (*icaA, icaB, icaC* and *icaD*); exotoxins, such as exfoliative toxin genes (*etA, etC* and *etD*) and toxic shock syndrome (*TSST*); enterotoxins (*sea, seb, sec, sed, see, seI* and *seH*); capsular polysaccharides (*cap5* and *cap8*); and genes encoding pore-forming leukotoxins (*lukED* and *luk*S/F-PV). Based on the annealing temperature and PCR product size of each primer, the reactions were grouped into different MPCRs. Namely: MPCR for *seH, icaD, TSST, etD, hlgCB* and *fnbB* genes; MPCR for *fnbB, icaA* and *agr III* genes; MPCR for *hlB, Agr I, agr II* and *agr IV* genes; MPCR for *icaC, icaB, etB* and *clfA* genes; MPCR for *clfB, lukED, cap5* and *cap8* genes; MPCR for seA, *seB, seC* and *seE* genes; MPCR for *seD, ACME-arcA* and *lukS/F-PV* genes; MPCR for *etA, sasX* and *hlA* genes. The gene *seI* was screened as a single PCR.

The MPCR master mixes were respectively prepared with 12.5 µL of Taq DNA Polymerase NZYTaq II2x Colourless Master Mix (MZYTech, Lisboa, Portugal), 1 µL of each forward and reverse primers (Table 2) (final concentration 0.3 µM), 2 µL of template DNA and sterile ultrapure water up to 25 µL of total volume. All primers commercially synthesized by STAB VIDA (STAB VIDA, Caparica, Portugal).

Genes encoding resistance to methicillin (*mecA*), penicillin (*blaZ*), erythromycin (*ermA, ermB and ermC*), and vancomycin (*vancA* and *vancB*) were screened by MPCR (Table 2). The MPCR master mix was prepared with 12.5 µL of Taq DNA Polymerase NZYTaq II2x Colourless Master Mix (MZTech, Lisboa, Portugal), 1 µL of each forward and reverse primers (Table 2) (final concentration 0.3 µM), 2 µL of template DNA, and sterile ultrapure water to

Table 2 Primers Used for the Detection of Antimicrobial Resistance Genes and Respective Sizes of Amplified Products

Target Gene	Primer sequence (5′–3′)	Size (bp)	Annealing Temperature (°C)	Reference
mecA	TCCAGATTACAACTTCACCAGG	180	52	Salamandane et al[17]
	CCACTTCATATCTTGTAACG			
blaZ	AAGAGATTTGCCTATGCTTC	170	60	Salamandane et al[43]
	GCTTGACCACTTTTATCAGC			
ermA	AAGCGGTAAACCCCTCTGA	190	55	
	TTCGCAAATCCCTTCTCAAC			
ermB	TCAAAACATAATATAGATAAA	642	55	Salamandane et al[29]
	GCTAATATTGTTTAAATCGTCAAT			
ermC	AATCGTCAATTCCTGCATGT	299	55	
	TAATCGTGGAATACGGGTTTG			
vancA	GGCAAGTCAGGTGAAGATG	713	55	Al-Amery et al[46]
	ATCAAGCGGTCAATCAGTTC			
vancB-F	GTGACAAACCGGAGGCGAGGA	430	50	
	CCGCCATCCTCCTGCAAAAAA			
dfrA	GACGAAGCGATGAGGAGAAG	632	55	Ho[47]
	TCGTTGTGAAGAACACGATCC			
dfrG	CAAAGGGACATCCGATAATA	310	55	Burgold-Voigt[48]
	AATACCTCATTCCATTCCTC			

complete 25 µL of total volume. MPCR conditions were performed as previous described.[17,29,43] All primers commercially synthesized by STAB VIDA (STAB VIDA, Caparica, Portugal).

All PCR reactions were performed in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Bio-Rad Laboratories, Segrate, Milan, Italy). PCR products were resolved on 2% (m/v) agarose gels in 1×TAE buffer, in an EC330 Thermo Fisher Scientific tank (Georgia, USA) at 8 V/cm for 60 min. Gels were stained with GelRed (Frilabo, Maia, Portugal) and analysed using a Gel Doc™ EZ System (Bio-Rad Laboratories, Segrate, Milan, Italy). To calculate the size of PCR products, the molecular marker 100 bp DNA Ladder (InVitrogen, California, USA) was used.

Results

Demographic Data

Demographic and lesion information, including age, sex, duration of DFI, occurrence of diabetes medication, lesion characteristics, and lesion location are shown in Table 3. The age of the patients ranged from 36 to 75 years (mean 65.8 ± 13.76 years) and 64.4% were female (Table 3). Most patients (93%) were taking medications to control diabetes. Most patients (55.6%) had DIF for more than three months (Table 3). The types of lesions among the patients were mainly deep ulcers (86.7%) with chronic wounds (77.8%). Most lesions were located on the right toe (37.8%) and plantar region (28.8%) (Table 3).

Table 3 Characterization of DFI Patients and Lesions

Characteristics of Patients and Lesions	Number (%)
Age (years)	36 to 75; mean 65.8 ± 13.76
Gender	
Male	16 (35.6%)
Female	29 (64.4%)
Diabetic medication	
Yes	41 (93%)
No	4 (7%)
Duration of foot infection	
Less than 60 days	6 (13.3%)
60–90 days	14 (31.1%)
More than 90 days	25 (55.6%)
Type of lesion	
Superficial ulcer	6 (13.3%)
Deep ulcer	39 (86.7%)
Chronic wounds	35 (77.8%)
Acute wounds	10 (22.2%)
Location of the foot ulcer	
Plantar	13 (28.8%)
Dorsal portion	8 (17.8%)
Toes (right foot)	17 (37.8%)
Toes (left foot)	7 (15.6%)
Patient weight profile	
Overweight	15 (33.3%)
Obesity	11 (24.4%)

The diabetic foot ulcers of the 45 patients evaluated in this study resulted in the identification of 45 isolates of *S. aureus*. These *S. aureus* isolates were subjected to analysis of the antibiotic resistance profile and the presence of virulence and antibiotic resistance genes.

Virulent Genes

To facilitate the recognition of the most relevant virulence genes in the 45*S. aureus* isolates, the genes were grouped according to their respective functions (Table 4). Thus, the most frequent virulence genes were those encoding exotoxins (*TSST*, 57.8% and *etA*, 35.6%), followed by genes encoding colonization (*clfB*, 37.8%) and regulators factors (*arcA*, 37.8%) (Table 4). Regarding leukocidin genes, *LukPV* was found in 26.7% and *LukED* in 15.6 of the isolates. The *cap5* (20%), *icaA* (17.8%) and *icaD* (11.1%) genes were the most representative adhesion genes. Among the seven genes investigated that encode staphylococcal enterotoxins, only the *seI* gene was found (13.3%) (Table 4). Among the 32

Table 4 Distribution of Virulent Genes by Function

Function	Gene	Frequency
Colonization: fibrinogen-binding adhesin	*clfB*	37.8%
	fnbB	4.4%
Hemolysin	*hlB*	8.9%
	hlgCB	2.2%
Exotoxin	*TSST*	57.8%
	etA	35.6%
Leukocidin	*LukPV*	26.7%
	LukED	15.6%
Regulator	*arcA*	37.8%
	Agr II	8.9%
	Agr I	2.2%
Adhesion: Biofilm+ Capsular factor	*cap5*	20%
	caA	17.8%
	icaD	11.1%
Enterotoxin	*seI*	13.3%

genes accessed, 15 (46.9%) were detected in at least one isolate (Table 5). Seventeen (*clfA, etB, etD, fnbA, sasX, hlA, seA, seB, seC, seD, seE, seh, icaB, icaC, agr III, agr IV* and *cap8*) of the 32 target genes were not found in any isolate.

Most isolates that present virulent genes contain more than one gene, related or not (Tables 4 and 5). Regarding similar virulence genes, the most predominant co-occurrences were observed with the exotoxin gene *TSST* and *etA* (31.1%), and the adhesion factors *icaA*/*icaD* and c*ap5* (13.3%). The co-occurrence of multiples (three or more) virulence genes involved in different functions was found in 42.2% of the isolates. On the other hand, 31.1% of the isolates presented two genes encoding different types of virulence. In five of the 45 isolates, none of the 32 virulent genes under analysis were detected (Table 5).

Table 5 Virulence Genes Detected in the 45 *Staphylococcus aureus* Isolates

Isolates	Gene function							
	Colonization	Hemolysin	Exotoxin	Leukocidin	Regulator	Biofilm	Capsular	Enterotoxin
FDB01	–	–	–	–	–	–	–	–
FDB02	–	–	*TSST*	–	*arcA*	–	–	–
FDB03	*clfB*	–	*TSST, etA*	*lukPV, lukED*	*agr I*	–	–	–
FDB04	*clfB*	–	*TSST, etA*	–	*arcA*	–	–	–
FDB05	*clfB*	–	*TSST, etA*	*lukPV*	–	*icaD*	–	–
FDB06	–	*hlgCB*	*TSST*	–	*arcA*	*icaD*	–	–
FDB07	–	–	*TSST*	*lukPV*	*arcA*	–	–	–

(*Continued*)

https://doi.org/10.2147/IDR.S521876

Table 5 (Continued).

Isolates	Gene function							
	Colonization	Hemolysin	Exotoxin	Leukocidin	Regulator	Biofilm	Capsular	Enterotoxin
FDB08	*clfB*	–	–	–	–	–	–	–
FDB09	*clfB*	–	*TSST*	–	–	–	–	–
FDB10	–	–	*TSST*	*lukPV*	–	*icaA*	–	–
FDB11	*clfB*	–	*TSST, etA*	*lukPV*	–	*IcaA*	*cap5*	–
FDB12	–	–	*TSST*	*lukPV*	–	*icaA*	*cap5*	–
FDB13	*clfB*	–	–	–	*arcA*	–	–	–
FDB14	–	–	–	–	–	–	–	–
FDB15	–	–	–	–	–	*icaA*	–	–
FDB16	–	*hlB*	*TSST, etA*	–	*arcA*	–	–	*sel*
FDB17	–	–	*TSST, etA*	–	*arcA*	–	–	–
FDB18	–	–	–	–	*arcA*	*icaA*	–	–
FDB19	–	–	–	–	–	–	–	–
FDB20	–	–	–	–	–	*icaA*	*cap5*	–
FDB21	*clfB*	–	*TSST, etA*	*lukED*	*arcA, agr II*	–	–	*sel*
FDB22	*clfB*	*hlB*	–	*lukED*	*arcA, agr II*	–	–	*sel*
FDB23	*fnbB*	–	–	*lukED*	–	–	–	–
FDB24	–	–	*TSST, etA*	–	–	*icaD*	*cap5*	–
FDB25	–	–	*etA*	–	–	–	–	–
FDB26	*clfB*	–	*TSST*	–	–	–	–	–
FDB27	–	–	*TSST*	*lukPV*	–	–	–	–
FDB28	*clfB*	–	*TSST, etA*	*lukPV*	–	–	–	–
FDB29	–	–	*TSST*	*lukPV*	–	–	–	–
FDB30	*clfB*	–	–	–	*arcA*	–	–	–
FDB31	–	–	–	–	–	–	–	–
FDB32	–	–	–	–	–	–	–	–
FDB33	–	*hlB*	*TSST, etA*	–	*arcA*	*IcaA, icaD*	*cap5*	*sel*
FDB34	–	–	*TSST, etA*	–	*arcA*	*IcaA, icaD*	*cap5*	–
FDB35	–	–	–	–	*arcA*	–	–	–
FDB36	–	–	*TSST*	*lukPV*	–	–	–	–
FDB37	*clfB*	–	–	–	*arcA*	–	–	–
FDB38	*clfB*	–	*TSST, etA*	*lukED*	*arcA, agr II*	–	–	*sel*
FDB39	*clfB*	*hlB*	–	*lukED*	*arcA, agr II*	–	–	*sel*

(*Continued*)

Table 5 (Continued).

Isolates	Gene function							
	Colonization	Hemolysin	Exotoxin	Leukocidin	Regulator	Biofilm	Capsular	Enterotoxin
FDB40	*fnbB*	–	–	*lukED*	–	–	–	–
FDB41	–	–	*TSST, etA*	–	–	–	*cap5*	–
FDB42	–	–	*etA*	–	–	–	*cap5*	–
FDB43	*clfB*	–	*TSST*	–	–	–	*cap5*	–
FDB44	–	–	*TSST*	*lukPV*	–	–	–	–
FDB45	*clfB*	–	*TSST, etA*	*lukPV*	–	–	–	–

Antibiotic Resistance

The most prevalent antimicrobial resistance profiles were penicillin (100%) and cefoxitin (53.3%) (Table 6). Among non-β-lactam antibiotics, the most frequent resistance profiles were to trimethoprim/sulfamethoxazole (40%), erythromycin (33.3%) and vancomycin (22.2%). Very few isolates showed resistance to chloramphenicol or gentamicin (8.9 and 4.4%, respectively) (Table 6). Among the 45 *S. aureus* isolates from DFI, 31 (68.9%) were multidrug-resistant as they showed resistance to cefoxitin and/or more than three groups of unrelated antibiotics (Table 7).

Regard the frequency of antibiotic resistance genes, the *blaZ* gene, which encodes penicillin resistance, was found in all 45 isolates analyzed (Table 6). The *mecA* gene, encoding methicillin/penicillin resistance, was found in 53.3% of the isolates. Two types of genes encoding vancomycin resistance were found (*vancA*, in 28.9% and *vancB*, 15.6%) (Table 6). Among the genes encoding erythromycin resistance, the *ermB* gene (15.6%) was the most common gene, followed by ermC (11.1%). Gene encoding resistance to trimethoprim/sulfamethoxazole *dfr*A (31.1%) and *dfr*G (17.8%) was found among 40% of isolates that showed resistance profile.

Table 6 Frequencies of Antibiotic Resistance Profile and Associated Genes

Antibiotic	Frequency of Resistance Profile (%)	Resistance Gene	Frequency of Gene (%)
Penicillin G (10U)	100	*blaZ*	100
Cefoxitin (30 μg)	53.3	*mecA*	53.3
Trimethoprim/sulfamethoxazole (23.75/1.25 μg)	40	*dfrA*	31.1
		dfrG	17.8
Erythromycin (16 μg)	33.3	*ermB*	15.6
		ermC	11.1
Vancomycin (5 μg)	22.2	*vancA*	28.9
		vancB	15.6
Tetracycline (30 μg)	15.6	N/A	–
Chloramphenicol (30 μg)	8.9	N/A	–
Gentamicin (10 μg)	4.4	N/A	–

Abbreviation: N/A, Not accessed.

https://doi.org/10.2147/IDR.S521876

Table 7 Antibiotic Resistance Profile and Presence of Genes Encoding Antibiotic Resistance in the 45 *Staphylococcus aureus* Isolates from DFI

Sample	FOX	P	VAN	CHL	TET	GEN	SXT	ERY	Antibiotic Resistance Gene
FDB01	R	R	S	S	R	S	R	R	*blaZ, mecA, vancB, ermA, dfrA*
FDB02	R	R	S	S	S	S	R	S	*blaZ, mecA, dfrG*
FDB03	R	R	S	S	S	S	S	S	*blaZ, mecA*
FDB04	R	R	R	S	S	S	R	R	*blaZ, mecA, vancA, ermC, dfrA*
FDB05	S	R	S	S	R	S	R	S	*blaZ, dfrA*
FDB06	S	R	S	S	S	S	S	R	*blaZ, vancA, vancB, ermC*
FDB07	S	R	S	S	S	S	S	S	*blaZ*
FDB08	S	R	R	S	S	S	R	S	*blaZ, vancA, dfrA, dfrG*
FDB09	S	R	R	R	I	S	S	R	*blaZ, vancA, ermA*
FDB10	S	R	S	S	S	S	S	R	*blaZ, ermB*
FDB11	R	R	S	S	S	R	R	S	*blaZ, mecA, dfrA*
FDB12	R	R	R	S	S	S	S	R	*blaZ, mecA, vancA, vancB, ermB*
FDB13	S	R	S	S	S	S	R	S	*blaZ, dfrA*
FDB14	R	R	S	S	S	S	S	S	*blaZ, mecA*
FDB15	S	R	S	S	S	S	S	S	*blaZ, vancA*
FDB16	S	R	S	S	R	S	S	S	*blaZ, vancB*
FDB17	R	R	S	S	S	S	R	S	*blaZ, mecA, dfrA*
FDB18	S	R	R	S	S	S	S	S	*blaZ, vancA*
FDB19	R	R	S	S	S	S	S	S	*blaZ, mecA*
FDB20	R	R	S	S	S	S	S	R	*blaZ, mecA, ermA*
FDB21	R	R	R	S	S	S	R	S	*blaZ, mecA, vancA, dfrA*
FDB22	S	R	S	S	S	S	S	S	*blaZ*
FDB23	S	R	S	S	S	S	S	S	*blaZ, vancA*
FDB24	R	R	S	R	R	S	R	R	*blaZ, mecA, ermB, dfrA*
FDB25	R	R	S	S	S	S	R	S	*blaZ, mecA, dfrA, dfrG*
FDB26	R	R	S	S	S	S	S	R	*blaZ, mecA, ermC*
FDB27	S	R	S	S	S	R	R	R	*blaZ, vancB, ermB, dfrG*
FDB28	R	R	S	S	S	S	S	R	*blaZ, mecA, ermC*
FDB29	R	R	R	S	R	S	S	S	*blaZ, mecA, vancA*
FDB30	S	R	S	S	S	S	R	S	*blaZ, mecA. dfrA*
FDB31	R	R	S	S	S	S	R	R	*blaZ, ermB, dfrA, dfrG*
FDB32	R	R	R	S	S	S	S	S	*blaZ, mecA, vancA*
FDB33	S	R	S	S	R	S	R	S	*blaZ*

(*Continued*)

Table 7 (Continued).

Sample	FOX	P	VAN	CHL	TET	GEN	SXT	ERY	Antibiotic Resistance Gene
FDB34	R	R	S	R	S	S	R	S	*blaZ, mecA, vancB, dfrG*
FDB35	S	R	S	S	S	S	S	S	*blaZ, vancA*
FDB36	R	R	S	S	S	S	S	S	*blaZ, mecA*
FDB37	R	R	S	S	S	S	S	R	*blaZ, mecA, ermB*
FDB38	S	R	S	S	R	S	I	S	*blaZ*
FDB39	R	R	S	S	S	S	R	S	*blaZ, mecA, dfrA*
FDB40	S	R	S	S	S	S	I	S	*blaZ, dfrG*
FDB41	R	R	S	R	I	S	S	S	*blaZ, mecA*
FDB42	S	R	R	S	S	S	S	R	*blaZ, VA, ermC*
FDB43	S	R	S	S	S	S	S	S	*blaZ*
FDB44	S	R	R	S	S	S	R	S	*blaZ, vancA, vancB, dfrA, dfrG*
FDB45	R	R	S	S	S	S	S	R	*blaZ, mecA, ermB*

Notes: Cefoxitin (FOX) 30 μg; penicillin G (P) 10U; vancomycin (VAN) 5 μg; chloramphenicol (CHL) 30 μg; tetracycline (TET) 30 μg; gentamicin (GEN) 10 μg; trimethoprim/sulfamethoxazole (SXT) 1.25/23.75 μg; erythromycin (ERY) 16 μg.
Abbreviations: R, Resistant; I, Intermediated; S, Susceptible.

Co-occurrence of resistance genes was observed in 84.4% of the isolates (Table 7). The most frequent co-occurrence was of *blaZ* and *mecA* (48.8%). In three of the 45 (6.7%) *S. aureus* isolates, all types of antibiotic resistance genes evaluated in this study were detected (*blaZ, mecA, vanc, dfr* and *erm*) (Table 7).

Beyond the high overall resistance to penicillin and cefoxitin, Table 7 shows a concerning trend of convergence between multidrug resistance and the presence of multiple virulence determinants in *S. aureus* isolates from DFI. Strains harboring both *mecA* and *blaZ* genes frequently co-expressed virulence genes such as *TSST, etA*, and leukocidin genes (*lukPV, lukED*), highlighting the potential for these strains to cause severe and hard-to-treat infections. Particularly notable were isolates like FDB33 and FDB34, which combined resistance to five or more antibiotics with genes associated with toxin production and biofilm formation (*icaA, icaD, cap5*), suggesting enhanced persistence and immune evasion capabilities.

Discussion

Methicillin-resistant *S. aureus* (MRSA) has been documented as the most dominant healthcare-associated *Staphylococcus* worldwide.[49,50] However, in Mozambique, there is no information on the characterization of *S. aureus* isolated from DIF. Hopefully, this study will contribute to fill this gap. In general, low genetic diversity was found among the isolates, demonstrated by the similarity of the virulent genes found. The results of this study highlight high resistance to penicillin (100%), cefoxitin (53.3%) and trimethoprim/sulfamethoxazole (40%).

Among diabetic patients with foot ulcers, MRSA has emerged as an important and commonly opportunistic pathogen, often community- and hospital-associated. MRSA can infect ulcers, skin, and soft tissues, making treatment very difficult.[51] Among the 53.3% (24/45) MRSA isolates identified in this study, 45.8% were resistant to trimethoprim/sulfamethoxazole, 41.7% to erythromycin and 29.2% to vancomycin. Similar results were found by other authors in Poland,[10] Egypt[16] and Brazil.[52] *S. aureus* recovered from DFI in a Tunisian hospital showed 100% resistance to methicillin and 33.3% to trimethoprim/sulfamethoxazole.[50] In Egypt, a high prevalence of MRSA strains (100%)[16] exhibiting resistance to erythromycin and vancomycin was noted, possibly reflecting different prescription habits and infection control policies. In Brazil, high resistance rates (61%) of MRSA were also observed.[52] However, the

socioeconomic factors, such as better access to healthcare and structured diabetes care programs, might partially mitigate the burden compared to the Mozambican context. In Mozambique, co-trimoxazole (trimethoprim/sulfamethoxazole (SXT)) plays a very important role in the treatment of opportunistic diseases in immunocompromised patients and, together with amoxicillin and clavamox (another penicillin antibiotics), is the most prescribed and recommended antibiotic by health professionals.[53]

The *mecA* gene was found in all isolates that showed resistance to cefoxitin. These isolates also carried the *blaZ* gene encoding penicillin resistance and 29.2% had the *vanc* and or *erm* gene. Several studies have found a high prevalence of antibiotic resistance and genes associated with antibiotic resistance in *S. aureus* recovered from clinical and food samples in Mozambique.[43,54,55] In food samples, Salamandane et al[43] reported the occurrence of the *blaZ* gene in 42.1% of *S. aureus* isolates from ready-to-eat foods, as well as the occurrence of the *mecA* gene in 36.8% and *vancA* in 31.6%. On the other hand, in clinical isolates, *blaZ* was found in 79.2% of samples and *mecA* in 100%.[55] Co-occurrence of multiple resistance genes further suggests potential for treatment failure and nosocomial spread.

In South Africa, MRSA prevalence among DFI patients has been reported at levels similar to or slightly higher than those observed in Mozambique, while studies from Nigeria and Kenya show even higher rates of multidrug resistance in *S. aureus* isolates.[56] Several factors as limitations in healthcare infrastructure, with reduced laboratory capacity for culture and sensitivity testing, leading to widespread empirical antibiotic use without proper microbiological diagnosis in different African countries could explain these differences.[57,58]

Regarding the genes encoding virulence factors, the most frequent were exotoxins *TSST* (57.8%), colonization factor *clfB* (40%), regulator factor *arcA* (37.8%) and exfoliative toxin *etA* (33.3%). A total of 53.8% of the isolates that presented *TSST* also presented the *etA* gene. Several studies found a low prevalence of *TSST* in *S. aureus* isolates from DFI.[50,59,60] In Costa Rica, Víquez-Molina et al detected the *TSST* gene in 5.2% of the samples,[59] and in India, Shettigar et al[60] found 13.9% of *TSST* in *S. aureus* recovered from individuals with foot ulcers. Combination of several factors, including high glucose, low oxygen levels resulting from the combination of neuropathy and poor circulation and indiscriminate use of a variety of antibiotics,[61] may have contributed to the high frequency of TSST genes in this work. Exfoliative toxins cause scalded skin syndrome, most commonly in newborns, young children and immunosuppressed individuals. Scalded skin syndrome is a staphylococcal infection that causes redness, blisters and peeling, causing the top layer of skin to peel off.[62]

The gene encoding Panton-Valentine Leucocidin (PVL) was found in 40% of the isolates. Of these, 11 isolates were positive for *lukPV*, six isolates for *lukED* and one isolate showed both genes (*lukPV* and *lukED*). Detection of *lukED* in MRSA strains associated with DFI has also been reported in Iran.[63] In Iran, *lukED* gene was found in 85% of *S. aureus* recovered from DIF. PVL is one of the main virulence factors associated with hospital-acquired pneumonia and skin infections. This toxin damages white blood cells, which are essential in fighting infection.[64] Meeren et al found 37.5% and 90.5% of the gene encoding this toxin PVL in hospital- and community-acquired *S. aureus* in Beira city, Mozambique.[54] Other relevant genes found in the present study are related to fibrinogen-binding adhesin of the host cell, such as *clfB* (37.8%) and *fnbB* (4.4%).

These genes encode proteins known as clumping factors, fibronectin-/fibrinogen-binding proteins, and adhesins,[65] which play an important role in biofilm formation. A recent study reported high biofilm formation in MRSA isolated from street food in Mozambique.[17] Regarding enterotoxins, only the *seI* gene was detected in 13.3% of the isolates. Although they are considered enterotoxins, *seI* and *seH* encode proteins that are also factors associated with the colonization of host tissues by *S. aureus*, due to their presumed participation in the infectious process.[66,67]

Some strains are of particular concern, as they are multidrug-resistant or multidrug-resistant MRSA, carry several virulent genes and isolated from high-risk patients. Namely, the MRSA isolates FDB03, that presented the *clfB, TSST, etA, lukPV, lukED* and *agr I* genes, and FDB04, with virulence genes *clfB, TSST, etA* and *arcA*, recovered from obese patients with ulcers for more than 90 days. Also, the MRSA isolates FDB11 and FDB39 recovered from ulcers older than 90 or 60 days, from elderly or obese patients, respectively, carried six virulence genes each. Although they were isolated from non-obese individuals with recent ulcers (less than 30 days), the multidrug-resistant isolate FDB33 carrying the *hlB, TSST, etA, arcA, IcaA, icaD, cap5* and *seI*; and the MRSA isolate FDB34, carrying the genes *TSST, etA, arcA, IcaA, icaD and cap5*, may be considered high risk, as they present genes related to biofilm formation. In fact, in the biofilm state,

bacteria are more resistant to antibiotics than their planktonic counterparts due to the multilevel protection conferred by the extracellular matrix (which hinders the penetration of antibiotics), altered metabolic states and lower growth rate.[17,28,68]

We did not find data on the prevalence of DFI in Mozambique. However, in South Africa, a country bordering Mozambique and sharing similarities in terms of prevalence of non-transmissible diseases, DIF is estimated to affect 28% of diabetic patients.[69] The prevalence of DFI in Zimbabwe was estimated to be 53%. Longer duration of diabetes, absence of pedal pulses, and peripheral neuropathy were considered risk factors for foot ulceration in these populations.[25,70–73]

Conclusions

This study aimed to investigate antibiotic resistance and the presence of virulence genes in *S. aureus* isolated from DFI. To the best of our knowledge, this is the first study addressing antibiotic resistance and virulence genes in *S. aureus* isolated from DFI patients in Mozambique. The results revealed alarming levels of antibiotic resistance, particularly to penicillin, cefoxitin, co-trimoxazole (trimethoprim/sulfamethoxazole) and vancomycin, as well as high percentages of multidrug-resistant strains. Furthermore, the study identified a high presence of virulent genes. The presence of multiple virulence genes, including *TSST, clfB*, and l*ukPV*, underscores the pathogenic potential of these strains and their capacity to cause severe infections. The presence of virulent genes in multidrug-resistant strains represents a worrying scenario, as it indicates the potential for greater severity of infections caused by these bacteria, making treatment difficult and increasing the risk of serious complications.

Abbreviations

BHI, Brain Heart Infusion; DFI, Diabetic foot infection; MSA, Mannitol Salt Agar; CLSI, Clinical Laboratory Standards Institute; MRSA, Methicillin-resistant *S. aureus*.

Data Sharing Statement

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Restrictions are applied for confidential personal data.

Ethics Approval and Informed Consent

The study was conducted according with the Declaration of Helsinki, and the protocol was approved by the National Committee for Bioethics in Health of Ministry of Health of Mozambique, protocol reference number 039/2022/DFI/BS/MSM. All participants provided written informed consent to take part in the study and confirmed their consent before participation.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

1. Khan MS, Azam M, Khan MN. et al. Identification of contributing factors, microorganisms and antimicrobial resistance involved in the complication of diabetic foot ulcer treatment. *Microb Pathog*. 2023;184:106363. doi:10.1016/j.micpath.2023.106363

2. Raspovic KM, Wukich DK. Self-reported quality of life and diabetic foot infections. *J Foot Ankle Surg*. 2014;53(6):716–719. doi:10.1053/j.jfas.2014.06.011
3. Gemechu FW, Curley CA, Curley CA. Diabetic Foot Infections. *Am Fam Physician*. 2013;88(3):177–184.
4. Wang X, Yuan C-X, Xu B, Yu Z. Diabetic foot ulcers: classification, risk factors and management. *World J Diabetes*. 2022;13(12):1049–1065. doi:10.4239/wjd.v13.i12.1049
5. Dunyach-Remy C, Essebe CN, Sotto A, Lavigne JP. Staphylococcus aureus toxins and diabetic foot ulcers: role in pathogenesis and interest in diagnosis. *Toxins*. 2016;8(7):1–20. doi:10.3390/toxins8070209
6. Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. *Nat Rev Microbiol*. 2015;13(9):529–543. doi:10.1038/nrmicro3521
7. Foster TJ, Fischetti VA, Novick RP. Surface Proteins of Staphylococcus aureus. *Microbiol Spectr*. 2019;7(4). doi:10.1128/microbiolspec.gpp3-0046-2018
8. Otto M. Staphylococcus aureus toxins. *Curr Opin Microbiol*. 2014;17:32–37. doi:10.1016/j.mib.2013.11.004
9. Ladhani S, Joannou CL, Lochrie DP, Evans RW, Poston SM. Clinical, Microbial, and Biochemical Aspects of the Exfoliative Toxins Causing Staphylococcal Scalded-Skin Syndrome. *Clin Microbiol Rev*. 1999;12(2):224–242. doi:10.1128/CMR.12.2.224
10. Kot B, Piechota M, Jakubczak A, et al. The prevalence of virulence determinants in methicillin-resistant Staphylococcus aureus isolated from different infections in hospitalized patients in Poland. *Sci Rep*. 2022;12(1). doi:10.1038/s41598-022-09517-x
11. Chen Q, Xie S. Genotypes, Enterotoxin Gene Profiles, and Antimicrobial Resistance of Staphylococcus aureus Associated with Foodborne Outbreaks in Hangzhou, China. *Toxins*. 2019;11(6):307. doi:10.3390/toxins11060307
12. Nasaj M, Saeidi Z, Tahmasebi H, Dehbashi S, Arabestani MR. Prevalence and distribution of resistance and enterotoxins/enterotoxin-like genes in different clinical isolates of coagulase-negative Staphylococcus. *Eur J Med Res*. 2020;25(1). doi:10.1186/s40001-020-00447-w
13. Boyanova L, Mitov I. Antibiotic resistance rates in causative agents of infections in diabetic patients: rising concerns. *Expert Rev Anti Infect Ther*. 2013;11(4):411–420. doi:10.1586/ERI.13.19
14. Abbas M, Uçkay I, Lipsky BA. In diabetic foot infections antibiotics are to treat infection, not to heal wounds. *Expert Opin Pharmacother*. 2015;16(6):821–832. doi:10.1517/14656566.2015.1021780
15. Feng S-H, Chu Y-J, Wang P-H, Jun X, Min D, Li X-M. Risk Factors and Gene Type for Infections of MRSA in Diabetic Foot Patients in Tianjin, China. *Int J Low Extrem Wounds*. 2013;12(2):106–112. doi:10.1177/1534734613489991
16. Abalkhail A, Elbehiry A. Methicillin-Resistant Staphylococcus aureus in Diabetic Foot Infections: protein Profiling, Virulence Determinants, and Antimicrobial Resistance. *Appl Sci*. 2022;12(21):10803. doi:10.3390/app122110803
17. Salamandane A, Correia J, Muetanene BA, Dos Santos M, Malfeito-Ferreira M, Brito L. Methicillin Resistance of Food-Borne Biofilm-Forming Staphylococci. *Appl Sci*. 2023;13(13):7725. doi:10.3390/app13137725
18. Arciola CR, Campoccia D, Ravaioli S, Montanaro L, Vasil ML, Otto M. Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. *Front Cell Infect Microbiol*. 2015;5(7). doi:10.3389/fcimb.2015.00007
19. Gajewska J, Chajecka-Wierzchowska W. Biofilm Formation Ability and Presence of Adhesion Genes among Coagulase-Negative and Coagulase-Positive Staphylococci Isolates from Raw Cow's Milk. *Pathogens*. 2020;9(8):654. doi:10.3390/pathogens9080654
20. Cue D, Lei MG, Lee CY. Genetic regulation of the intercellular adhesion locus in staphylococci. *Front Cell Infect Microbiol*. 2012;2. doi:10.3389/fcimb.2012.00038.
21. Sohail MU, Mashood F, Oberbach A, Chennakkandathil S, Schmidt F. The role of pathogens in diabetes pathogenesis and the potential of immunoproteomics as a diagnostic and prognostic tool. *Front Microbiol*. 2022;13. doi:10.3389/fmicb.2022.1042362.
22. Ranjalkar J, Chandy S. India's National Action Plan for antimicrobial resistance – an overview of the context, status, and way ahead. *J Family Med Prim Care*. 2019;8(6):1828. doi:10.4103/jfmpc.jfmpc_275_19
23. Pezzani MD, Mazzaferri F, Compri M, et al. Linking antimicrobial resistance surveillance to antibiotic policy in healthcare settings: the COMBACTE-Magnet EPI-Net COACH project. *J Antimicrob Chemother*. 2020;75(Supplement_2):ii2–ii19. doi:10.1093/jac/dkaa425
24. Trivedi KK, Pollack LA. The Role of Public Health in Antimicrobial Stewardship in Healthcare. *Clinl Infect Dis*. 2014;59(suppl_3):S101–S103. doi:10.1093/cid/ciu544
25. Silva-Matos C, Gomes A, Azevedo A, Damasceno A, Prista A, Lunet N. Diabetes in Mozambique: prevalence, management and healthcare challenges. *Diabetes Metab*. 2011;37(3):237–244. doi:10.1016/j.diabet.2010.10.006
26. Li M, Du X, Villaruz AE, et al. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. *Nat Med*. 2012;18(5):816–819. doi:10.1038/nm.2692
27. EUROSIS. *Relatório da Avaliação Anual de Desempenho do Município da Beira*. Beira: EUROSIS; 2020.
28. Salamandane A, Cahango G, Muetanene BA, Malfeito-Ferreira M, Brito L. Multidrug Resistance in Enterococci Isolated from Cheese and Capable of Producing Benzalkonium Chloride-Resistant Biofilms. *Biology*. 2023;12(10):1353. doi:10.3390/biology12101353
29. Salamandane A, Leech J, Almeida R, et al. Metagenomic analysis of the bacterial microbiome, resistome and virulome distinguishes Portuguese Serra da Estrela PDO cheeses from similar non-PDO cheeses: an exploratory approach. *Food Res Int*. 2024;189:114556. doi:10.1016/j.foodres.2024.114556
30. CLSI. *M100 Performance Standards for Antimicrobial Susceptibility Testing an Informational Supplement for Global Application Developed Through the Clinical and Laboratory Standards Institute Consensus Process*. 2021.
31. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268–281. doi:10.1111/j.1469-0691.2011.03570.x
32. Peacock SJ, Moore CE, Justice A, et al. Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. *Infect Immun*. 2002;70(9):4987–4996. doi:10.1128/IAI.70.9.4987-4996.2002
33. Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G. Use of multiplex PCR to identify Staphylococcus aureus adhesins involved in human hematogenous infections. *J Clin Microbiol*. 2003;41(9):4465–4467. doi:10.1128/JCM.41.9.4465-4467.2003
34. Campbell SJ, Deshmukh HS, Nelson CL, et al. Genotypic characteristics of Staphylococcus aureus isolates from a multinational trial of complicated skin and skin structure infections. *J Clin Microbiol*. 2008;46(2):678–684. doi:10.1128/JCM.01822-07

35. Lin T, Li Q, Jin D, Liu W, Tang C, Zhang X. Investigation of Virulence Genes of Staphylococcus aureus Isolated from Sterile Body Fluid Samples and Their Correlation with Clinical Symptoms and Outcomes. *Can J Infect Dis Med Microbiol*. 2021;2021:1–8. doi:10.1155/2021/5354747
36. Jarraud S, Mougel C, Thioulouse J, et al. Relationships between Staphylococcus aureus genetic background, virulence factors, agr groups (alleles), and human disease. *Infect Immun*. 2002;70(2):631–641. doi:10.1128/IAI.70.2.631-641.2002
37. Kumar JD, Negi YK, Gaur A, Khanna D. Detection of virulence genes in Staphylococcus aureus isolated from paper currency. *Inter J Infect Dis*. 2009;13(6):e450–e455. doi:10.1016/j.ijid.2009.02.020
38. Růžičková V, Voller J, Pantůček R, Petráš P, Doškař J. Multiplex PCR for detection of three exfoliative toxin serotype genes inStaphylococcus aureus. *Folia Microbiol*. 2005;50(6):499–502. doi:10.1007/BF02931437
39. Li X, Fang F, Zhao J, et al. Molecular characteristics and virulence gene profiles of Staphylococcus aureus causing bloodstream infection. *Braz J Infect Dis*. 2018;22(6):487–494. doi:10.1016/j.bjid.2018.12.001
40. Strommenger B, Braulke C, Pasemann B, Schmidt C, Witte W. Multiplex PCR for rapid detection of Staphylococcus aureus isolates suspected to represent community-acquired strains. *J Clin Microbiol*. 2008;46(2):582–587. doi:10.1128/JCM.01600-07
41. Becker K, Roth R, Peters G. Rapid and specific detection of toxigenic Staphylococcus aureus: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *J Clin Microbiol*. 1998;36(9):2548–2553. doi:10.1128/JCM.36.9.2548-2553.1998
42. Solati SM, Tajbakhsh E, Khamesipour F, Gugnani HC. Prevalence of virulence genes of biofilm producing strains of Staphylococcus epidermidis isolated from clinical samples in Iran. *AMB Express*. 2015;5(1):47. doi:10.1186/s13568-015-0134-3
43. Salamandane A, Oliveira J, Coelho M, et al. Enterotoxin- and Antibiotic-Resistance-Encoding Genes Are Present in Both Coagulase-Positive and Coagulase-Negative Foodborne Staphylococcus Strains. *Appl Microbiol*. 2022;2(2):367–380. doi:10.3390/applmicrobiol2020028
44. Moura GS, Mota RA, Marques MFS, et al. Gangrenous mastitis in sheep caused by multidrug-resistant Staphylococcus haemolyticus. *Pesquisa Veterinária Brasileira*. 2020;40(12):947–954. doi:10.1590/1678-5150-pvb-6658
45. Acosta AC, Oliveira PRF, Albuquerque L, et al. Frequency of Staphylococcus aureus virulence genes in milk of cows and goats with mastitis. *Pesquisa Veterinária Brasileira*. 2018;38(11):2029–2036. doi:10.1590/1678-5150-pvb-5786
46. Al-Amery K, Elhariri M, Elsayed A, et al. Vancomycin-resistant Staphylococcus aureus isolated from camel meat and slaughterhouse workers in Egypt. *Antimicrob Resist Infect Control*. 2019;8(129). doi:10.1186/s13756-019-0585-4
47. Ho YM, Sun Y-J, Wong S-Y, Lee ASG. Contribution of dfrA and inhA Mutations to the Detection of Isoniazid-Resistant Mycobacterium tuberculosis Isolates. *Antimicrob Agents Chemother*. 2009;53(9):4010–4012. doi:10.1128/AAC.00433-09
48. Burgold-Voigt S, Monecke S, Simbeck A, et al. Characterisation and Molecular Analysis of an Unusual Chimeric Methicillin Resistant Staphylococcus Aureus Strain and its Bacteriophages. *Front Genet*. 2021;12:723958. doi:10.3389/fgene.2021.723958
49. Zhou S, Hu X, Wang Y, Fei W, Sheng Y, Que H. The Global Prevalence of Methicillin-Resistant Staphylococcus Aureus in Patients with Diabetic Foot Ulcers: a Systematic Review and Meta-Analysis. *Diabetes Metabolic Syndrome Obesity*. 2024;17:563–574. doi:10.2147/DMSO.S446911
50. Arfaoui A, Sallem R, Fernández-Fernández R. Methicillin-Resistant Staphylococcus aureus from Diabetic Foot Infections in a Tunisian Hospital with the First Detection of MSSA CC398-t571. *Antibiotics*. 2022;11(12):1755. doi:10.3390/antibiotics11121755
51. Chen Y, Yang J, Wang Y, et al. Community-associated methicillin-resistant Staphylococcus aureus infection of diabetic foot ulcers in an eastern diabetic foot center in a tertiary hospital in China: a retrospective study. *BMC Infect Dis*. 2023;23(1):652. doi:10.1186/s12879-023-08631-z
52. Perim MC, Celeste SRC, Orsolin EF, et al. Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. *Rev Soc Bras Med Trop*. 2015;48(5):546–554. doi:10.1590/0037-8682-0146-2015
53. Rodrigues CF. Self-medication with antibiotics in Maputo, Mozambique: practices, rationales and relationships. *Palgrave Commun*. 2020;6 (1):1–12. doi:10.1057/s41599-019-0385-8
54. Van der Meeren BT, Millard PS, Scacchetti M, et al. Emergence of methicillin resistance and Panton-Valentine leukocidin positivity in hospital- and community-acquired Staphylococcus aureus infections in Beira, Mozambique. *Tropical Medicine and International Health*. 2014;19(2):169–176. doi:10.1111/tmi.12221
55. Vubil D, Garrine M, Ruffing U, et al. Molecular Characterization of Community Acquired Staphylococcus aureus Bacteremia in Young Children in Southern Mozambique, 2001–2009. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.00730.
56. Wada FW, Mekonnen MF, Sawiso ED, et al. Bacterial profile and antimicrobial resistance patterns of infected diabetic foot ulcers in sub-Saharan Africa: a systematic review and meta-analysis. *Sci Rep*. 2023;13(1):14655. doi:10.1038/s41598-023-41882-z
57. Ehsan H. Antibiotic Resistance in Developing Countries: emerging Threats and Policy Responses. *Public Health Challenges*. 2025;4(1). doi:10.1002/puh2.70034
58. Iskandar K, Molinier L, Hallit S, et al. Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. *Antimicrob Resist Infect Control*. 2021;10(1):63. doi:10.1186/s13756-021-00931-w
59. Víquez-Molina G, Aragón-Sánchez J, Pérez-Corrales C, Murillo-Vargas C, López-Valverde ME, Lipsky BA. Virulence Factor Genes in Staphylococcus aureus Isolated From Diabetic Foot Soft Tissue and Bone Infections. *Int J Low Extrem Wounds*. 2018;17(1):36–41. doi:10.1177/1534734618764237
60. Shettigar K, Jain S, Bhat DV, et al. Virulence determinants in clinical Staphylococcus aureus from monomicrobial and polymicrobial infections of diabetic foot ulcers. *J Med Microbiol*. 2016;65(12):1392–1404. doi:10.1099/jmm.0.000370
61. Zhao H, Xu S, Yang H, et al. Molecular Typing and Variations in Amount of tst Gene Expression of TSST-1-Producing Clinical Staphylococcus aureus Isolates. *Front Microbiol*. 2019;10. doi:10.3389/fmicb.2019.01388.
62. Mishra AK, Yadav P, Mishra A. A Systemic Review on Staphylococcal Scalded Skin Syndrome (SSSS): a Rare and Critical Disease of Neonates. *Open Microbiol J*. 2016;10(1):150–159. doi:10.2174/1874285801610010150
63. Kananizadeh P, Moghadam SO, Sadeghi Y, Foroushani AR, Adibi H, Pourmand MR. Molecular characteristics of methicillin-resistant staphylococcus aureus (MRSA) isolated from diabetic foot infection. *Iran J Pathol*. 2019;14(4):329–337. doi:10.30699/IJP.2019.101092.2035
64. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis*. 2003;9(8):978–984. doi:10.3201/eid0908.030089
65. Abraham NM, Jefferson KK. Staphylococcus aureus clumping factor B mediates biofilm formation in the absence of calcium. *Microbiology*. 2012;158(6):1504–1512. doi:10.1099/mic.0.057018-0

66. Ortega E, Abriouel H, Lucas R, Gálvez A. Multiple Roles of Staphylococcus aureus Enterotoxins: pathogenicity, Superantigenic Activity, and Correlation to Antibiotic Resistance. *Toxins*. 2010;2(8):2117–2131. doi:10.3390/toxins2082117
67. Roetzer A, Gruener C, Haller G, Beyerly J, Model N, Eibl M. Enterotoxin Gene Cluster-Encoded SEI and SEIN from Staphylococcus aureus Isolates are Crucial for the Induction of Human Blood Cell Proliferation and Pathogenicity in Rabbits. *Toxins*. 2016;8(11):314. doi:10.3390/toxins8110314
68. Silva V, Almeida L, Gaio V, et al. Biofilm formation of multidrug-resistant mrsa strains isolated from different types of human infections. *Pathogens*. 2021;10(8):970. doi:10.3390/pathogens10080970
69. Turner MJ, van Vuuren S, Leigh-De Rapper S. Analysing patient factors and treatment impact on diabetic foot ulcers in South Africa. *S Afr J Sci*. 2024;120(3–4). doi:10.17159/sajs.2024/16301
70. Kuguyo O, Mukona DM, Chikwasha V, Gwanzura L, Chirenda J, Matimba A. Prevalence and risk factors for diabetic foot complications among people living with diabetes in Harare, Zimbabwe: a cross-sectional study. *BMC Public Health*. 2024;24(1):677. doi:10.1186/s12889-023-17610-7
71. Madede T, Damasceno A, Lunet N, et al. Changes in prevalence and the cascade of care for type 2 diabetes over ten years (2005-2015): results of two nationally representative surveys in Mozambique. *BMC Public Health*. 2022;22(1). doi:10.1186/s12889-022-14595-7
72. Fontes F, Damasceno A, Jessen N, et al. Prevalence of overweight and obesity in Mozambique in 2005 and 2015. *Public Health Nutr*. 2019;22(17):3118–3126. doi:10.1017/S1368980019002325
73. Nutor JJ, Duodu PA, Agbadi P, Duah HO, Oladimeji KE, Gondwe KW. Predictors of high HIV+ prevalence in Mozambique: a complex samples logistic regression modeling and spatial mapping approaches. *PLoS One*. 2020;15(6). doi:10.1371/journal.pone.0234034