Characterisation of Methicillin-Resistant *Staphylococcus aureus* from Alexandria, Egypt

Stefan Monecke [1,2,3,*], Amira K. Bedewy [4], Elke Müller [1,2], Sascha D. Braun [1,2], Celia Diezel [1,2], Amel Elsheredy [4], Ola Kader [4], Martin Reinicke [1,2], Abeer Ghazal [4], Shahinda Rezk [4,†] and Ralf Ehricht [1,2,5,†]

[1] Leibniz Institute of Photonic Technology (IPHT), 07745 Jena, Germany
[2] InfectoGnostics Research Campus, 07743 Jena, Germany
[3] Institute for Medical Microbiology and Virology, Dresden University Hospital, 01307 Dresden, Germany
[4] Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria 5424041, Egypt
[5] Institute of Physical Chemistry, Friedrich-Schiller University, 07743 Jena, Germany
* Correspondence: stefan.monecke@leibniz-ipht.de
† These authors contributed equally to this work.

Abstract: The present study aims to characterise clinical MRSA isolates from a tertiary care centre in Egypt's second-largest city, Alexandria. Thirty isolates collected in 2020 were genotypically characterised by microarray to detect their resistance and virulence genes and assign them to clonal complexes (CC) and strains. Isolates belonged to 11 different CCs and 14 different strains. CC15-MRSA-[V+*fus*] (n = 6), CC1-MRSA-[V+*fus*+*tir*+*ccrA/B-1*] (PVL+) (n = 5) as well as CC1-MRSA-[V+*fus*+*tir*+*ccrA/B-1*] and CC1153-MRSA-[V+*fus*] (PVL+) (both with n = 3) were the most common strains. Most isolates (83%) harboured variant or composite SCC*mec* V or VI elements that included the fusidic acid resistance gene *fusC*. The SCC*mec* [V+*fus*+*tir*+*ccrA/B*-1] element of one of the CC1 isolates was sequenced, revealing a presence not only of *fusC* but also of *blaZ*, *aacA-aphD* and other resistance genes. PVL genes were also common (40%). The hospital-acquired MRSA CC239-III strain was only found twice. A comparison to data from a study on strains collected in 2015 (Montelongo et al., 2022) showed an increase in *fusC* and PVL carriage and a decreasing prevalence of the CC239 strain. These observations indicate a diffusion of community-acquired strains into hospital settings. The beta-lactam use in hospitals and the widespread fusidic acid consumption in the community might pose a selective pressure that favours MRSA strains with composite SCC*mec* elements comprising *mecA* and *fusC*. This is an unsettling trend, but more MRSA typing data from Egypt are required.

Keywords: *Staphylococcus aureus*; DNA microarrays; MRSA; hospital-acquired MRSA; community-acquired MRSA; Panton-Valentine-leukocidin; *mecA*; *fusC*; fusidic acid resistance

1. Introduction

Staphylococcus aureus (*S. aureus*) is a human and animal pathogen that is a global cause of morbidity and mortality. Antimicrobial resistance in *S. aureus* is frequently associated with mobile genetic elements, including plasmids, transposons, and staphylococcal cassette chromosome (SCC) elements that act as carrier to exchange genetic information between *Staphylococcus* strains. Methicillin resistance in staphylococci is based on the production of altered penicillin-binding proteins with a low affinity for beta-lactam antibiotics. These proteins are encoded by different *mec* genes (*mecA* or *mecC*), out of which *mecA* is the most common and widespread one [1]. SCC*mec* elements carry *mec* genes along with the genes that control their expression. There are three basic genetic units within SCC*mec*: the *ccr* recombinase gene complex, the *mec* gene complex and the joining region (J region) [2–6]. Other SCC elements might carry fusidic acid resistance (mediated by the *fusC* gene), heavy metal resistance, or certain virulence factors such as *tirS* or a phenol-soluble modulin (PSM-*mec*) [7,8].

MRSA has been recognized for decades as a common cause of nosocomial infections, resulting in increased mortality, longer hospitalisations, and higher costs to healthcare systems. Healthcare-associated MRSA (HA-MRSA) are *S. aureus* isolates obtained from patients two or more days after hospitalisation or from patients with a history of recent hospitalisation, surgery, dialysis, or residence in a long-term care facility with an indwelling medical device at the time of culture [9]. However, some MRSA strains can disseminate among otherwise healthy individuals, leading to community-acquired infections [10–19]. Community-associated MRSA (CA-MRSA) differ from typical HA-MRSA strains in not only epidemiological background, but also in their antibiotic susceptibility profile and in genotypic features. These include the presence of the Panton–Valentine leukocidin (PVL, [17–21]) and the carriage of SCC*mec* IV or V elements. However, strains or lineages traditionally considered "community-associated" can also cause nosocomial outbreaks, and there are strains associated with medical care outside hospitals. In addition, parts of the world—including India, the Middle East, and North Africa—are currently plagued by an emergence of PVL-positive, multi-resistant strains harbouring SCC*mec* IV and V elements, which can be found both in hospitals as well as in the community. Thus, distinctions between HA-MRSA and CA-MRSA become increasingly blurry, and epidemiological considerations are only sometimes compatible with a genotype-based definition of CA-MRSA [22].

Although molecular typing data for *S. aureus* and MRSA are abundantly available for Western Europe, North America and Australia, comparatively few studies were conducted to describe the *S. aureus*/MRSA epidemiology in the rest of the world [23–27]. For the Middle East, previous work showed a high rate of MRSA, a high diversity of different clonal complexes and strains, and a high rate of PVL carriage and of resistance to fusidic acid, either mediated by plasmids or by SCC*mec* elements harbouring *fusC* [28–35]. A detailed molecular characterisation of clinical *S. aureus* isolates from Africa has been largely neglected in the past [36]. Some localised studies and analysis of cases of *S. aureus*-related infections in returning travellers have suggested that African *S. aureus* might have a different genetic background and might be more virulent than isolates from Europe with Africa being an endemic region for PVL-positive *S. aureus* [37–39].

At the crossroads of Africa and the Middle East, Egypt is among the countries where only anecdotal typing data are available. Some work focused on livestock animals and contact persons [40–42], but in general, the epidemiology of human MRSA infection, regardless of whether HA or CA, needs to be better understood [42]. This is regrettable, as MRSA appears to be abundant. A recent study revealed an extremely high MRSA rate, with about 80% of total *S. aureus* isolates being MRSA [43]. A recently published sequencing study included clinical methicillin-susceptible *S. aureus* (MSSA) and MRSA isolates from Alexandria [44], collected in 2015. In this study, the most common MRSA strains were the pandemic hospital-associated ST239-MRSA-III, CC80-MRSA-IV (a PVL-positive strain frequently found in Mediterranean and Middle Eastern countries as well as in Western European tourists returning from there) and a variety of CC1 strains.

Various molecular techniques have been developed to study the genetic diversity of *S. aureus* strains and, in particular, MRSA strains [45,46]. DNA microarray technology allows the simultaneous detection of many molecular targets, including resistance genes and virulence factors. The overall hybridization profile could also be used as a fingerprint, or a dataset, that allows elucidating relatedness between different isolates and allocating them to strains [23,47] based on a framework of clonal complexes (CC), as initially defined by multilocus sequence typing (MLST; [48–50]), and on their SCC*mec* types. The present study aimed to characterise clinical MRSA isolates from a tertiary care centre in Egypt's second-largest city, Alexandria.

2. Results

Thirty-four clinical isolates of *S. aureus* were obtained from routine diagnostic procedures at the microbiology laboratory of the Medical Research Institute, Alexandria

University over five months in 2020. Most MRSA isolates were obtained from aspirated pus followed by wound swabs (Table 1). Four isolates were excluded from further analysis based on PCR and microarray experiments. The *mecA* gene was absent in one isolate that phenotypically was tested as cefoxitin-resistant; three isolates were PCR-negative for *femA* and were by microarray categorised as coagulase-negative, albeit *mecA*-positive, staphylococci.

Table 1. Sample types and isolates.

Sample Type	Number of MRSA Isolates	Percent
Aspirated pus	18	60.0
Wound swab	4	13.3
Blood culture	2	6.7
Bone marrow aspirate	1	3.3
Catheter tip	1	3.3
Peritoneal fluid	1	3.3
Sputum	1	3.3
Throat swab	1	3.3
Urine	1	3.3

2.1. Resistance Genes and Antibiotic Resistance, SCCmec Elements

MRSA isolates were tested against 19 antimicrobial discs (Table 2). Apart from cefoxitin, the isolates exhibited the highest rates of resistance to gentamicin (90%), tobramycin (90%), and fusidic acid (86.7%).

Table 2. Susceptibility of MRSA isolates to antibiotics using the Kirby–Bauer disk diffusion method according to CLSI guidelines or if no interpretive criteria are recommended by the CLSI, according to EUCAST (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf; as accessed 1 December 2022).

Antibiotic Compound	N (Suscept.)	% (Suscept.)	N (Intermed.)	% (Intermed.)	N (Resistant)	% (Resistant)
Cefoxitin	0	0.0	0	0.0	30	100.0
Vancomycin	30	100.0	0	0.0	0	0.0
Gentamicin	1	3.3	2	6.7	27	90.0
Amikacin *	1	3.3	13	43.3	16	53.3
Tobramycin *	0	0.0	3	10.0	27	90.0
Erythromycin	1	3.3	17	56.7	12	40.0
Doxycycline	0	0.0	16	53.3	14	46.7
Tigecycline *	14	46.7	12	40.0	4	13.3
Ciprofloxacin	3	10.0	14	46.7	13	43.3
Levofloxacin	2	6.7	20	66.7	8	26.7
Ofloxacin	0	0.0	14	46.7	16	53.3
Norfloxacin	0	0.0	1	3.3	0	0.0
Clindamycin	9	30.0	11	36.7	10	33.3
Trimethoprim+ sulfamethoxazole	4	13.3	9	30.0	17	56.7
Chloramphenicol	0	0.0	23	76.7	7	23.3
Rifampin	21	70.0	5	16.7	4	13.3
Linezolid	28	93.3	0	0.0	2	6.7
Fusidic acid *	0	0.0	4	13.3	26	86.7
Nitrofurantoin	0	0.0	1	3.3	0	0.0

* EUCAST criteria used.

All isolates harboured *mecA* as part of various SCC*mec* elements. SCC*mec* elements I, II, VT, VII, VIII, IX, X and XI (including *mecC*) were not found. Two isolates harboured composite SCC*mec* III elements. Another three isolates carried "plain" SCC*mec* IVa elements. All others had composite elements that included SCC*mec* IV, V, or VI and SCC*fus*. Accordingly, the SCC-associated *fusC* gene was found in 25 isolates, i.e., in 83%.

In contrast, the plasmid-borne fusidic acid resistance gene *far*-1 was not detected. This gene is usually associated with the PVL-positive CC80-MRSA-IV, which was conspicuously absent. The mupirocin resistance gene *mupA* was detected once in a CC1-MRSA-[V+*fus*+*tir*+*ccrAB1*] isolate that also harboured *cfr* and *aadD*. The multidrug resistance gene *cfr* was found once, although no linezolid resistance was observed phenotypically. Vancomycin resistance genes were not detected which was in accordance to the phenotypic glycopeptide susceptibility of all isolates. The gene *fexA* was detected in two CC5 isolates, and both were phenotypically resistant to chloramphenicol. SCC*mec* markers and resistance genes, as detected by array hybridisation, are listed in Table 3.

Table 3. Markers used for SCC*mec* subtyping and resistance genes.

Marker	Description/Gene Product	Ref.	n	%
mecA	Gene encoding a modified penicillin-binding protein (PBP2a)	[23,47]	30	100.0
mecC	Alternate gene encoding a modified penicillin-binding protein, SCC*mec* XI	[51,52]	0	0.0
Delta *mecR1*	Truncated methicillin resistance operon repressor 1. Truncated *mec*R1 is present in SCC*mec* I, IV, V, VI, VII	[23,47]	7	23.3
mecR1	Methicillin resistance operon repressor 1. Un-truncated sequence in SCC*mec* II, III, VIII	[23,47]	2	6.7
mecI	Gene encoding a methicillin-resistance regulatory protein. Present in SCC*mec* II, III, VIII	[23,47]	2	6.7
xylR=mecR2	Methicillin resistance operon repressor 2, homolog of xylose repressor. Present in SCC*mec* II, III, VIII	[23,47]	2	6.7
ugpQ	Gene encoding glycerophosphoryl diester phosphodiesterase. Accompanies *mecA* in nearly all SCC*mec* sequences	[23,47]	30	100.0
pls-SCC	Gene encoding Plasmin-sensitive surface protein	[23,47]	0	0.0
cstB-SCC	CsoR-like sulfur transferase-regulated gene B. Used to distinguish SCC*mec* IVa from other SCC*mec* IV subtypes	[52]	3	10.0
kdpA+B+D-SCC	SCC-borne ATP-driven potassium transport (KDP) system, SCC*mec* II	[23,47]	0	0.0
D1GU38	Putative protein. Used for identification of SCC*mec* VT, SCC*mec* ZH47, SCC*mec* VII because of an association with (additional/second) *ccrC* copies	[52]	0	0.0
B2Y834	Abortive phage resistance protein. Used for identification of SCC*mec* IV A, G, c and SCC*mec* MRSAZH47	[52]	0	0.0
B6VQU0	Putative protein. Used for identification of SCC*mec* IVh/j	[52]	0	0.0
Q3YK51	*Putative* protein. Subtyping SCC*mec* IV, i.e., identification of SCC*mec* IV g	[52]	0	0.0
tirS	Staphylococcal TIR-protein binding protein	[52]	11	36.7
arcA+B+D-SCC	Genes encoding the arginine metabolic operon from ACME-1/-2 elements	[23,47]	0	0.0
opp3B and *speG*	Genes encoding oligopeptide permease and spermidine N-acetyltransferase. Associated with ACME or composite SCC*mec*/ACME elements	[52]	0	0.0
ccrA-1+ccrB-1	Cassette chromosome recombinase genes, type 1	[23,47]	8	26.7
ccrA-2+ccrB-2	Cassette chromosome recombinase genes, type 2	[23,47]	3	10.0
ccrA-3+ccrB-3	Cassette chromosome recombinase genes, type 3	[23,47]	2	6.7
ccrAA+ccrC	Cassette chromosome recombinase gene C and associated *ccr* homologue	[23,47]	24	80.0
ccrA-4+ccrB-4	Cassette chromosome recombinase genes, type 4	[23,47]	2	6.7
Q9XB68-*dcs*	Located at the terminus of SCC*mec* directly next to *orfX*	[23,47]	0	0.0
merA+merB	Genes from the mercury resistance operon	[23,47]	0	0.0
czrC	Cadmium and zinc resistance gene C, heavy metal translocating P-type ATPase. Frequently associated with livestock MRSA	[52]	0	0.0
cadD (R35)	SCC-borne cadmium resistance gene, used for subtyping CC239-MRSA-III clades	[52]	2	6.7
blaZ+blaI+blaR	Penicillinase operon (excluding the SCC*mec* XI-associated allele)	[23,47]	28	93.3
blaZ(SCC*mec* XI)	Gene encoding beta-lactamase, from SCC*mec* XI	[51,52]	0	0.0

Table 3. *Cont.*

Marker	Description/Gene Product	Ref.	n	%
erm(A)	rRNA adenine N-6-methyl-transferase conferring erythro-/clindamycin resistance	[23,47]	1	3.3
erm(B)	rRNA adenine N-6-methyl-transferase, erythro-/clindamycin resistance	[23,47]	1	3.3
erm(C)	rRNA adenine N-6-methyl-transferase, erythro-/clindamycin resistance	[23,47]	4	13.3
lnu(A)	Lincosamide-nucleotidyltransferase (=*linA*)	[23,47]	5	16.7
lsa-E	Lincosamide ABC transporter	[52]	0	0.0
msrA	Macrolide resistance ABC transporter, ATP-binding protein	[23,47]	5	16.7
mefA	Macrolide efflux protein A	[23,47]	0	0.0
mph(C)	Macrolide 2′-phosphotransferase II (=*mpbBM*)	[23,47]	0	0.0
vat(A), *vat*(B)	Acetyltransferase inactivating streptogramin A, virginiamycin	[23,47]	0	0.0
vga(A), *vgb*	Streptogramin A resistance genes	[23,47]	0	0.0
aacA-aphD	Bifunctional enzyme Aac/Aph (6′-aminoglycoside N-acetyltransferase and 2″-aminoglycoside phosphotransferase), gentamicin/tobramycin resistance	[23,47]	25	83.3
aadD	Aminoglycoside adenyltransferase, tobramycin resistance	[23,47]	6	20.0
aphA3	3′5′-aminoglycoside phosphotransferase, neo-/kanamycin resistance	[23,47]	12	40.0
sat	Streptothricine acetyltransferase	[23,47]	9	30.0
dfrA	Dihydrofolate reductase type 1	[23,47]	4	13.3
fusC	SCC-associated fusidic acid resistance gene (=Q6GD50)	[23,47]	25	83.3
far1	Plasmid borne fusidic acid resistance gene (=*fusB*)	[23,47]	0	0.0
mupA	(High level) mupirocin resistance protein	[23,47]	1	3.3
tet(K)	Tetracycline resistance gene	[23,47]	9	30.0
tet(L)	Tetracycline resistance gene	[52]	9	30.0
tet(M)	Tetracycline resistance gene	[23,47]	4	13.3
cat	Chloramphenicol acetyltransferase	[23,47]	1	3.3
cfr	23S rRNA methyltransferase encoding resistance towards Lincosamides, Oxazolidinones, Pleuromutilins, Streptogramin A etc.	[23,47]	1	3.3
fexA	Chloramphenicol/florfenicol exporter	[23,47]	2	6.7
qacA, *qacC*	Quaternary ammonium compound resistance proteins A and C	[23,47]	0	0.0
vanA, *vanB*, *vanZ*	Glycopeptide resistance genes	[23,47]	0	0.0

2.2. Virulence Factors

Regarding virulence factors (Table 4), 12 isolates (40%) were Panton–Valentine leucocidin (PVL)-positive, of which five belonged to CC1, three to CC 1153, two to CC 152, one to CC 121 and one to CC 30. The enterotoxin gene cluster (*egc*, consisting of *seg*, *sei*, *selm*, *seln*, *selo* and *selu*) was found in all CC5, CC22, CC30, and CC121 isolates, representing 20% of all the tested isolates. The toxic shock toxin gene (*tst1*) was found in all CC22 isolates (6.7%). Enterotoxin genes *sec* and *sel* as well as the exfoliative toxins genes *etB* and *etD* were not detected. However, *etD2*=*etE* yielded signals in two isolates belonging to CC152 (6.7%). The epidermal cell differentiation inhibitor gene *edinB* was also found in these two isolates. The *edinA* gene was not identified in any of the isolates, and *edinC* was present in only one isolate belonging to CC5. Various combinations of the immune evasion complex (IEC) genes (*sea*, *sak*, *chp* and *scn*) were also detected in most isolates. Amongst the IEC-positive isolates, IEC type D (*sea*, *sak*, and *scn*) predominated (36.6%). Amongst the individual IEC genes, the most predominant gene was *scn* (in all but one CC1 isolate). The gene encoding surface-anchored protein X, *sasX*=*sesI*, was not found, neither in the two CC239 isolates (which represent the lineage from which it was initially described) nor elsewhere.

Table 4. Virulence factors.

Marker	Description/Gene Product	Ref.	n	%
lukF-PV+lukS-PV	Phage-borne Panton-Valentine leukocidin	[23,47]	12	40.0
lukM+lukF-P83	Phage-borne LukM/F-P83 leukocidin, associated with disease in ungulates	[23,47]	0	0.0
lukD+lukE	Genomic-Island-borne leukocidin	[23,47]	21	70.0
tst1	Toxic shock syndrome toxin	[23,47]	2	6.7
sea	Gene encoding enterotoxin A	[23,47]	13	43.3
sea (N315)=*sep*	Allele of the enterotoxin A gene, frequently found in CC5 and CC7	[23,47]	0	0.0
seb	Gene encoding enterotoxin B	[23,47]	2	6,7
sec, *see*, *sel*	Genes encoding enterotoxins C, E and L	[23,47]	0	0.0
sed	Gene encoding enterotoxin D	[23,47]	1	3.3
seh	Gene encoding enterotoxin H, associated, e.g., with CC1, CC10 and CC34	[23,47]	9	30.0
sej	Gene encoding enterotoxin J	[23,47]	1	3.3
sek	Gene encoding enterotoxin K	[23,47]	12	40.0
seq	Gene encoding enterotoxin Q	[23,47]	12	40.0
ser	Gene encoding enterotoxin R	[23,47]	1	3.3
egc	Enterotoxin gene cluster consisting of *seg*, *sei*, *selm*, *seln*, *selo* and *selu*	[23,47]	6	20.0
ORF CM14	Enterotoxin gene homologue, associated, e.g., with CCs 93, 121 and 705	[23,47]	1	3.3
sak	Staphylokinase	[23,47]	24	80.0
chp	Chemotaxis-inhibiting protein (CHIPS)	[23,47]	9	30.0
scn	Staphylococcal complement inhibitor	[23,47]	29	96.7
etA, *etB*, *etD*	Genes encoding Exfoliative Toxins A, B. D	[23,47]	0	0.0
etD2/etE/etE2	Exfoliative Toxin homologue *	[51,52]	2	6.7
edinA	Epidermal cell differentiation inhibitor	[23,47]	1	3.3
edinB	Epidermal cell differentiation inhibitor B	[23,47]	2	6.7
edinC	Epidermal cell differentiation inhibitor C	[23,47]	0	0.0
cap 5	Capsule type 5 (summary of probes for *capH*5, *capJ*5, *capK*5)	[23,47]	10	33.3
cap 8	Capsule type 8 (summary of probes for *capH*8, *capI*8, *capJ*8, *capK*8)	[23,47]	20	66.7
cna	Gene encoding collagen adhesion factor	[23,47]	18	60.0
sasG	*Staphylococcus aureus surface* protein G	[23,47]	26	86.7
sasX=sesI	Surface-anchored protein X, used for subtyping CC239-MRSA-III clades	[52]	0	0.0
agr I	Accessory gene regulator, group (variant/allele) 1	[23,47]	8	26.7
agr II	Accessory gene regulator, group (variant/allele) 2	[23,47]	11	36.7
agr III	Accessory gene regulator, group (variant/allele) 3	[23,47]	10	33.3
agr IV	Accessory gene regulator, group (variant/allele) 4	[23,47]	1	3.3
hld	Haemolysin Delta, small peptide whose gene is located next to *agr*	[23,47]	30	100.0
tirS	Staphylococcal TIR-protein binding protein	[52]	11	36.7
arcA+B+D-SCC	Genes encoding the arginine metabolic operon from ACME-1/-2 elements	[23,47]	0	0.0

* Weak and/or irregular signals (one out of two probes) attributable to the presence of a novel *etD/E* homologue in CC152 strains, as recently recognised in [53].

2.3. Strain Affiliations

This study identified 11 different CCs and 14 strains (as defined by CC affiliation, toxin gene carriage, and SCC*mec* subtype; Table 5). A recently published sequencing described MRSA from Alexandria [44] collected in 2015. Forty-six genomes from this study were analysed (see Section 4) and assigned to strains allowing a direct comparison of strain prevalence in 2015 and 2020.

Table 5. Affiliations to CCs and strains, comparison to the data from the 2015 study [44].

Clonal Complex	Strain	n in 2020	% in 2020	n in 2015	% in 2015
CC1	CC1-MRSA-[V+*fus*+*tir*+ccrA/B-1]	3	10.0	2	4.3
	CC1-MRSA-[V+*fus*+*tir*+ccrA/B-1] (PVL+)	5	16.7	4	8.7
	CC1-MRSA-[V+*fus*+*tir*]	1	3.3	0	0
CC5	CC5-MRSA-[V+cas], "WA MRSA-123"	0	0.0	2	4.3
	CC5-MRSA-[VI+*fus*+*tir*]	2	6.7	1	2.2
CC6	CC6-MRSA-IVa, "WA MRSA-51"	0	0.0	1	2.2
	CC6-MRSA-[V+*fus*]	1	3.3	0	0
CC15	CC15-MRSA-[V+*fus*]	6	20.0	0	0
CC22	CC22-MRSA-IVa (tst1+), "Gaza Epidemic Strain"	2	6.7	3	6.5
CC30	CC30-MRSA-IVa (PVL+), "WSPP/Southwest Pacific Clone"	1	3.3	0	0
CC80	CC80-MRSA-IVc	0	0.0	1	2.2
	CC80-MRSA-IVc (PVL+)	0	0.0	3	6.5
CC88	CC88-MRSA-IV	0	0.0	1	2.2
CC97	CC97-MRSA-IVc, "WA MRSA-54/63"	0	0.0	3	6.5
	CC97-MRSA-V	0	0.0	1	2.2
	CC97-MRSA-[V+*fus*]	1	3.3	2	4.3
CC121	CC121-MRSA-[V+*fus*] (PVL+)	1	3.3	0	0
CC152	CC152-MRSA-[V+*fus*] (PVL+)	2	6.7	1	2.2
CC239	CC239-MRSA-[III+Cd/Hg+*ccrC*] (*sasX*-positive), "Southeast Asian Clade"	0	0.0	1	2.2
	CC239-MRSA-[III+Cd+*ccrC*] (*sasX*-negative), "Middle Eastern Cluster"	1	3.3	18	39.1
	CC239-MRSA-[III+Cd] (*sasX*-negative)	1	3.3	2	4.3
CC1153	CC1153-MRSA-[V+*fus*] (PVL+)	3	10.0	0	0

2.4. The SCCmec Element in CC1-MRSA-[V+fus+tir+ccrA/B-1]

Eight isolates harboured SCC*mec* elements described according to the array profiles as SCC*mec* [V+*fus*+*tir*+*ccrA/B*-1]. All these isolates belonged to CC1. To the best of our knowledge, no contiguous sequence of such an element was yet available, as related MUM475 (GenBank AZSG01000015.1 plus AZSG01000034) and MRSA1_ST20130096 (FSRY01000032.1) carry SCC*mec* [VT+*fus*+*tir*+*ccrA/B*-1] elements. Thus, one representative isolate (the PVL-negative Alexandria_2020-19) was fully sequenced using nanopore technology. This confirmed affiliation to CC1, Sequence Type 1 and the absence of PVL from that particular isolate (although another prophage inhabited the integration site usually occupied by PVL phages).

Its SCC*mec* element spanned 72,298 bp. Its sequence and a detailed list of all identified genes are provided as supplemental files S2/S3, and a schematic representation is provided in Figure 1.

It comprised the markers of a typical SCC*mec* V element (*mecA*, *ugpQ*, *ccrAA*, *ccrC*, *mvaS*-SCC), but it lacked the additional *ccrAA/ccrC* and D1GU38 genes that define SCC*mec* VT as they are present in the previously released CC1 sequence MUM475 (GenBank AZSG01000015.1 plus AZSG01000034).
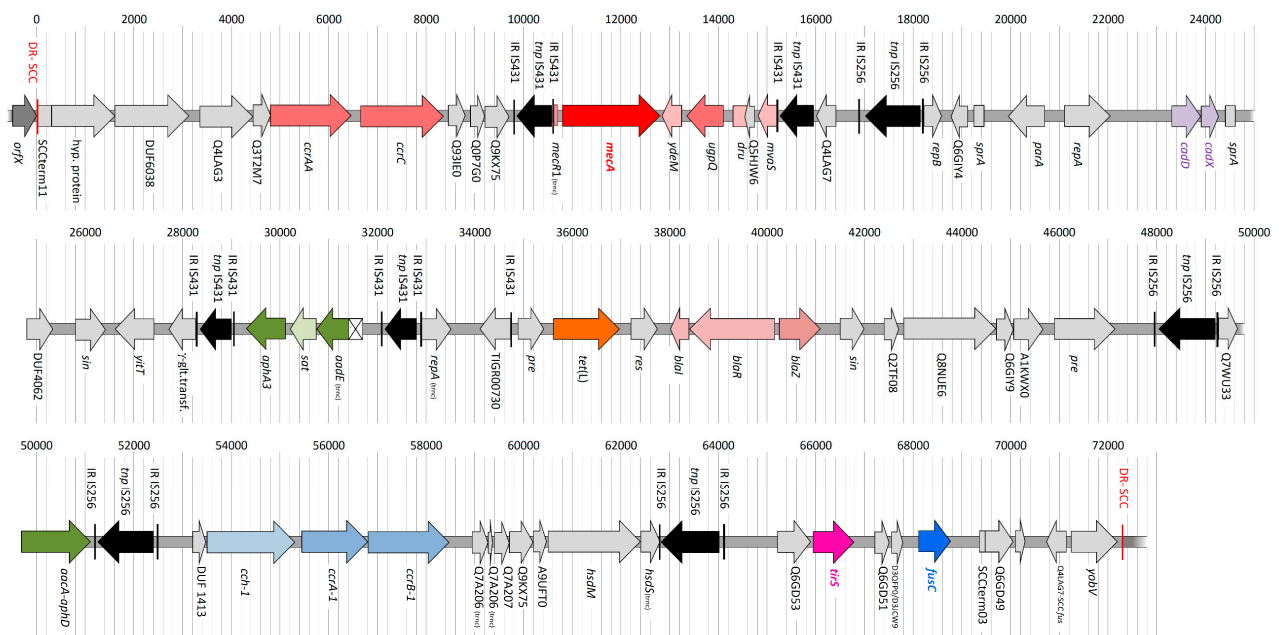
Figure 1. Schematic representation of the SCC*mec* [V+*fus*+*tir*+*ccrA/B*-1] element from a CC1 isolate (GenBank CP113244.1).

The SCC*mec* V genes were combined with several transposase genes and integrated mobile genetic elements that, among various "putative proteins", also included heavy metal resistance genes *cadD*+*cadX*. The aminoglycoside and streptothricin resistance genes *aphA3* and *sat* were also present. The gene *aadE* (that typically accompanies these two genes) was found to be truncated from 909 bp to 624 bp. This truncation was also present in other Egyptian CC1 sequences (JAEOUR010000048.1 and JAEOWJ010000028.1 [44]), but it can also be found in entirely unrelated strains such as TCH1516 (CP000731.1, pos. 15,611 to 16,252). Furthermore, there was the tetracycline resistance gene *tet*(L), the penicillinase operon *blaI*+*blaR*+*blaZ* and the gentamicin/tobramycin resistance gene *aacA-aphD*.

Then, *ccrA/B-1* recombinase genes and a truncated gene cluster encoding an incomplete type I restriction–modification system followed. The *hsdM* gene was still present there, *hsdS* was truncated and *hsdR* has been replaced by yet another transposase copy which also removed the first gene (for a "putative protein" Q6GD54) from the actual *fusC*-associated complex. This complex included, among other genes, *tirS*, *fusC* and *yobV*; it was with regard to gene content as well as to allelic variants most closely related to the one in other CC1 strains (MSSA476, BX571857 and KT/314250, AOCP01000013) and thus it could be assigned to the previously defined [35] *fusC*-complex class "A". The association of a *fusC*-complex class "A" with *ccrA/B-1* recombinase genes is typical for, but not restricted to, CC1 strains [35].

3. Discussion

With regard to antimicrobial resistance, the most remarkable observation of the present study was a presence of *fusC* in diverse lineages of *S. aureus* and its extremely high prevalence. It was as high as 83%%, while in 2015 [44], the rate of genotypically fusidic acid (FA)-resistant strains (e.g., positive either for *fusC* or *far1*) was 30,4%. This could indicate an alarming trend, although several caveats (low numbers, absence of data from other hospitals, other towns and provinces, and predominance of skin/soft tissue infections among study samples) apply. As many genotypically different strains were involved, a local outbreak situation as a cause for this observation can likely be ruled out. A high and possibly increasing rate of genotypic FA resistance is likely to be related to a high rate of consumption of that drug, as observed in other countries such as New Zealand, where an increase in FA consumption led to a parallel increase in MRSA with composite SCC[*mec*+*fus*]

elements [54]. Indeed, FA is over-the-counter available in Egyptian pharmacies, without prescription, as ointment, cream, or eye-drops. It is extensively misused and/or overused as monotherapy, even for non-infectious skin conditions or for prophylaxis. It is inexpensive, with prices as low as 15 to 25 Egyptian Pounds (*ca.* 0.75 to 1.30 Euro) for 15 g crème with 2% FA content. MRSA with composite SCC[*mec+fus*] elements can be expected to have a clear evolutionary advantage under such conditions in both ecological niches, in hospitals and in the community. In hospitals, they thrive because of their beta-lactam resistance (with about half of all antibiotics used being beta-lactams; see [55]). The high consumption of FA in the community poses a selective pressure favouring *fusC*-positives. When *mecA* and *fusC* are located on the same mobile genetic element, outpatient use of FA promotes MRSA in the same way as an in-hospital use of beta-lactams favours FA resistance. This might be a reason for a blurring of the distinction between CA- and HA-MRSA. It also means that the excessive use of FA in the community eventually endangers the lives of Egyptian hospital patients. Therefore, the use of FA should be curtailed, e.g., by requiring a prescription by a physician, as has been recently done in the U.A.E.

Another interesting observation was the high rate of carriage of PVL genes. Similar observations were made at other study sites in Egypt [56] and the Middle East [57,58]. A previous study [56] from another Egyptian city (Cairo) observed, in 20202, an even higher PVL rate of 75% (29% in hospital- and 92% in community-acquired infection) clearly indicating that the rate observed here was no outlier. In general, Middle Eastern and Northern African studies indicate that PVL-MRSA are no longer restricted to the "community" (if they ever were in this part of the world) but also thoroughly infiltrated hospital settings. The high PVL prevalence in our study, as well as in others from the region, cannot be attributed to a single outbreak strain, simply because of a high genotypic diversity of PVL-positive strains. However, the high proportion of wound and pus samples could in our case have caused a bias towards PVL-positive strains. The PVL rate for MRSA collected in Alexandria in 2015 was much lower 17% [44], and it cannot be ruled out that the lower rate observed in this study could be related to an outbreak of a PVL-negative strain (CC239).

The recent publication of genomes of MRSA isolates also from Alexandria [44] allowed to look at temporal changes affecting population structure, as defined by affiliations to CCs.

CC1 strains with SCC*mec* V or VT (as in MUM475, GenBank AZGS) elements that additionally harbour *fusC* and the virulence factor *tirS* have frequently been observed in various countries. However, in most cases, whether they originated from SCC*mec* V or VT elements was not determined. Some previously described isolates [52,59] with Middle Eastern or Eastern African provenance indeed harboured composite elements based on SCC*mec* V rather than on SCC*mec* VT, as all Egyptian isolates described herein or in the earlier study [44] did. As discussed above, one isolate was sequenced to characterise its SCC*mec* element. It was found to harbour *mecA*, *fusC*, and several other resistance genes (including *aacA-aphD*), fitting into a broader trend of increasing "multi-resistance" in supposedly community-associated SCC*mec* IV/V strains. Another aminoglycoside resistance gene in this strain's SCC*mec* element, *aadE*, appeared to be truncated in our sequence. This was not a sequencing artifact, as corresponding contigs of previously sequenced CC1 strains [44] and strains from unrelated lineages (e.g., TCH1516, CC8 or TW20, CC239) showed the same. This likely indicated that this truncation predated the acquisition of the mobile genetic element carrying *aphA3/sat/aadE* by diverse MRSA strains.

Egyptian CC5-MRSA-[VI+*fus+tir*] can be discerned from a similar strain from Portugal (HDE288; AF411935,3) based on differences in its SCC*mec* element, including an absence of *dcs*, but isolates match a strain that was repeatedly found in Middle Eastern countries (Kuwait: [60]; K.S.A.: [61]; U.A.E.: [62]).

Not much is known about CC6-MRSA-[V+*fus*], although a similar or related strain was observed in Kuwait [33]. In general, CC6 MRSA are common and widespread in the Middle East, but previously described strains usually differ in having SCC*mec* type IV [61–64].

CC15-MRSA-[V+*fus*] is a remarkable strain given that it is nearly the only MRSA strain that emerged from the globally spread and common lineage CC15 [65–67]. It has been found in humans in Saudi Arabia and other Gulf countries and in livestock and camel meat [68–71]. It was also observed in chickens from Egypt (unpubl. communication with Dr. Hotzel, Jena, Germany), and it was detected in a farmer from the Nile Delta region in Egypt [42]. In 2015, this MRSA strain was not found in Alexandria [44].

CC22-MRSA-IVa carrying the *tst1* gene has been frequently observed around the Mediterranean Sea [72], in Middle Eastern counties [69,73–76], and among refugees from the Middle East after it was first from Gaza [77–79]. It was also found in Egypt, in livestock, and in farm personnel [42], as well as in an Alexandrian hospital [44]. Thus, its detection is not surprising, but it was remarkable for being one of three lineages that did not harbour SCC-encoded fusidic acid resistance.

PVL-positive CC30-MRSA-IV (PVL+) has been dubbed the "WSPP/Southwest Pacific Clone" after an initial outbreak among New Zealanders and Samoans [80,81]. Meanwhile, such strains can be found globally, but different SCC*mec* subtypes might indicate a polyphyletic emergence. SCC*mec* IVa, as in the present isolate, has been observed in WSPP-like isolates from Europe and the Middle East [52,62] and the U.S. (GenBank CP026066).

CC97-MRSA-[V+*fus*] has been found in Europe and the Middle East [69], and it was detected some years ago in chicken meat brought from Egypt to Germany [82], indicating both a prolonged presence in Egypt as well as a possible livestock connection. Other, *fusC*-negative, CC97-MRSA have also been observed in Egypt [44].

CC121-MRSA-[V+*fus*] (PVL+) is another rare MRSA strain emerging from a globally spread and common MSSA lineage [65,83–86]. The authors have observed related or similar strains in the Middle East [33,42,62].

CC152-MRSA-[V+*fus*] belongs to a lineage known to be common in Africa, but these isolates are usually MSSA. The particular MRSA strain was observed in Egyptian livestock and contact persons [42], and again, the presence of PVL could be seen as an indication of transmission from humans to animals. Other observations came from the Arabian Gulf [62] and Egypt [44]. CC152 was recently shown to carry a novel *etD/E* homologue, *etE2* [53]. The observation of weak and/or irregular signals for *etD2* likely can be attributed to a presence of this gene.

CC239-MRSA is a comparatively ancient, truly pandemic lineage of hospital-acquired MRSA with a core genome that can be described as a chimera of CC8 and CC30 [87] harbouring the large and distinct SCC*mec* III element [3]. Many variants of that strain largely correlate with geographic regions of origin [88–92]. The two isolates found in our study were not identical. One isolate belonged to a clade previously identified in various Middle Eastern countries or people from there [88]. The other isolate matched a group of strains and sequences from Western Europe (Portugal), the U.S. (ATCC33592), and Russia [88], as well as from Egypt [44]. Since it differed only in the absence of *ccrAA/C* recombinase genes from the abovementioned clade, this entire group, or single specimens out of it, might be a mere deletion variant of the Middle Eastern clade. CC239-MRSA was previously found in Alexandria [44], and then (in 2015) it was the most common strain, comprising nearly half of the MRSA isolates characterised, i.e., 23 out of 47 isolates (49%) that belonged to various variants of the ST239-MRSA-III strain.

The issue of the receding CC239 clone might, although the numbers of typed strains are low, suggest a profound change in the MRSA population structure, blurring the distinction between HA- and CA-MRSA. As mentioned in the Introduction, there was an idea of distinguishing CA- and HA-MRSA by molecular means, e.g., based on PVL status, type of SCC*mec* elements, and affiliation to "unusual" clonal complexes. This concept might still apply in countries such as China (where CC239-MRSA-III at least until recently predominated in hospitals and CC59-MRSA-IV or -V in the community [93,94]) or the USA (where CC5-MRSA-II used to be common in hospitals, while PVL-positive CC8-MRSA-IV prevail in the community). However, among the Egyptian sample analysed herein, only two out of 30 hospital isolates could be assigned to the CC239 strain traditionally associated with

a hospital-acquired infection. In contrast, all others belonged to various strains with features associated with CA-MRSA (PVL, SCC*mec* IV/V/VI elements). Similar observations were also made in Middle Eastern countries [37]. No molecular marker can be used in these settings anymore as a surrogate for the assignment to hospital- versus community-acquired infections. In order to discern these, one must thoroughly interview the patient and assess the case history.

CC1153-MRSA-[V+*fus*] (PVL+) is a strain that we previously found in a patient of Egyptian origin in Germany [35] and Dubai [58]. Furthermore, isolates for which it was not determined whether they harboured SCC[*mec* V+*fus*] or SCC[*mec* VT+*fus*] elements have been observed in the United Arab Emirates, Saudi Arabia, and Kuwait [35]. PVL-positive MSSA from this lineage has also been found in Egypt's livestock [40]. Given the pathogenetic role of PVL in humans, this might be attributed to an anthropozoonotic transition. However, this observation raises the question whether ancestral, susceptible CC1153 strains might already have circulated in Egypt prior to the emergence of MRSA from this lineage. In 2015, no CC153 MRSA was found [44], possibly indicating a recent emergence.

Finally, the PVL-positive CC80-MRSA-IV strain, widespread in the Mediterranean and the Middle East [30,73,95–99] and previously found in Alexandria [44], was not observed. Whether this is due to the small sample or a recent decline still needs to be established.

A limitation of the study is of course the small sample size, resulting from opportunistic sampling at a single location. The predominance of isolates from swab/pus specimens might have caused a bias towards *fusC*- and/or PVL-positive isolates that might be less common in other types of samples.

More comprehensive surveys into *S. aureus*/MRSA populations in Egypt and elsewhere in Africa and the Greater Middle East are urgently needed as well as studies on a possible impact of new antibiotics such as daptomycin and fifth generation cephalosporins. A two-pronged approach of array-based typing followed by genome sequencing of "interesting" or conspicuous strains, as described herein, might help to gather more typing data for these parts of the world.

4. Materials and Methods

4.1. Isolates

Clinical samples (see Table 1) were cultured routinely on blood agar (Oxoid Ltd., Basingstoke, Hampshire, UK) and MacConkey agar and were incubated at 37 °C under aerobic conditions for 16–24 h. Subculturing was aided by microscopy; Gram-positive cocci were further identified by biochemical tests, including Catalase test and Coagulase test (Remel-Oxoid, Basingstoke, Hampshire, UK) and they were subjected to antibiotic susceptibility testing by disc diffusion (Oxoid Ltd., Basingstoke, Hampshire, UK) using CLSI methodology and breakpoints (CLSI; https://clsi.org/media/3481/m100ed30_sample.pdf; accessed on 1 December 2022). In addition, identification as *S. aureus*/MRSA was confirmed by PCR (see below). For long-term storage at the laboratory in Egypt, one ml of fresh saturated bacterial culture grown on Luria Bertani (LB) broth was added to one ml of sterile glycerol solution in screw capped glass tubes. The tubes were stored at −20 °C. Strains at the German laboratory were stored at −80 °C using microbank tubes (Fisher Scientific GmbH, Schwerte, Germany/Pro-Lab Diagnostics, Richmond Hill, ON, Canada) according to the manufacturer's instructions. For re-culturing, one loop of bacterial material was streaked over blood agar and incubated overnight at 37 °C.

4.2. PCR for S. aureus/MRSA

Multiplex PCR was used for genotypically identifying *S. aureus* and methicillin resistance by amplification of *femA* and *mecA* genes, respectively. Nucleotide sequences of primers (Biosearch Technologies, Inc., Petaluma, CA, USA) used in this investigation are shown in Table 6.

Table 6. PCR primers used.

Primer	Sequence	Tm (°C)	Amplicon Size (bp)	Reference
femA FWR	CTTACTTACTGCTGTACCTG	58	686	[100,101]
femA REV	ATCTCGCTTGTTATGTGC	56		
mecA FWR	TGGCTATCGTGTCACAATCG	58.08	304	[100,102]
mecA REV	CTGGAACTTGTTGAGCAGAG	56.3		

DNA was extracted from MRSA isolates by boiling method [103]. Multiplex PCR was performed using 12 μL as total volume, consisting of 6 μL of mastermix (MyTaq HS Red Mix 2X; BioLine, London, UK), 0.5 μL of each of the diluted DNA extract, *femA*, and *mecA* primers, and 3.5 μL of PCR grade water. A negative control was prepared by adding the same contents to the tubes with water placed instead of the DNA extract. PCR cycling conditions were as follows: 4 min of initial denaturation at 95 °C, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 52 °C for 15 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. PCR was performed using a Veriti Thermal Cycler (Applied Biosystems, Foster, CA, USA). The PCR products were loaded on 1.5% agarose gel and analysed by gel electrophoresis (Mupid-exU, ADVANCE Co., Ltd., Tokyo, Japan).

4.3. Array Procedures

For this study, a new experimental microarray was used. It was based on a previously described system [23,47]. However, it has been modified by adding probes for detecting some recently described markers and for a more detailed typing of SCC elements. These probes have already been used in earlier work when they were, among others, localised on another second array [52]. Experimental procedures were performed as described for earlier versions [23,47]; primer and probe sequences have been disclosed thence ([23,47,51,52]; see also Tables 1–3 and Supplemental Table S1 for individual target genes).

Isolates were cultured overnight at 37 °C on Columbia blood agar. Harvested cells were digested enzymatically [104]. DNA was purified using Qiagen columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The assay relied on a linear multiplex primer elongation using one primer per target. During amplification, biotin-16-dUTP was incorporated into the amplicons which were then hybridised to the array. After washing and blocking, horseradish–peroxidase–streptavidin was added binding to the biotin and causing local precipitation of a dye in case of a positive reaction. Finally, an array image was recorded and analysed using a designated reader and software (Arraymate, Iconoclust, both by Alere Technologies/Abbott, Jena, Germany).

4.4. Nanopore Sequencing

The Oxford Nanopore MinION platform was used to sequence the genome of one MRSA isolate (Alexandria_2020-19). Library preparation was done using the 1D genomic DNA by ligation kit (SQK-LSK109, version GDE_9063_v109_revX_14Aug2019; ONT) following the manufacturer's instruction for flongles (FLO-FLG001 containing an R9.4.1 pore). Before library preparation, size selection was performed using AMPure-beads (Beckman Coulter) in a ratio of 1:1 (*v/v*) with the isolated DNA sample. The flongle flow cell was loaded with ca. 200 ng DNA (measured by Qubit4 Fluorometer; Thermo Fisher Scientific, Waltham, WA, USA). The sequencing ran for 48 h using the MinKNOW software version 20.10.3 starting with a total of 65 active pores.

The Guppy basecaller (version 4.4.2+9623c1626, Oxford Nanopore Technologies, Oxford, UK) translated the MinION raw reads (FAST5) into quality tagged sequence reads (4000 reads per FASTQ-file) using the barcode trimming option. Flye (version 2.8.3-b1695) was used to assemble each strain's quality tagged sequence reads into one big circular contig. The polishing of assemblies was divided into two steps. At first, racon (v1.4.17) was

iteratively used four times with the following parameter: match 8; mismatch 6; gap 8, and windows-lengths 500. Afterwards, medaka (version 1.4.3) ran on the last racon (version 1.4.21) polished assembly using the model r941_min_high_g360. This corrected assembly was used for further analysis.

4.5. Analysis of Previously Published Genome Sequences

Published genome sequences from the previously published paper on MRSA from Alexandria [44] were considered (GenBank JAEOUR, JAEOUS, JAEOUU, JAEOUV, JAEOUW, JAEOUX, JAEOUY, JAEOUZ, JAEOVA, JAEOVB, JAEOVC, JAEOVD, JAEOVE, JAEOVF, JAEOVG, JAEOVH, JAEOVI, JAEOVJ, JAEOVK, JAEOVL, JAEOVM, JAEOVO, JAEOVP, JAEOVQ, JAEOVR, JAEOVS, JAEOVT, JAEOVU, JAEOVV, JAEOVX, JAEOVY, JAEOVZ, JAEOWA, JAEOWB, JAEOWC, JAEOWE, JAEOWG, JAEOWH, JAEOWI, JAEOWJ, JAE-OWK, JAEOWL, JAEOWM, JAEOWN, JAEOWP, JAEOWQ, JAEOWU). Sequences were analysed for the presence of the known probe sequences and their reverse complement sequences. A perfect match was assigned a score of 0.9. In case of one or two mismatches, a score of 0.4 was assigned. Probes with no hits, or with more than two mismatches, got a score of 0.0. Thus, a list of probes with corresponding scores was generated for each sequence and this list was analysed in the same way as the measurements from array experiments. This allowed us to assign previously sequenced isolates to strains using the same nomenclature and criteria for array experiments and directly compare results from both approaches. Two isolates were described as SCC*mec* un-typable (JAEOUU and JAEOWG) but were identified as CC97 and CC1 MRSA, respectively. This contradiction cannot be resolved here, but results for these sequences were included in Table 4. Another sequence (JAEOWU) included *mecA* (as also mentioned in the supplemental file to [44]) but lacked any other SCC-associated genes; it was excluded.

5. Conclusions

In conclusion, the population structure of MRSA from Alexandria, 2020, was characterised by the presence of many genotypically diverse strains. Most (83%) harboured SCC*mec* elements that included a fusidic acid resistance gene, and PVL was also common (40%). A comparison to an earlier study [44] from the same city in 2015 suggested a dramatic increase in the prevalence of fusidic acid resistance and of PVL carriage while a long-known strictly hospital-associated strain (CC239-MRSA-III) was receding. These observations indicate a diffusion of community-acquired strains into hospital settings and a selective pressure by beta-lactam use in hospitals and fusidic acid consumption in the community that favours MRSA strains with composite SCC*mec* elements comprising *mecA* and *fusC*. This could indicate an unsettling trend, but more data are needed to assess the current situation.

Author Contributions: S.M.: Conceptualization, formal analysis, visualization, writing—original draft preparation; A.K.B.: Conceptualization, resources, investigations, writing—original draft preparation, funding acquisition; E.M.: methodology/investigations; S.D.B.: methodology/investigations, formal analysis; C.D.: methodology/investigations, formal analysis; A.E.: resources, investigations; O.K.: resources, project administration; M.R.: methodology/investigations, formal analysis; A.G.: supervision, investigations; R.E.: writing—review and editing, supervision, project administration, funding acquisition; S.R.: writing—review and editing, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lowy, F.D. Antimicrobial resistance: The example of *Staphylococcus aureus*. *J. Clin. Investig.* **2003**, *111*, 1265–1273. [CrossRef] [PubMed]
2. Ito, T.; Hiramatsu, K.; Tomasz, A.; de Lencastre, H.; Perreten, V.; Holden, M.T.G.; Coleman, D.C.; Goering, R.; Giffard, P.M.; Skov, R.L.; et al. Guidelines for Reporting Novel *mecA* Gene Homologues. *Antimicrob. Agents Chemother.* **2012**, *56*, 4997–4999. [CrossRef] [PubMed]
3. Ito, T.; Katayama, Y.; Asada, K.; Mori, N.; Tsutsumimoto, K.; Tiensasitorn, C.; Hiramatsu, K. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2001**, *45*, 1323–1336. [CrossRef] [PubMed]
4. Ito, T.; Ma, X.X.; Takeuchi, F.; Okuma, K.; Yuzawa, H.; Hiramatsu, K. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, ccrC. *Antimicrob. Agents Chemother.* **2004**, *48*, 2637–2651. [CrossRef] [PubMed]
5. Ito, T.; Okuma, K.; Ma, X.X.; Yuzawa, H.; Hiramatsu, K. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: Genomic island SCC. *Drug Resist. Updates* **2003**, *6*, 41–52. [CrossRef]
6. Liu, J.; Chen, D.; Peters, B.M.; Li, L.; Li, B.; Xu, Z.; Shirliff, M.E. Staphylococcal chromosomal cassettes *mec* (SCC*mec*): A mobile genetic element in methicillin-resistant *Staphylococcus aureus*. *Microb. Pathog.* **2016**, *101*, 56–67. [CrossRef]
7. Queck, S.Y.; Khan, B.A.; Wang, R.; Bach, T.H.; Kretschmer, D.; Chen, L.; Kreiswirth, B.N.; Peschel, A.; Deleo, F.R.; Otto, M. Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. *PLoS Pathog.* **2009**, *5*, e1000533. [CrossRef]
8. Patot, S.; Imbert, P.; Baude, J.; Martins Simões, P.; Campergue, J.-B.; Louche, A.; Nijland, R.; Bès, M.; Tristan, A.; Laurent, F.; et al. The TIR Homologue Lies near Resistance Genes in *Staphylococcus aureus*, Coupling Modulation of Virulence and Antimicrobial Susceptibility. *PLoS Pathog.* **2017**, *13*, e1006092. [CrossRef]
9. Naimi, T.S.; LeDell, K.H.; Como-Sabetti, K.; Borchardt, S.M.; Boxrud, D.J.; Etienne, J.; Johnson, S.K.; Vandenesch, F.; Fridkin, S.; O'Boyle, C.; et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *J. Clin. Microbiol.* **2003**, *290*, 2976–2984.
10. Boyle-Vavra, S.; Daum, R.S. Community-acquired methicillin-resistant *Staphylococcus aureus*: The role of Panton-Valentine leukocidin. *Lab. Investig.* **2007**, *87*, 3–9. [CrossRef]
11. Buescher, E.S. Community-acquired methicillin-resistant *Staphylococcus aureus* in pediatrics. *Curr. Opin. Pediatr.* **2005**, *17*, 67–70. [CrossRef] [PubMed]
12. Center for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*-Minnesota and North Dakota. *Morb. Mortal Wkly. Rep.* **1999**, *282*, 1123–1125.
13. Collignon, P.; Gosbell, I.; Vickery, A.; Nimmo, G.; Stylianopoulos, T.; Gottlieb, T. Community-acquired meticillin-resistant *Staphylococcus aureus* in Australia. Australian Group on Antimicrobial Resistance. *Lancet* **1998**, *352*, 145–146. [CrossRef]

14. Liassine, N.; Auckenthaler, R.; Descombes, M.C.; Bes, M.; Vandenesch, F.; Etienne, J. Community-acquired methicillin-resistant *Staphylococcus aureus* isolated in Switzerland contains the Panton-Valentine leukocidin or exfoliative toxin genes. *J. Clin. Microbiol.* **2004**, *42*, 825–828. [CrossRef] [PubMed]

15. Linde, H.; Wagenlehner, F.; Strommenger, B.; Drubel, I.; Tanzer, J.; Reischl, U.; Raab, U.; Holler, C.; Naber, K.G.; Witte, W.; et al. Healthcare-associated outbreaks and community-acquired infections due to MRSA carrying the Panton-Valentine leucocidin gene in southeastern Germany. *Eur. J. Clin. Microbiol. Infect. Dis.* **2005**, *24*, 419–422. [CrossRef]

16. Ma, X.X.; Ito, T.; Tiensasitorn, C.; Jamklang, M.; Chongtrakool, P.; Boyle-Vavra, S.; Daum, R.S.; Hiramatsu, K. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob. Agents Chemother.* **2002**, *46*, 1147–1152. [CrossRef] [PubMed]

17. McCaskill, M.L.; Mason, E.O., Jr.; Kaplan, S.L.; Hammerman, W.; Lamberth, L.B.; Hulten, K.G. Increase of the USA300 clone among community-acquired methicillin-susceptible *Staphylococcus aureus* causing invasive infections. *Pediatr. Infect. Dis. J.* **2007**, *26*, 1122–1127. [CrossRef]

18. Munckhof, W.J.; Schooneveldt, J.; Coombs, G.W.; Hoare, J.; Nimmo, G.R. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infection in Queensland, Australia. *Int. J. Infect. Dis.* **2003**, *7*, 259–264. [CrossRef]

19. Vandenesch, F.; Naimi, T.; Enright, M.C.; Lina, G.; Nimmo, G.R.; Heffernan, H.; Liassine, N.; Bes, M.; Greenland, T.; Reverdy, M.E.; et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: Worldwide emergence. *Emerg. Infect. Dis.* **2003**, *9*, 978–984. [CrossRef]

20. Baba, T.; Takeuchi, F.; Kuroda, M.; Yuzawa, H.; Aoki, K.; Oguchi, A.; Nagai, Y.; Iwama, N.; Asano, K.; Naimi, T.; et al. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* **2002**, *359*, 1819–1827. [CrossRef]

21. Enany, S.; Yaoita, E.; Yoshida, Y.; Enany, M.; Yamamoto, T. Molecular characterization of Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* isolates in Egypt. *Microbiol. Res.* **2010**, *165*, 152–162. [CrossRef] [PubMed]

22. Mediavilla, J.R.; Chen, L.; Mathema, B.; Kreiswirth, B.N. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). *Curr. Opin. Microbiol.* **2012**, *15*, 588–595. [CrossRef] [PubMed]

23. Monecke, S.; Coombs, G.; Shore, A.C.; Coleman, D.C.; Akpaka, P.; Borg, M.; Chow, H.; Ip, M.; Jatzwauk, L.; Jonas, D.; et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS ONE* **2011**, *6*, e17936. [CrossRef]

24. Falagas, M.E.; Karageorgopoulos, D.E.; Leptidis, J.; Korbila, I.P. MRSA in Africa: Filling the global map of antimicrobial resistance. *PLoS ONE* **2013**, *8*, e68024. [CrossRef] [PubMed]

25. Goering, R.V.; Shawar, R.M.; Scangarella, N.E.; O'Hara, F.P.; Amrine-Madsen, H.; West, J.M.; Dalessandro, M.; Becker, J.A.; Walsh, S.L.; Miller, L.A.; et al. Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from global clinical trials. *J. Clin. Microbiol.* **2008**, *46*, 2842–2847. [CrossRef]

26. Holden, M.T.G.; Hsu, L.-Y.; Kurt, K.; Weinert, L.A.; Mather, A.E.; Harris, S.R.; Strommenger, B.; Layer, F.; Witte, W.; de Lencastre, H.; et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res.* **2013**, *23*, 653–664. [CrossRef]

27. Rasigade, J.-P.; Laurent, F.; Lina, G.; Meugnier, H.; Bes, M.; Vandenesch, F.; Etienne, J.; Tristan, A. Global Distribution and Evolution of Panton- Valentine Leukocidin-Positive Methicillin-Susceptible *Staphylococcus aureus*, 1981–2007. *J. Infect. Dis.* **2010**, *201*, 1589–1597. [CrossRef]

28. O'Brien, F.G.; Price, C.; Grubb, W.B.; Gustafson, J.E. Genetic characterization of the fusidic acid and cadmium resistance determinants of *Staphylococcus aureus* plasmid pUB101. *J. Antimicrob. Chemother.* **2002**, *50*, 313–321. [CrossRef]

29. Udo, E.E.; Sarkhoo, E. Genetic analysis of high-level mupirocin resistance in the ST80 clone of community-associated meticillin-resistant *Staphylococcus aureus*. *J. Med. Microbiol.* **2010**, *59*, 193–199. [CrossRef]

30. Nejma, M.B.; Mastouri, M.; Jrad, B.B.H.; Nour, M. Characterization of ST80 Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* clone in Tunisia. *Diagn. Microbiol. Infect. Dis.* **2013**, *77*, 20–24. [CrossRef]

31. Ellington, M.J.; Reuter, S.; Harris, S.R.; Holden, M.T.; Cartwright, E.J.; Greaves, D.; Gerver, S.M.; Hope, R.; Brown, N.M.; Torok, M.E.; et al. Emergent and evolving antimicrobial resistance cassettes in community-associated fusidic acid and meticillin-resistant *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* **2015**, *45*, 477–484. [CrossRef] [PubMed]

32. Boswihi, S.S.; Udo, E.E.; Al-Sweih, N. Shifts in the Clonal Distribution of Methicillin-Resistant *Staphylococcus aureus* in Kuwait Hospitals: 1992–2010. *PLoS ONE* **2016**, *11*, e0162744. [CrossRef] [PubMed]

33. Boswihi, S.S.; Udo, E.E.; Monecke, S.; Mathew, B.; Noronha, B.; Verghese, T.; Tappa, S.B. Emerging variants of methicillin-resistant *Staphylococcus aureus* genotypes in Kuwait hospitals. *PLoS ONE* **2018**, *13*, e0195933. [CrossRef] [PubMed]

34. Senok, A.; Slickers, P.; Hotzel, H.; Boswihi, S.; Braun, S.D.; Gawlik, D.; Müller, E.; Nabi, A.; Nassar, R.; Nitschke, H.; et al. Characterisation of a novel SCCmec VI element harbouring *fusC* in an emerging *Staphylococcus aureus* strain from the Arabian Gulf region. *PLoS ONE* **2019**, *14*, e0223985. [CrossRef]

35. Monecke, S.; Müller, E.; Braun, S.D.; Armengol-Porta, M.; Bes, M.; Boswihi, S.; El-Ashker, M.; Engelmann, I.; Gawlik, D.; Gwida, M.; et al. Characterisation of *S. aureus*/MRSA CC1153 and review of mobile genetic elements carrying the fusidic acid resistance gene *fusC*. *Sci. Rep.* **2021**, *11*, 8128. [CrossRef]

36. Schaumburg, F.; Alabi, A.S.; Peters, G.; Becker, K. New epidemiology of *Staphylococcus aureus* infection in Africa. *Clin. Microbiol. Infect.* **2014**, *20*, 589–596. [CrossRef]

37. Ahoyo, T.A.; Martin-Odoom, A.; Bankole, H.S.; Baba-Moussa, L.; Zonon, N.; Loko, F.; Prevost, G.; Sanni, A.; Dramane, K. Epidemiology and prevention of nosocomial pneumonia associated with Panton-Valentine Leukocidin (PVL) producing *Staphylococcus aureus* in Departmental Hospital Centre of Zou Collines in Benin. *Ghana Med. J.* **2012**, *46*, 234–240.

38. Okon, K.O.; Basset, P.; Uba, A.; Lin, J.; Oyawoye, B.; Shittu, A.O.; Blanc, D.S. Co-occurrence of Predominant PVL-positive (ST 152) and Multidrug-resistant (ST 241) *Staphylococcus aureus* clones in Nigerian hospitals. *J. Clin. Microbiol.* **2009**, *47*, 3000–3003. [CrossRef]

39. Rasigade, J.P.; Trouillet-Assant, S.; Breurec, S.; Antri, K.; Lina, G.; Bes, M.; Tristan, A.; Badiou, C.; Bernelin, M.; Fall, C.; et al. The levels of antibodies to Panton-Valentine leukocidin (PVL) vary with PVL prevalence along a north-to-south gradient. *Eur. J. Clin. Microbiol. Infect. Dis.* **2015**, *34*, 927–933. [CrossRef]

40. El-Ashker, M.; Gwida, M.; Monecke, S.; El-Gohary, F.; Ehricht, R.; Elsayed, M.; Akinduti, P.; El-Fateh, M.; Maurischat, S. Antimicrobial resistance pattern and virulence profile of *S. aureus* isolated from household cattle and buffalo with mastitis in Egypt. *Vet. Microbiol.* **2020**, *240*, 108535. [CrossRef]

41. El-Ashker, M.; Gwida, M.; Tomaso, H.; Monecke, S.; Ehricht, R.; El-Gohary, F.; Hotzel, H. Staphylococci in cattle and buffaloes with mastitis in Dakahlia Governorate, Egypt. *J. Dairy Sci.* **2015**, *98*, 7450–7459. [CrossRef] [PubMed]

42. El-Ashker, M.; Monecke, S.; Gwida, M.; Saad, T.; El-Gohary, A.; Mohamed, A.; Reißig, A.; Frankenfeld, K.; Gary, D.; Müller, E.; et al. Molecular characterisation of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* clones isolated from healthy dairy animals and their caretakers in Egypt. *Vet. Microbiol.* **2022**, *267*, 109374. [CrossRef] [PubMed]

43. Alfeky, A.E.; Tawfick, M.M.; Ashour, M.S.; El-Moghazy, A.A. High Prevalence of Multi-drug Resistant Methicillin-Resistant *Staphylococcus aureus* in Tertiary Egyptian Hospitals. *J. Infect. Dev. Ctries.* **2022**, *16*, 795–806. [CrossRef]

44. Montelongo, C.; Mores, C.R.; Putonti, C.; Wolfe, A.J.; Abouelfetouh, A.; Mkrtchyan, H.V. Whole-Genome Sequencing of *Staphylococcus aureus* and *Staphylococcus haemolyticus* Clinical Isolates from Egypt. *Microbiol. Spectr.* **2022**, *10*, e02413–e02421. [CrossRef]

45. Mehndiratta, P.L.; Bhalla, P. Typing of Methicillin resistant *Staphylococcus aureus*: A technical review. *Indian J. Med. Microbiol.* **2012**, *30*, 16–23. [CrossRef] [PubMed]

46. Dunne, W.M.; Pouseele, H.; Monecke, S.; Ehricht, R.; van Belkum, A. Epidemiology of transmissible diseases: Array hybridization and next generation sequencing as universal nucleic acid-mediated typing tools. *Infect. Genet. Evol.* **2018**, *63*, 332–345. [CrossRef] [PubMed]

47. Monecke, S.; Slickers, P.; Ehricht, R. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. *FEMS Immunol. Med. Microbiol.* **2008**, *53*, 237–251. [CrossRef]

48. Enright, M.C.; Day, N.P.; Davies, C.E.; Peacock, S.J.; Spratt, B.G. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J. Clin. Microbiol.* **2000**, *38*, 1008–1015. [CrossRef] [PubMed]

49. Pritchard, J.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef]

50. Robinson, D.; Enright, M. Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.* **2004**, *10*, 92–97. [CrossRef]

51. Monecke, S.; Gavier-Widen, D.; Mattsson, R.; Rangstrup-Christensen, L.; Lazaris, A.; Coleman, D.C.; Shore, A.C.; Ehricht, R. Detection of mecC-positive *Staphylococcus aureus* (CC130-MRSA-XI) in diseased European hedgehogs (*Erinaceus europaeus*) in Sweden. *PLoS ONE* **2013**, *8*, e66166. [CrossRef] [PubMed]

52. Monecke, S.; Jatzwauk, L.; Müller, E.; Nitschke, H.; Pfohl, K.; Slickers, P.; Reissig, A.; Ruppelt-Lorz, A.; Ehricht, R. Diversity of SCC*mec* elements in *Staphylococcus aureus* as observed in South-Eastern Germany. *PLoS ONE* **2016**, *11*, e0162654. [CrossRef] [PubMed]

53. Sabat, A.J.; Wouthuyzen-Bakker, M.; Rondags, A.; Hughes, L.; Akkerboom, V.; Koutsopetra, O.; Friedrich, A.W.; Bathoorn, E. Case Report: Necrotizing fasciitis caused by *Staphylococcus aureus* positive for a new sequence variant of exfoliative toxin E. *Front. Genet.* **2022**, *13*, 964358. [CrossRef] [PubMed]

54. Williamson, D.A.; Monecke, S.; Heffernan, H.; Ritchie, S.R.; Roberts, S.A.; Upton, A.; Thomas, M.G.; Fraser, J.D. High usage of topical fusidic acid and rapid clonal expansion of fusidic acid-resistant *Staphylococcus aureus*: A cautionary tale. *Clin. Infect. Dis.* **2014**, *59*, 1451–1454. [CrossRef]

55. Talaat, M.; Saied, T.; Kandeel, A.; El-Ata, G.A.; El-Kholy, A.; Hafez, S.; Osman, A.; Razik, M.A.; Ismail, G.; El-Masry, S.; et al. A Point Prevalence Survey of Antibiotic Use in 18 Hospitals in Egypt. *Antibiotics* **2014**, *3*, 450–460. [CrossRef]

56. El-Baghdady, K.Z.; El-Borhamy, M.I.; Abd El-Ghafar, H.A. Prevalence of resistance and toxin genes in community-acquired and hospital-acquired methicillin-resistant *Staphylococcus aureus* clinical isolates. *Iran J. Basic Med. Sci.* **2020**, *23*, 1251–1260. [CrossRef]

57. Monecke, S.; Muller, E.; Buechler, J.; Rejman, J.; Stieber, B.; Akpaka, P.E.; Bandt, D.; Burris, R.; Coombs, G.; Hidalgo-Arroyo, G.A.; et al. Rapid detection of Panton-Valentine leukocidin in *Staphylococcus aureus* cultures by use of a lateral flow assay based on monoclonal antibodies. *J. Clin. Microbiol.* **2013**, *51*, 487–495. [CrossRef]

58. Senok, A.; Monecke, S.; Nassar, R.; Celiloglu, H.; Thyagarajan, S.; Müller, E.; Ehricht, R. Lateral Flow Immunoassay for the Detection of Panton-Valentine Leukocidin in *Staphylococcus aureus* from skin and soft tissue infections in the United Arab Emirates. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 754523. [CrossRef]

59. Albrecht, N.; Jatzwauk, L.; Slickers, P.; Ehricht, R.; Monecke, S. Clonal replacement of epidemic methicillin-resistant *Staphylococcus aureus* strains in a German university hospital over a period of eleven years. *PLoS ONE* **2011**, *6*, e28189. [CrossRef]

60. Boswihi, S.S.; Udo, E.E.; AlFouzan, W. Antibiotic resistance and typing of the methicillin-resistant *Staphylococcus aureus* clones in Kuwait hospitals, 2016–2017. *BMC Microbiol.* **2020**, *20*, 314. [CrossRef]

61. Senok, A.; Somily, A.M.; Nassar, R.; Garaween, G.; Kim Sing, G.; Müller, E.; Reissig, A.; Gawlik, D.; Ehricht, R.; Monecke, S. Emergence of novel methicillin-resistant *Staphylococcus aureus* strains in a tertiary care facility in Riyadh, Saudi Arabia. *Infect. Drug Resist.* **2019**, *12*, 2739–2746. [CrossRef] [PubMed]

62. Senok, A.; Nassar, R.; Celiloglu, H.; Nabi, A.; Alfaresi, M.; Weber, S.; Rizvi, I.; Muller, E.; Reissig, A.; Gawlik, D.; et al. Genotyping of methicillin resistant *Staphylococcus aureus* from the United Arab Emirates. *Sci. Rep.* **2020**, *10*, 18551. [CrossRef] [PubMed]

63. Udo, E.E.; Al-Sweih, N. Emergence of Methicillin-Resistant *Staphylococcus aureus* in the Maternity Hospital, Kuwait. *Med. Princ. Pract.* **2013**, *22*, 535–539. [CrossRef] [PubMed]

64. Udo, E.E.; Al-Lawati, B.A.H.; Al-Muharmi, Z.; Thukral, S.S. Genotyping of methicillin-resistant *Staphylococcus aureus* in the Sultan Qaboos University Hospital, Oman reveals the dominance of Panton–Valentine leucocidin-negative ST6-IV/t304 clone. *New Microbes New Infect.* **2014**, *2*, 100–105. [CrossRef] [PubMed]

65. Egyir, B.; Guardabassi, L.; Sorum, M.; Nielsen, S.S.; Kolekang, A.; Frimpong, E.; Addo, K.K.; Newman, M.J.; Larsen, A.R. Molecular epidemiology and antimicrobial susceptibility of clinical *Staphylococcus aureus* from healthcare institutions in Ghana. *PLoS ONE* **2014**, *9*, e89716. [CrossRef] [PubMed]

66. Lozano, C.; Mari, A.; Aspiroz, C.; Gomez-Sanz, E.; Ceballos, S.; Fortuno, B.; Barcenilla, F.; Jover-Saenz, A.; Torres, C. Nasal carriage of coagulase positive staphylococci in patients of a Primary-Healthcare-Center: Genetic lineages and resistance and virulence genes. *Enferm. Infecc. Microbiol. Clin.* **2015**, *33*, 391–396. [CrossRef]

67. Monecke, S.; Luedicke, C.; Slickers, P.; Ehricht, R. Molecular epidemiology of *Staphylococcus aureus* in asymptomatic carriers. *Eur. J. Clin. Microbiol. Infect. Dis.* **2009**, *28*, 1159–1165. [CrossRef]

68. Raji, M.A.; Garaween, G.; Ehricht, R.; Monecke, S.; Shibl, A.M.; Senok, A. Genetic Characterization of *Staphylococcus aureus* Isolated from Retail Meat in Riyadh, Saudi Arabia. *Front. Microbiol.* **2016**, *7*, 911. [CrossRef]

69. Senok, A.; Ehricht, R.; Monecke, S.; Al-Saedan, R.; Somily, A. Molecular characterization of methicillin-resistant *Staphylococcus aureus* in nosocomial infections in a tertiary-care facility: Emergence of new clonal complexes in Saudi Arabia. *New Microbes New Infect.* **2016**, *14*, 13–18. [CrossRef]

70. Senok, A.C.; Somily, A.M.; Slickers, P.; Raji, M.A.; Garaween, G.; Shibl, A.; Monecke, S.; Ehricht, R. Investigating a rare methicillin-resistant *Staphylococcus aureus* strain: First description of genome sequencing and molecular characterization of CC15-MRSA. *Infect. Drug Resist.* **2017**, *10*, 307–315. [CrossRef]

71. Udo, E.E.; Boswihi, S.S.; Mathew, B.; Noronha, B.; Verghese, T.; Al-Jemaz, A.; Al Saqer, F. Emergence of Methicillin-Resistant *Staphylococcus aureus* Belonging to Clonal Complex 15 (CC15-MRSA) in Kuwait Hospitals. *Infect. Drug Resist.* **2020**, *13*, 617–626. [CrossRef] [PubMed]

72. Geraci, D.M.; Bonura, C.; Giuffrè, M.; Aleo, A.; Saporito, L.; Graziano, G.; Valenti, R.M.; Mammina, C. *tst1*-positive ST22-MRSA-IVa in healthy Italian preschool children. *Infection* **2014**, *42*, 535–538. [CrossRef] [PubMed]

73. Al-Bakri, A.G.; Al-Hadithi, H.; Kasabri, V.; Othman, G.; Kriegeskorte, A.; Becker, K. The epidemiology and molecular characterization of methicillin-resistant staphylococci sampled from a healthy Jordanian population. *Epidemiol. Infect.* **2013**, *141*, 2384–2391. [CrossRef]

74. Senok, A.; Somily, A.; Raji, A.; Gawlik, D.; Al-Shahrani, F.; Baqi, S.; Boswihi, S.; Skakni, L.; Udo, E.E.; Weber, S.; et al. Diversity of methicillin-resistant *Staphylococcus aureus* CC22-MRSA-IV from Saudi Arabia and the Gulf region. *Int. J. Infect. Dis.* **2016**, *51*, 31–35. [CrossRef] [PubMed]

75. Udo, E.E.; Boswihi, S.S.; Al-Sweih, N. High prevalence of toxic shock syndrome toxin-producing epidemic methicillin-resistant *Staphylococcus aureus* 15 (EMRSA-15) strains in Kuwait hospitals. *New Microbes New Infect.* **2016**, *12*, 24–30. [CrossRef]

76. Ababneh, Q.; Jaradat, Z.; Khanfar, M.; Alnohoud, R.; Alzu'bi, M.; Makahleh, S.; Abulaila, S. Methicillin-resistant *Staphylococcus aureus* contamination of high-touched surfaces in a university campus. *J. Appl. Microbiol.* **2022**, *132*, 4486–4500. [CrossRef] [PubMed]

77. Chang, Q.; Abuelaish, I.; Biber, A.; Jaber, H.; Callendrello, A.; Andam, C.P.; Regev-Yochay, G.; Hanage, W.P. Genomic epidemiology of meticillin-resistant *Staphylococcus aureus* ST22 widespread in communities of the Gaza Strip, 2009. *Euro Surveill.* **2018**, *23*, 1700592. [CrossRef] [PubMed]

78. Biber, A.; Abuelaish, I.; Rahav, G.; Raz, M.; Cohen, L.; Valinsky, L.; Taran, D.; Goral, A.; Elhamdany, A.; Regev-Yochay, G.; et al. A Typical Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* Clone Is Widespread in the Community in the Gaza Strip. *PLoS ONE* **2012**, *7*, e42864. [CrossRef]

79. Laham, N.A.; Mediavilla, J.R.; Chen, L.; Abdelateef, N.; Elamreen, F.A.; Ginocchio, C.C.; Pierard, D.; Becker, K.; Kreiswirth, B.N. MRSA Clonal Complex 22 Strains Harboring Toxic Shock Syndrome Toxin (TSST-1) Are Endemic in the Primary Hospital in Gaza, Palestine. *PLoS ONE* **2015**, *10*, e0120008. [CrossRef]

80. Adhikari, R.P.; Cook, G.M.; Lamont, I.; Lang, S.; Heffernan, H.; Smith, J.M. Phenotypic and molecular characterization of community occurring, Western Samoan phage pattern methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **2002**, *50*, 825–831. [CrossRef]

81. Smith, J.M.; Cook, G.M. A decade of community MRSA in New Zealand. *Epidemiol. Infect.* **2005**, *133*, 899–904. [CrossRef]

82. Muller, A.; Seinige, D.; Jansen, W.; Klein, G.; Ehricht, R.; Monecke, S.; Kehrenberg, C. Variety of Antimicrobial Resistances and Virulence Factors in *Staphylococcus aureus* Isolates from Meat Products Legally and Illegally Introduced to Germany. *PLoS ONE* **2016**, *11*, e0167864. [CrossRef] [PubMed]

83. Masiuk, H.; Kopron, K.; Grumann, D.; Goerke, C.; Kolata, J.; Jursa-Kulesza, J.; Giedrys-Kalemba, S.; Broker, B.M.; Holtfreter, S. Association of Recurrent Furunculosis with Panton-Valentine Leukocidin and the Genetic Background of *Staphylococcus aureus*. *J. Clin. Microbiol.* **2010**, *48*, 1527–1535. [CrossRef] [PubMed]

84. Yeap, A.D.; Woods, K.; Dance, D.A.B.; Pichon, B.; Rattanavong, S.; Davong, V.; Phetsouvanh, R.; Newton, P.N.; Shetty, N.; Kearns, A.M. Molecular epidemiology of *Staphylococcus aureus* skin and soft tissue infections in the Lao People's Democratic Republic. *Am. J. Trop. Med. Hyg.* **2017**, *97*, 423–428. [CrossRef] [PubMed]

85. Vorobieva, V.; Bazhukova, T.; Hanssen, A.M.; Caugant, D.A.; Semenova, N.; Haldorsen, B.C.; Simonsen, G.S.; Sundsfjord, A. Clinical isolates of *Staphylococcus aureus* from the Arkhangelsk region, Russia: Antimicrobial susceptibility, molecular epidemiology, and distribution of Panton-Valentine leukocidin genes. *Apmis* **2008**, *116*, 877–887. [CrossRef]

86. Monecke, S.; Slickers, P.; Ellington, M.; Kearns, A.; Ehricht, R. High diversity of Panton-Valentine leucocidin-positive, methicillin-susceptible isolates of *Staphylococcus aureus* and implications for the evolution of community-associated MRSA. *Clin. Microbiol. Infect.* **2007**, *13*, 1157–1164. [CrossRef]

87. Robinson, D.A.; Enright, M.C. Evolution of *Staphylococcus aureus* by Large Chromosomal Replacements. *J. Bacteriol.* **2004**, *186*, 1060–1064. [CrossRef]

88. Monecke, S.; Slickers, P.; Gawlik, D.; Müller, E.; Reissig, A.; Ruppelt-Lorz, A.; Akpaka, P.; Bandt, D.; Bes, M.; Boswihi, S.; et al. Molecular typing of ST239-MRSA-III from diverse geographic locations and the evolution of the SCCmec III element during its intercontinental spread. *Front. Microbiol.* **2018**, *9*, 1436. [CrossRef]

89. Shang, W.; Hu, Q.; Yuan, W.; Cheng, H.; Yang, J.; Hu, Z.; Yuan, J.; Zhang, X.; Peng, H.; Yang, Y.; et al. Comparative Fitness and Determinants for the Characteristic Drug Resistance of ST239-MRSA-III-t030 and ST239-MRSA-III-t037 Strains Isolated in China. *Microb. Drug Resist.* **2016**, *22*, 185–192. [CrossRef]

90. Smyth, D.S.; McDougal, L.K.; Gran, F.W.; Manoharan, A.; Enright, M.C.; Song, J.H.; de Lencastre, H.; Robinson, D.A. Population structure of a hybrid clonal group of methicillin-resistant *Staphylococcus aureus*, ST239-MRSA-III. *PLoS ONE* **2010**, *5*, e8582. [CrossRef]

91. Wang, Z.; Zhou, H.; Wang, H.; Chen, H.; Leung, K.K.; Tsui, S.; Ip, M. Comparative genomics of methicillin-resistant *Staphylococcus aureus* ST239: Distinct geographical variants in Beijing and Hong Kong. *BMC Genom.* **2014**, *15*, 529. [CrossRef] [PubMed]

92. Yamamoto, T.; Takano, T.; Higuchi, W.; Iwao, Y.; Singur, O.; Reva, I.; Otsuka, Y.; Nakayashiki, T.; Mori, H.; Reva, G.; et al. Comparative Genomics and Drug Resistance of a Geographic Variant of ST239 Methicillin-Resistant *Staphylococcus aureus* Emerged in Russia. *PLoS ONE* **2012**, *7*, e29187. [CrossRef]

93. Chen, H.; Yin, Y.; van Dorp, L.; Shaw, L.P.; Gao, H.; Acman, M.; Yuan, J.; Chen, F.; Sun, S.; Wang, X.; et al. Drivers of methicillin-resistant *Staphylococcus aureus* (MRSA) lineage replacement in China. *Genome Med.* **2021**, *13*, 171. [CrossRef] [PubMed]

94. Jin, Y.; Zhou, W.; Zhan, Q.; Zheng, B.; Chen, Y.; Luo, Q.; Shen, P.; Xiao, Y. Genomic Epidemiology and Characterization of Methicillin-Resistant *Staphylococcus aureus* from Bloodstream Infections in China. *mSystems* **2021**, *6*, e0083721. [CrossRef]

95. Kechrid, A.; Perez-Vazquez, M.; Smaoui, H.; Hariga, D.; Rodriguez-Banos, M.; Vindel, A.; Baquero, F.; Canton, R.; Del Campo, R. Molecular analysis of community-acquired methicillin-susceptible and resistant *Staphylococcus aureus* isolates recovered from bacteraemic and osteomyelitis infections in children from Tunisia. *Clin. Microbiol. Infect.* **2011**, *17*, 1020–1026. [CrossRef]

96. Monecke, S.; Skakni, L.; Hasan, R.; Ruppelt, A.; Ghazal, S.S.; Hakawi, A.; Slickers, P.; Ehricht, R. Characterisation of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. *BMC Microbiol.* **2012**, *12*, 146. [CrossRef]

97. Stegger, M.; Wirth, T.; Andersen, P.S.; Skov, R.L.; De Grassi, A.; Simoes, P.M.; Tristan, A.; Petersen, A.; Aziz, M.; Kiil, K.; et al. Origin and evolution of European community-acquired methicillin-resistant *Staphylococcus aureus*. *mBio* **2014**, *5*, e01044-14. [CrossRef]

98. Abou Shady, H.M.; Bakr, A.E.; Hashad, M.E.; Alzohairy, M.A. *Staphylococcus aureus* nasal carriage among outpatients attending primary health care centers: A comparative study of two cities in Saudi Arabia and Egypt. *Braz. J. Infect. Dis.* **2015**, *19*, 68–76. [CrossRef]

99. Elhani, D.; Gharsa, H.; Kalai, D.; Lozano, C.; Gomez, P.; Boutheina, J.; Aouni, M.; Barguellil, F.; Torres, C.; Ben Slama, K. Clonal lineages detected amongst tetracycline-resistant meticillin-resistant *Staphylococcus aureus* isolates of a Tunisian hospital, with detection of lineage ST398. *J. Med. Microbiol.* **2015**, *64*, 623–629. [CrossRef]

100. Vannuffel, P.; Gigi, J.; Ezzedine, H.; Vandercam, B.; Delmee, M.; Wauters, G.; Gala, J.L. Specific detection of methicillin-resistant Staphylococcus species by multiplex PCR. *J. Clin. Microbiol.* **1995**, *33*, 2864–2867. [CrossRef]

101. Ardic, N.; Sareyyupoglu, B.; Ozyurt, M.; Haznedaroglu, T.; Ilga, U. Investigation of aminoglycoside modifying enzyme genes in methicillin-resistant staphylococci. *Microbiol. Res.* **2006**, *161*, 49–54. [CrossRef] [PubMed]

102. Shokravi, Z.; Mehrad, L.; Ramazani, A. Detecting the frequency of aminoglycoside modifying enzyme encoding genes among clinical isolates of methicillin-resistant *Staphylococcus aureus*. *Bioimpacts* **2015**, *5*, 87–91. [CrossRef]

103. Louie, L.; Matsumura, S.O.; Choi, E.; Louie, M.; Simor, A.E. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. *J. Clin. Microbiol.* **2000**, *38*, 2170–2173. [CrossRef] [PubMed]
104. Monecke, S.; Kuhnert, P.; Hotzel, H.; Slickers, P.; Ehricht, R. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Vet. Microbiol.* **2007**, *125*, 128–140. [CrossRef] [PubMed]