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RESEARCH ARTICLE

Dissemination of epidemic ST239/ST241-t037-*agr*I-SCC*mec*III methicillin-resistant *Staphylococcus aureus* in a Tunisian trauma burn intensive care unit

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen causing health care-infections in the world, especially in burns. The aim of this study was to assess the extent of dissemination of MRSA isolated from burn patients in Burn Intensive Care Unit in Tunisia and to evaluate the frequency of virulence and antibiotics resistance genes. Among the 72 *S. aureus* isolates analyzed in the study, 54% were MRSA. The majority of MRSA (94.8%) were multidrug resistant and they had a high resistance rates to kanamycin (94.8%), tobramycin (90%), tetracycline (94.8%) and ciprofloxacin and rifampicin (87%, each). The gene *aac(6′)-Ie-aph(2″)-Ia* conferring resistance to kanamycine and tobtamycin were detected in all isolates and the *aph(3′)-Ia* gene conferring resistance to gentamicin were detected in 2.8% of resistant isolates. Tetracycline resistance genes *tet(M), tet(K)* and *tet(L)* were detected in 100%, 10.8% and 2.8% of the isolates, respectively. The SCC*mec* type III and the *agr* type I were the most predominant (69.2% and 90%, respectively). The 27 SCC*mec*III-*agr*I isolates were clustered into two PFGE types A and B. The two representative isolates of PFGE clusters A and B belonged to ST239-t037 and ST241-t037 respectively. As conclusion, our results showed a high prevalence of MRSA in trauma burn intensive care unit belonging to two multidrug resistant clones ST239/ST241-*agr*I-t037-SCC*mec*III MRSA. We also demonstrated that MRSA was disseminated between burn patients.

KEYWORDS

MRSA, burn patients, ST239/ST241, Intensive Care Unit, dissemination

INTRODUCTION

Staphylococcus aureus is a commensal bacterium of the skin and mucous flora. It can be responsible for many different types of infections in humans that can vary from simple cutaneous lesions to potentially life threating infections [1]. The pathogenicity of *S. aureus* is due to several virulence factors such as enterotoxins, Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin 1 (TSST-1) and biofilm production. Burn patients are at greater risk of exposure to *S. aureus* infections due to the loss of protective barriers and the frequent use of

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intravenous vascular catheters. Moreover, the emergence of methicillin resistant strains can worsen the prognosis of these infections and leads to increased healthcare costs [1].

Methicillin-resistance is mainly encoded by the *mecA* gene, carried by the Staphylococcal Chromosomal Cassette *mec* (SCC*mec*), classified into twelve types (I–XII) [2]. Previous international epidemiologic studies showed that methicillin-resistant *S. aureus* (MRSA) dissemination was associated to number of clones harboring specific genetics background. In the 1990s, molecular studies described the emergence of new clones which were able to rapidly disseminate between hospitals with a high rate of transfer between patients [1]. The presence of these clones increased considerably in the last two decades with important geographical variations. Five clonal complexes (CC) (CC5, CC8, CC22, CC30 and CC45) were associated to healthcare-MRSA (HA-MRSA) in Europe, New York/Japan Clone and Brazilian/Hungarian are associated to HA-MRSA, respectively, in Asia and Africa [3].

In this study, we aimed to assess the extent of dissemination of MRSA in burn patients isolated at Burn Intensive Care Unit (BICU) of the Trauma and Burn Centre of Ben Arous in Tunisia. Furthermore, we intended to evaluate the frequency and the distribution of virulence and antibiotics resistance genes in this species.

MATERIAL AND METHODS

Setting and bacterial isolates

This study was conducted in Traumatology and Great Burned Center of Ben Arous, an university hospital with168 beds. It includes several wards, such as plastic surgery, neurosurgery, orthopedic and burn intensive care units. The BICU is the largest one in Tunisia and has a 20-bed capacity: 10 single-bedrooms, two rooms with three beds and two rooms with two beds. The healthcare worker-to-patient ratio was 1:3. The average period of hospitalization in the BICU was 21 days. From January to December 2017, a total of 72 *S. aureus strains* were collected from hospitalized burn patients at the BICU of Traumatology and Great Burned Center. Isolates were collected from skin samples (SK; $n = 37$), blood (BL; $n = 18$), intravenous vascular catheters (IVC; $n = 11$) and respiratory samples (RES; $n = 6$). Skin samples were done systematically during dressing change in burn wound areas. Blood, intravenous vascular catheters and respiratory samples were performed according to the patient condition. One isolate per patient was considered. Species were identified using conventional phenotypic methods and Api ID32 STAPH (Bio-Mérieux S.A, Marcy l'Etoile). Isolates were stored in Brain Heart Infusion Broth (BHIB) (Bio-Rad, Marnes-la-Coquette, France) with 10% glycerol at −70 °C.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was determined by the disk diffusion method on Mueller Hinton (MH) agar (Bio-Rad, Marnes-la-Coquette, France) according to Antibiogram Committee of the French Society of Microbiology (CA-SFM) guidelines [4]. A total of eleven antibiotics (Bio-Rad, Marnes-la-Coquette, France) were tested using the following discs: penicillin (P, 10UI), oxacillin (OXA, 1 µg), cefoxitin (CXI, 30 µg), erythromycin (ERY, 15 µg), gentamicin (GEN, 10 µg), fosfomycin (FOS, 50 µg), teicoplanin (TEI, 30 µg), tetracycline (TET, 30 µg), rifampicin (RIF, 5 µg), fusidic acid (FUS, 10 µg), ciprofloxacin (CIP, 5 µg). The minimal inhibitory concentrations (MICs) for teicoplanin and vancomycin were determined using broth dilution method (UMIC Biocenric®) and interpreted as recommended by CA-SFM breakpoints. The strain *S. aureus* ATCC25923 was included as a quality control for the antimicrobial susceptibility testing. Isolates that presented resistance to three or more classes of antibiotics, other than beta-lactams, were classified as having a multidrug resistance profile [4].

DNA extraction

Genomic DNA was extracted by Instagene™ Matrix (Bio-Rad, California,USA) and was utilized for molecular characterization of MRSA isolates.

Detection of antimicrobial resistance genes

MRSA isolates were submitted to polymerase chain reaction (PCR) amplification assays to detect the following genes: *mecA, bla(Z), aph(3′)-Ia,aac(6′)-Ie-aph(2″)-Ia, ermA, ermB, ermC, tet(K), tet(L) and tet(M)* as previously described [5–8] and using the primers listed in Table1.

Detection of virulence genes

The presence of genes encoding for staphylococcal enterotoxines (*sea, seb, sec, sed, see*), leucocidins (*lukF-lukS*-PV [Panton–Valentine leucocidin (PVL) determinant]), exfoliative toxins (*eta* and *etb*) and toxic shock syndrome toxin I (*tsst-1*) was performed by multiplex PCR as previously reported methodologies [9, 10] (Table1).

Detection of *ica* genes

The presence of *icaA* gene which encodes for biofilm production was analyzed by PCR [11] (Table1).

SCC*mec* typing

The SCC*mec* structural type was determined by multiplex PCR strategy as described by Zhang et *al.* [12] For non-typeable (no PCR amplification occurred for any of the primers pairs used) isolates, the structure of *ccr* (*ccrAB1, ccrAB2, ccrAB3 and ccrC1*) and *mec* complex were determined as described by Okuma et al. [13] SCC*mec* types were classified using the guidelines proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) [14].

agr typing

The presence of the accessory gene regulator, *agr*, was determined by PCR amplification of the hypervariable domain of the *agr* locus using previously described primers [15].

Table 1. Primers used for PCR reactions

Gene	Sequence (5′-3′)	Size (pb)	References
BlaZ	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173	[6]
ermA	TATCTTATCGTTGAGAAGGGATT CTACACTTGGCTTAGGATGAAA	139	[6]
ermB	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142	[6]
ermC	CTTGTTGATCACGATAATTTCC ATCTTTTAGCAAACCCGTATTC	190	[6]
mecA	AACAGGTGAATTATTAGCACTTGTAAG ATTGCTGTTAATATTTTTTGAGTTGAA	174	[6]
aph(3′)-IIIa	CTGATCGAAAAATACCGCT ACAATCCGATATGTCGATGGAG	354	[7]
aac(6′)-aph(2″)	CCAAGAGCAATAAGGGCATACC ACCCTCAAAAACTGTTGTTGC	675	[7]
tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360	[8]
tetL	ATAAATTGTTTCGGGTCGGTAAT AACCAGCCAACTAATGACAAGAT	1,077	[8]
tetM	GCTCATGTTGATGCAGGAAATCCCATTGTTCA	550	[9]
Sea	CCTTTGGAAACGGTTAAAACG TCTGAACCTTCCCATCAAAAAC	127	[10]
Seb	TCGCATCAAACTGACAAACG GCAGGTACTCTATAAGTGCCTGC	477	[10]
Sec	CTCAAGAACTAGACATAAAAGCTAGG TCAAAATCGGATTAACATTATCC	271	[10]
sed	CTAGTTTGGTAATATCTCCTTTAAACG TTAATGCTATATCTTATAGGGTAAACATC	319	[10]
See	CAGTACCTATAGATAAAGTTAAAACAAGC TAACTTACCGTGGACCCTTC	187	[10]
lukS-PV/lukF-PV	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAASTGTATTGGATAGCAAAAGC	433	[11]
eta	GCAGGTGTTGATTTAGCATT AGATGTCCCTATTTTTGCTG	93	[11]
etb	ACAAGCAAAAGAATACAGCG GTTTTTGGCTGCTTCTCTTG	226	[11]
Tsst-1	ACCCCTGTTCCCTTATCATC TTTTCAGTATTTGTAACGCC	326	[11]
icaA	CCTAACTAACGAAAGGTAG AAGATATAGCGATAAGTGC	1,315	[12]

PFGE typing

The genetic relationship of the MRSA isolates was determined by PFGE as described in a previous study [16]. PFGE was performed for isolates (n = 27) with the same *agr* type and harbored the same SCC*mec* type. The resulting restriction band patterns were analyzed by visual interpreted according to the criteria proposed by Tenover et al. [17]

MLST and *spa* typing

Multi-locus sequence typing (MLST) and *spa* typing were performed for representative MRSA isolates belonging to different PFGE types. *Spa* typing was performed by PCR amplification and sequencing of the polymorphic X region of the protein A gene (*spa*) [18]. *Spa* types were assigned by using RidomStaph-Type software (version 1.4; Ridom GmbH, Wuürzburg, Germany) as described by Harmsen et al. [19]. MLST was carried out by amplifying and sequencing the seven housekeeping genes. The sequence types (ST) were assigned according to the MLST database (http://www.mlst.net).

RESULTS

MRSA were prevalent in the BICU

Among the 72 *S. aureus* isolates analyzed in the study, 54% were MRSA as confirmed by genotypic (PCR detection of *mecA*) method. MRSA isolates were recovered from various clinical specimens. The highest prevalence was observed in skin (54%), followed by blood (23%), intravenous vascular catheter (16%) and respiratory tract (7%).

MRSA in BICU were multidrug resistant

A total of 39 MRSA isolates were analyzed by SCC*mec* typing. Three SCC*mec* types (III, IV, and V) were found and their distribution is shown in Table 2. Among these, the SCC*mec* type III was the most common type being found in 69.2% (27/39) of the MRSA isolates. The remaining isolates carried SCC*mec* types IV or V (15.4% each).

The MRSA isolates tested showed high rates of phenotypic resistance to kanamycin (94.8%), tobramycin (94.8%), gentamicin (90%), tetracycline (94.8%), ciprofloxacin and

Table 2. Phenotypic and genotypic characterization of 39 MRSA isolated from burn patients

Sample ID	Isolation source[a]	Isolation Date	Profile of phenotypic resistance[b]	Antibiotic resistance genes[c]	Virulence genes[d]	*mecA*[e]	SCC*mec* type	*Agr* type	PFGE type	*Spa* type	ST[f]
29	RES	05/10/2017	CIP, KAN, GEN,TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	A_1	t037	ST239
3	SK	17/02/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	A_1	t037	ST239
5	SK	21/03/2022	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	A_1	t037	ST239
23	SK	14/07/2017	CIP, KAN, GEN, TET, RIF, FUS	*tet(M), aac6-aph3*	*sea*	+	III	I	A_1	t037	ST239
36	IVC	10/11/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea*	+	III	I	A_1	t037	ST239
37	SK	18/11/2107	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea*	+	III	I	A_1	t037	ST239
11	BL	16/04/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	A_2	–	–
16	SK	03/06/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	A_1	t037	ST239
1	IVC	04/01/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(K), tet(M), aac6-aph3*	*sea, icaA*	+	III	I	A_1	t037	ST239
27	BL	21/07/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(K), tet(M), aac6-aph3*	*sea, icaA*	+	III	I	A_1	t037	ST239
9	BL	06/04/2017	CIP, KAN, TET, RIF, FOS	*tet(M), aph3*	*sea, eta, icaA*	+	III	I	A_1	t037	ST239
18	SK	06/06/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, eta, tsst-1, icaA*	+	III	I	A_1	t037	ST239
28	RS	28/08/2017	CIP, KAN, GEN, TET, FOS	*tet(M), aac6-aph3*	*sea, eta*	+	III	I	A_1	t037	ST239
14	RS	27/04/2017	KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	A_1	t037	ST239
31	SK	16/10/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	B_1	t037	ST241
32	SK	30/10/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	B_1	t037	ST241
35	SK	02/11/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	B_1	t037	ST241
20	RS	21/06/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	B_3	–	–
22	SK	12/07/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	B_1	t037	ST241
26	SK	22/08/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	B_1	t037	ST241
30	BL	15/08/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	B_1	t037	ST241
8	IVC	03/04/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	B_2	–	–
19	SK	07/06/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	B_1	t037	ST241
39	BL	18/11/2107	CIP, KAN, GEN, TET, RIF	*tet(K), tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	B_2	–	–
17	IVC	05/06/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(K), tet(M), aac6-aph3*	*sea, eta, icaA*	+	III	I	B_1	t037	ST241
10	BL	07/04/2017	CIP, KAN, GEN, TET	*tet(M), aac6-aph3*	*sea, icaA*	+	III	I	B_1	t037	ST241
34	SK	01/11/2017	CIP,KAN,GEN,TET,RIF	*tet(M), aac6-aph3*	*sea*	+	III	I	B_1	t037	ST241
25	SK	21/08/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	V	I	–	–	–
12	SK	18/04/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	V	I	–	–	–
2	SK	23/01/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*sea, icaA*	+	V	I	–	–	–
38	BL	13/11/2017	–	-	*sea, icaA*	+	V	I	–	–	–
21	SK	06/07/2017	CIP, KAN, GEN, ERY, TET, RIF, FOS, FUS	*tet(M), aac6-aph3, erm(A)*	*sea, icaA*	+	V	I	–	–	–
24	IVC	23/07/2017	KAN, GEN, TET, RIF	*tet(M), tet(L), aac6-aph3*	*pvl, sea, icaA*	+	V	III	–	–	–
4	SK	17/02/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, icaA,*	+	IV	I	–	–	–
15	BL	12/05/2017	CIP, KAN, GEN, TET, RIF, FOS	*tet(M), aac6-aph3*	*sea, icaA,*	+	IV	I	–	–	–
7	SK	29/03/2017	CIP, KAN, GEN, TET, RIF	*tet(M), aac6-aph3*	*pvl, sea, icaA*	+	IV	I	–	–	–
13	SK	20/04/2017	–	-	-	+	IV	I	–	–	–
33	SK	30/10/2017	CIP, KAN, GEN, TET, RIF, FUS	*tet(M), aac6-aph3*	*sea*	+	IV	III	–	–	–
6	IVC	23/03/2017	KAN, TET, FUS	*tet(M), aph3*	*pvl, sea, icaA*	+	IV	II	–	–	

[a]SK: Skin, BL: Blood, IVC: Intravenous vascular catheter, RS: respiratory tract; [b]KAN: Kanamycin, GEN: Gentamicin, CIP: Ciprofloxacin, TET: Tetracycline, ERY: Erythromycin, RIF: Rifampicin, FOS: Fosfomycin, FUS: Fusidic acid; [b,c,d] – Absence; [e] + Presence; [f]ST: Sequence type; 29,31: Representative strain of each pulsotype

rifampicin (87% each) and fosfomycin (46%) (Table 3). There were no isolates resistant to vancomycin and teicoplanin. Moreover, the majority of MRSA isolates (94.8%) showed a multidrug resistant (MDR) profile (Table 2) being resistant to four or more antimicrobial classes. The most common MDR profiles included resistance to ciprofloxacin, kanamycin, gentamicin, tetracycline and rifampicin (35.9%) or ciprofloxacin, kanamycin, gentamicin, tetracycline, rifampicin and fosfomycin (33.3%).

To understand which was the genetic content of the MRSA in antibiotic resistance determinants, PCR amplification was performed for selected antibiotic classes. We found that MRSA isolates carried genes conferring resistance to beta-lactams (*blaZ,* 100%), macrolide-lincosamide-streptogramins determinants (*ermB*, 100%), kanamycin (*aph(3′)-Ia,*2.8%), gentamicin (*aac(6′)-Ie-aph(2″)Ia*, 100%) and tetracyclines (*tet(M)*, 100%; *tet(K),* 10.8%*; tet(L),* 2.8%). The *tet(M)* was found in association with *tet(K)* in four isolates and with *tet(L)* in a single isolate (Table 2).

MRSA in BICU had a high pathogenic potential

The results of virulence gene analysis showed that MRSA isolates carried some virulence genes, namely *sea*, encoding staphylococcal enterotoxins (97%), *eta,* encoding for exfoliative toxin A (28%), *tsst-1*, encoding for toxic shock syndrome toxin I (2.5%) and *pvl*, encoding for leucocidins (7.5%) (Table 3). The *icaA* gene was found in 32 isolates (82%). The MRSA isolates displayed different virulence profiles ($n = 5$) with considerable variability. The *sea icaA* was the most frequent virulence profile (48%) mainly in skin, followed by *sea eta icaA* (23%), *sea* (12%), *pvl sea icaA* (7.5%) and *sea eta tsst-1*and *icaA* (2.5%). Additionally, we observed that toxins were mainly enriched in isolates collected from skin when compared to blood, intravenous vascular catheter, and respiratory tract (Table 2).

The *agr*, which encodes a quorum-sensing system that acts as a master virulence regulator and as a central player in the pathogenicity of *S. aureus* [15], was present in all the MRSA isolates. The *agr* type I was the most predominant (92%) followed by type III (5%) and only one strain harbored the type II.

Evidence of dissemination of MRSA in BICU

The PFGE analysis grouped the 27 SCC*mec*III and *agr*I isolates into two clusters A ($n = 14$ isolates) and B ($n = 13$ isolates). PFGE types A and B were subdivided into two (A1-A2) and three (B1–B3) subtypes, respectively based on minor band variations (Table 2). The two clusters A and B belonged to ST239 and ST241 (clonal complex CC8) respectively and shared the same *spa* type t037. Twenty-three MRSA isolates shared the same PFGE subtypes (subtype A1; $n = 13$ and subtype B1; $n = 10$) suggesting the occurrence of at least 23 events of cross-transmission of ST239/ST241 within the BICU.

Within each of the two clusters, we identified MRSA isolates that shared the same SCC*mec*, *agr* types, resistance and virulence phenotypic profiles and gene content. In particular,

Table 3. Prevalence of Antibiotics resistance and virulence genes in methicillin-resistant *S. aureus* strains isolated from burn patients

	Antibiotics resistance								virulence genes				
Cluster (Nb)	CIP	Kan	Gen	TET	RIF	FOS	FUS	ERY	*sea*	*eta*	*icaA*	*pvl*	*Tsst-1*
A1 (13)	12	13	12	13	12	11	1	0	13	5	9	0	1
A2 (1)	1	1	1	1	1	1	0	0	1	1	1	0	0
B1 (10)	10	10	10	10	9	2	0	0	10	3	9	0	0
B2 (2)	2	2	2	2	2	0	0	0	2	2	2	0	0
B3 (1)	1	1	1	1	1	1	0	0	1	0	1	0	0
Other strains (12)	8	10	9	10	9	3	3	1	11	0	10	3	0
Total (39)	**34**	**37**	**35**	**37**	**34**	**18**	**4**	**1**	**38**	**11**	**32**	**3**	**1**
%	**87%**	**94.8%**	**90%**	**94.8%**	**87%**	**46%**	**10%**	**2.5%**	**97%**	**28%**	**82%**	**7.5%**	**2.5%**

Nb: Number, KAN: Kanamycin, GEN: Gentamicin, CIP: Ciprofloxacin, TET: Tetracycline, ERY: Erythromycin, RIF: Rifampicin, FOS: Fosfomycin, FUS: Fusidic acid.

we found that three pairs of strains 1/27 (IVC-BL), 3/29 (SK-RES) and 36/37 (IVC-SK) belonging to the same PFGE subtype A1 carried similar antibiotic resistance and virulence genes content as well as *SCCmec* and *agr* types (Table 2). Moreover we found that isolates 22, 26, 31, 32 and 35 from skin, subtype B1,were phenotypically resistant to the same antibiotics (ciprofloxacin, kanamycin, gentamicin, tetracycline, rifampicin), carried the same resistance and virulence genes (*tetM,aac6-aph3, sea, icaA*) and had the same SCC*mec* type (SCC*mec*III) and *agr* type (*agrI*). Additionally, the pair 29/30 (RES-SK) grouped within subtypes A1/B1 were undisguisable regarding their antibiotic resistance and virulence profile, shared the same SCC*mec*, *spa* and *agr* and belonging to same clonal complex. Overall, the results suggest the existence of frequent cross-transmission of MRSA in the BICU. We observed that MRSA isolates had the ability to persist over time since we could recover isolates from skin that were related (same PFE subtypes A1 or B1) and could persist for 10 months. This the case of isolates 3 and 37 (PFGE subtype A1) and isolates 35/22 (PFGE subtype B1).

DISCUSSION

Infections caused by MRSA are a major challenge to burn patients. They are associated with high rates of morbidity and mortality. In this study, we found a high prevalence of MRSA from trauma burn intensive care unit to be 54%. This rate was much higher than rates reported in a Tunisian multicentric study (19.3%) [20] and in countries surrounding the Mediterranean area (<50%) [20]. However, the prevalence detected in this study was lower than that reported in several study in burn patients, which can exceed 75% [21, 22]. These discrepancies in the prevalence of MRSA among various studies has been linked to many factors including the diverse antibiotic use patterns or beta-lactam usage pressure in ward and hospital, different infection control policies and luck of supervision on antibiotic use.

Moreover, the risk factors of MRSA acquisition in burn patients were associated with the site, the size and the mechanism of burn, and the length of hospitalization in intensive care unit [23]. In this study, all the patients had occupied different beds throughout the ward, had important (large) burns and hospitalized for long periods of time (21 days). A weekly surveillance in burn unit in a Canadian hospital showed that on admission only 2.5% of patients were colonized by MRSA, whereas 17.9% became colonized during the hospitalization [24]. Furthermore, in another study, a meta-analysis showed that rates of colonization with MRSA increased from 4.1% on admission to 21.2% after 3 days in burn unit [23]. In this study, there was no decolonization protocol applied during the hospitalization at the Traumatology and Great Burned Center. Previous studies have shown the role of decolonization protocols in the decrease of MRSA acquisition [23], which was a result of transmission of strains from the environment to patient [23] or from patient to patient through the healthcare workers [23, 25].

Additionally, we found that more than 50% of MRSA were isolated from the skin followed by the blood and the intravenous catheters. These rates were similar to those previously reported in Tunisia and other countries [2, 3, 26]. The fact that *S. aureus* is a commensal bacterium of the skin, skin glands and mucous membranes, after burn, necrotic tissue can serve as a favorite environment for growth of this bacteria and causes skin and soft tissue infections as well as invasive infections [27]. A previous study has estimated that patients which were colonized by MRSA have 3.9 times more risk to develop bacteremia than patient which colonized by MSSA [23]. MRSA was the third most common species encountered in blood cultures in a South African intensive care burn unit with 17% of the patients with MRSA-positive blood cultures died [28].

Most of the MRSA isolated in the current study was found to be SCC*mec* type III. Previous studies in burn patients also detected SCC*mec* type III as a most prevalent type in China [29] and Brazil [30]. The high prevalence of SCC*mec* type III in our center suggests the nosocomial origin of the isolates, and thus emphasizing the importance of disinfection of burn wards environment.

We showed that MRSA were disseminated in the entire BICU among patients with large burns. The contamination with MRSA probably occurred in the unit setting, due to patient-to-patient transmission promoted by healthcare workers or surrounding environment-to-patient transmission because of the poor hygiene. Moreover, we clearly observed cross-transmission of isolates and we identified similar MRSA isolates that shared the same PFGE subtypes (subtype A1or B1), suggesting the occurrence of at least 23 events of cross-transmission of ST239/ST241(CC8) in burn patients hospitalized within BICU with no decolonization protocol applied during the hospitalization period and with healthcare worker-to-patient ratio of 1:3. These isolates shared also the same SCC*mec* and *agr* types, resistance and virulence phenotypic profiles and gene content. Cross-transmission risk within healthcare settings, mainly burn units, is high [25] Healthcare personal who has the most direct contact with patients are frequently reported as critical vectors for MRSA transmission [31]. In addition, healthcare worker carriage has the ability to complicate the control of MRSA in hospital setting because skin carriage is important and single episodes of contact are sufficient for MRSA transmission to occur. Also, in burn units, MRSA may be transmitted through the air environment which can become heavily colonized with MRSA and may serve as an important reservoir for continued transmission of this bacteria [32] because when exposed to dry surfaces, MRSA have the potential to remain viable for months and can remain virulent and capable of causing infection for at least 10 days [33]. In burn units, the proportion of surfaces contaminated with MRSA in patient rooms can range from few percent to 64% [34].

In our study, the finding of isolates with the same PFGE subtypes in different patients and in the same or different samples suggests that the cross-transmission observed can be either due to the contamination of the room environment and/or to the healthcare workers. In the absence of adequate hygiene, cleaning and decontamination process, which the

case in the BICU, MRSA may persist over time within the burn unit. In this study, MRSA isolates had the ability to persist over time which could be related to the formation of biofilms as 82% of the isolates harbored the *icaA* gene or to resident microbiota of the burn unit.

The majority of MRSA (94.8%) isolates exhibited a multidrug resistance phenotype, and the most resistance profiles found mainly in skin included resistance to ciprofloxacin, kanamycin, gentamicin, tetracycline, rifampicin and fosfomycin. It was reported that *S. aureus* or MRSA multidrug resistant strains in burn patients have been increasing. This is due to the excessive and widespread use of broad-spectrum antibiotics, particularly cephalosporins and carbapenems in hospital setting [24, 27, 35] In agreement with previous findings [35, 36], we also found that MRSA in the BICU were highly resistant to many antimicrobials, including kanamycin, gentamicin, tetracycline, rifampicin, and ciprofloxacin and indicating the harsh situation of antimicrobial resistance in burn patients. Almost all of these antibiotics such as β-lactams, teicoplanin, gentamicin, ciprofloxacin and rifampicin, together with amikacin and fosfomycin, are commonly used in the treatment and prevention of infection in burn patients in BICU in our Center [37] which could explain the high resistance rates to these drugs detected in MRSA isolated in this study.

Besides being MDR resistant, MRSA isolates carried several virulence genes including enterotoxin genes (*sea*), exotoxin genes (*eta, tst* and *pvl*) and adhesion associated genes (*icaA*). The *sea icaA* was the most frequent virulence profile mainly detected in skin. The great majority of MRSA isolates harbored the staphylococcal enterotoxins encoding gene (*sea*) which is described to be carried by a temperate bacteriophage [14] and is the most common enterotoxin recovered from food-poisoning outbreaks [15, 16]. Thus, the high detection rate of the *sea* gene in our unit may be due to the prevalence of bacteriophages carrying this gene. However, whether the *sea* gene plays a significant role in causing human infections requires further investigation. Three MRSA isolates were *pvl* positive in our study. The *pvl* prevalence in *S. aureus* is highly variable worldwide and more associated to CA-MRSA [2]. The carrying rate of the *icaA* gene encoding for biofilm production, was high. The *icaA* gene is frequently detected in clinical *S. aureus* isolates in burn units [38]. Moreover, several previous studies had reported that the ST239/241-SCC*mec*III clone, to which belonged 59% of our MRSA isolates has high biofilm formation capacity [2]. In accordance with other studies [2, 3], the *agrI* was the most common in MRSA isolates.

In the current study, the great majority (59%) of MRSA isolates belong to ST239/ST241-t037-*agrI*-SCC*mec*III which belong to the CC8 which could be transferred from neighboring countries [2, 3]. The ST239/241-SCCmecIII-t037 is a major HA-MRSA predominating in Morocco (95%), in Europe (78%) and in Taiwan (73%) [2, 3]. In Tunisia, the first report of ST239-t03-agrI-SCC*mec*III (CC8) and ST241-t125-agrI-SCC*mec*III (CC8) was at Charles Nicolle Hospital of Tunisia in 2013 with low prevalence (10% and 7%) in a comparison to CC80-t70-agrIII-SCC*mec*IVc (51.2%) [39].

CONCLUSION

Overall, our results showed high prevalence of MRSA in trauma burn intensive care unit belonging to two multidrug resistant clones ST239/ST241-t037-agrI-SCC*mec*III. Moreover, we demonstrated that MRSA was disseminated between burn patients. To better control infection and monitor the eventual spread of MRSA, more attention should be attached to nosocomial infection in Traumatology and Great Burned Center and surveillance should be reinforced in burn unit.

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Ethics approval: This study was performed with approval from the Local Medical Committee of Charles Nicolle Hospital, Tunis, Tunisia. Since the strains were de-identified prior to analysis and the strains, not human, were studied anonymously, this is exempt from human research committee approval according to the regulations of the Tunisian Local Medical Committee of Charles Nicolle Hospital and informed consent is not required according to the Ethical Committee.

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