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Antimicrobial Susceptibility and Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Tunisia: Results of a Multicenter Study

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Abstract

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) as a major cause of infections in hospital and community settings is a global health concern. The purpose of this study was to determine the antimicrobial susceptibility and the molecular characteristics of MRSA strains causing community-acquired (CA) and hospital-acquired (HA) infections in Tunisia.

Methods: A total of 135 non-duplicate MRSA strains were consecutively collected from five Tunisian hospitals. Antimicrobial susceptibility was done by disc diffusion method and by MIC. The presence of *pvl* (Panton Valentine Leukocidin) and *tst-1* (toxic shock syndrome toxin 1) Genes were determined by PCR method. Strains were typed by *agr*, SCC*mec* typing, PFGE and *spa* typing.

Results: Forty-nine strains (36.3%) were CA. HA strains showed significantly higher rates of resistance than the CA strains. One HA strain was resistant to teicoplanin (MIC = 4 µgml⁻¹). The *pvl* gene was detected in 83.7% and 32.6% of CA and HA strains, respectively. Only eight strains were *tst-1* positive. PFGE revealed 61 pulsotypes among HA strains and 20 pulsotypes among CA strains. Twenty-four *spa* types were identified. *spa* type t044 was the most common, representing 69.4% and 25.6% among CA and HA strains respectively. Most of t044 strains was *pvl*-positive, harbored *agr3* and SCC*mec* IV and were resistant to kanamycin, tetracycline and fusidic acid. t037, *agr1* and SCC*mec* III was the most prevalent among HA-MRSA.

Conclusions: Genetically diverse MRSA strains were circulating in our hospitals with relatively high prevalence of *spa* type t044 and t037. Regular surveillance studies on MRSA are needed to monitor the evolution of antimicrobial susceptibility, to better elucidate the distribution of existing MRSA clones and to detect the emergence of new MRSA clones.

Keywords

Methicillin-Resistant *Staphylococcus aureus*, Epidemiology, *pvl*, *agr*, SCC*mec*, *spa* type

Introduction

Over the last six decades, methicillin-resistant *Staphylococcus aureus* (MRSA) spread over the whole

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world and has become a global public health threat. Primarily MRSA was restricted to hospitals (hospital-acquired MRSA, HA-MRSA) and generally affect patients with predisposing risk factors such as recent hospitalisation, presence of indwelling catheters, admission to intensive care unit, exposure to a patient who is colonised or infected with MRSA, prolonged antibacterial therapy and surgery. For the last three decades, MRSA could also be found outside the hospital, in the community (community-acquired MRSA, CA-MRSA) and generally affect healthy and younger people without such the aforementioned risk factors [1,2]. Moreover, HA-MRSA and CA-MRSA belong to distinct genetic lineages. HA-MRSA strains are mostly multidrug resistant and carry the larger staphylococcal cassette chromosome *mec* (SCC*mec*) types I,II,III, however CA-MRSA strains frequently carry smaller SCC*mec* elements, usually type IV and V, and are resistant to fewer classes of antimicrobials. Also, CA-MRSA strains are strongly associated with virulence factors such as Panton Valentine leukocidin (PVL) which is thought to contribute to their pathogenicity [1-3]. Importantly, in recent years, the distinction between HA-MRSA and CA-MRSA has become increasingly blurred. In fact, CA-MRSA strains have been increasingly identified as a cause of HA-infections. On the other hand, HA-clones have been described to cause CA-infections suggesting that certain clones have the ability to cross barriers between hospitals and the community [3-7]. Recently, another development was the emergence of so-called livestock-associated MRSA (LA-MRSA) linked with mainly pig farming and is therefore found in people with contact to animals. These strains mainly belonged to the sequence type (ST) 398 [8]. In 2011, a novel *mecA* homologue, mec_{LGA251}, named *mec*C located in a new SCC*mec* cassette designed SCC*mec* XI, has been reported from humans and animals in the Germany [9], UK and Denmark [10], Spain [11] and Austria [12]. These isolates mainly belonged to ST 130 [9-12].

Fortunately, whereas HA-MRSA isolates are generally multidrug resistant, CA and livestock-associated -MRSA tend to be resistant to fewer classes of antibiotics. The global emergence and spread of multidrug resistant MRSA limits the effectiveness of therapeutic options. Vancomycin has been the mainstay of treatment for MRSA infections and the emergence of resistance is rare and worrying [13,14].

Few studies have focused on molecular epidemiology of MRSA in Tunisia, so we conducted this retrospective multicenter study to investigate the molecular characteristics and the resistance profiles of MRSA causing community-acquired and hospital-acquired infections in Tunisia.

Material and Methods

Bacterial strains

From March 2011 to March 2012, 135 clinical non-duplicate consecutive MRSA strains recovered from patients in five Tunisian university hospitals were studied: Habib Bourguiba hospital of Sfax which drains the south of Tunisia and it is located 270 km from the capital, Tunis (49 strains), Fattouma Bourguiba hospital of Monastir located in the center, 160 km from Tunis (25 strains), Institute Kassab of Tunis (26 strains), Charles Nicolle hospital of Tunis (22 strains) and military hospital of Tunis (13 strains). For each strain, demographic and clinical informations were recorded. CA-MRSA infection was defined as a positive culture of MRSA in patients with no history of hospitalization, surgery or outpatient care, or alternatively, if the signs of infection were present on admission. HA-MRSA infection was assigned when the strain was obtained at least 78h after hospitalization and when the infection was not the reason for admission [1].

The strains were identified by conventional methods: Gram-positive cocci, catalase positive, mannitol fermenting, DNase-positive and producing clumping factor (staphyslide test, bioMérieux).

Antimicrobial susceptibility testing

The antibiotic susceptibilities of the strains were performed by a disc diffusion method on Mueller-Hinton agar (BioRad Laboratories, France) according to the CA-SFM criteria (http://www.sfm.asso.fr).

Methicillin resistance was confirmed by the detection of *mecA* gene by PCR as described elsewhere [15]. *S. aureus* ATCC 43300 was used as positive control (MRSA).

Minimal inhibitory concentrations (MICs) of vancomycin, teicoplanin, linezolid and tigecycline were determined by the broth microdilution method and were interpreted according to the CA-SFM criteria. Detection of heteroresistant vancomycin intermediate *S. aureus* strains (h-VISA) was performed by the Macromethod Etest on BHI agar for all of MRSA strains with a MIC ≥ 1 µgml^{-1} for vancomycin or teicoplanin, as described previously [16]. The strains h-VISA-Mu3 and Mu50 and the vancomycin-susceptible *S. aureus* ATCC 29213 were tested in parallel as positive and negative controls, respectively.

Molecular study

DNA extraction: Genomic DNA used for polymerase chain reaction (PCR) was extracted using Instagène Matrix (BioRad) according to the manufacturer's instructions.

Toxin gene detection: All strains were screened for the Panton Valentine Leukocidin, *pvl* (*lukS-PV,lukF-PV*) and toxic shock syndrome toxin 1, *tst-1* genes by PCR simplex, as described previously [17,18]. *S. aureus* ATCC 49775 and S19 were used as controls for detection of *pvl* and *tst-1* genes respectively.

Determination of *agr* groups: The identification

of *agr* groups was performed by multiplex PCR amplification of the hypervariable domain of the *agr* locus using primers specific for each of the four major specificity groups (forward primer, pan *agr*; and four reverse primers) [19]. *S. aureus* RN 6390 (*agr* group 1), RN 6607 (*agr* group 2), RN 8462/5 (*agr* group 3), and RN4850 (*agr* group 4) were used as controls for *agr* group identification.

SCC*mec* typing: The SCC*mec* types (I-IV) were detected by using the method described by Oliveira and de Lencastre [20]. Strains NCTC10442, N315, 85/2082, 4744, and WIS harboring, respectively, SCC*mec* type I-V were used as controls.

PFGE typing: PFGE of chromosomal DNA after *smaI* macrorestriction was performed using the GenePath system (Bio-Rad, France) as described previously [21]. PFGE patterns were compared by using Finger Printing 2 Software (Biorad). Similarity matrice and dendrograms were obtained using arithmetic average (UPGMA). Similarity coefficients were calculated according to the dice method. Strains clustering above 80% similarity were considered the same clone.

***spa* typing:** *spa* typing was performed as previously described [22]. The X region of the *spa* gene was amplified by PCR. Purified *spa* PCR products were sequenced. *spa* types were determined with the Ridom Staph Type Software (Ridom, Gmbh Würzburg, Germany) which automatically detects spa repeats and assigns a *spa* type according to http://spaserver.ridom.de/.

Statistical analysis

X^2 test was used to analyse the qualitative variables. A *p* value of < 0.05 was considered statistically significant.

Results

Among the 135 strains studied, 49 (36.3%) were CA and 86 (63.7%) were HA. Clinical characteristics of patients with MRSA infection according to the origin of infection are summarized in Table 1.

Antimicrobial resistance

The rates of resistance to antibiotics were low in CA-MRSA strains except for kanamycin (83.7%), tetracycline (75.5%), and fusidic acid (75.5%) (Table 2).

Vancomycin, teicoplanin, linezolid and tigecycline have demonstrated excellent *in vitro* activity against

Table 1: Demographic and clinical characteristics of patients with MRSA infection according to the origin of infection.

Characteristics	Total n = 135	CA-MRSA n = 49	HA-MRSA n = 86	*p -value*
Demographic informations				
Age (years)				
Mean	40.07	29.81	45.98	*< 0.001*
Range	01-85	01-77	01-85	
Sex ratio (M/F)	1.65	1.3	1.89	0.31
Diagnosis, No. (%) of strains				
Skin and soft tissue infections	69 (51.1)	38 (77.6)	31 (36)	*< 0.001*
Panaris	17	17	0	
Cutaneous abscesses	7	3	4	
Phlegmon	7	5	2	
Furuncle	3	3	-	
Cellulitis	1	1	-	
Wound infection	5	-	5	
Burn/ulcer	29	9	20	
Bacteremia	25 (18.5)	1 (2)	24 (27.9)	*< 0.001*
Respiratory tract infection	11 (8.1)	-	11 (10.8)	*0.008*
ORL infection	10 (7.4)	6 (12.2)	4 (4.6)	0.37
Bone and joint infection	7 (5.2)	1 (2)	6 (7)	0.23
Osteomyelitis	1	1	-	
Osteoarthritis	2	-	2	
Orthopedic implant infection	4	-	4	
Catheter related infection	6 (4.4)	-	6 (7)	0.03
Endocarditis	2 (1.4)	2 (4)	-	0.17
Urinary tract infection	2 (1.4)	-	2 (2.3)	0.5
Other	3 (2.2)	1 (2)	2 (2.3)	1

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Table 2: Resistance rates of community-acquired (CA) and Hospital-acquired (HA) MRSA in Tunisia.

| | No. (%) of strains | | | |
	Total (n = 135)	CA-MRSA (n = 49)	HA-MRSA (n = 86)	*P-value*
Kanamycin	110 (81.5)	41 (83.7)	69 (80.2)	*< 0.001*
Tobramycin	46 (34.1)	2 (4.1)	44 (51.2)	*< 0.001*
Gentamicin	41 (30.4)	1 (2)	10 (46.5)	*< 0.001*
Erythromycin	60 (44.4)	11 (22.4)	49 (57)	*< 0.001*
Lincomycin	32 (23.7)	1 (2)	31 (36)	*< 0.001*
Pristinamycin	4 (2.9)	0	4 (4.6)	0.5
Chloramphenicol	23 (17)	1 (2)	22 (25.6)	*< 0.001*
Tetracycline	102 (75.6)	37 (75.5)	65 (75.6)	1
Ofloxacin	47 (34.8)	5 (10.2)	42 (48.8)	*< 0.001*
Rifampicin	28 (20.7)	2 (4.1)	26 (30.2)	*< 0.001*
Trimethoprim-sulfamethoxazole	12 (8.9)	2 (4.1)	10 (11.6)	0.3
Fosfomycin	3 (2.2)	0	3 (3.5)	0.6
Fusidic acid	68 (50.4)	37 (75.5)	31 (36)	*< 0.001*

Table 3: MIC distributions and activities of vancomycin, teicoplanin, linezolid and tigecycline against the 135 MRSA strains.

| | No. of strains with MIC (μg ml^{-1}) | | | | | | | MIC$_{50}$ | MIC$_{90}$ | % S |
	0.064	0.125	0.25	0.5	1	2	4			
Vancomycin	0	0	2	59	72	2	0	1	1	100
Teicoplanin	0	2	45	51	28	8	1	0.5	1	99.2
Linezolid	0	0	1	12	55	59	8	1	2	100
Tigecycline	17	46	43	28	1	0	0	0.25	0.5	99.2

MIC$_{50/90}$, minimum inhibitory concentration which 50%, 90%, respectively of the strains were inhibited.
S: Susceptible.

MRSA strains (Table 3). Only one HA-MRSA and multidrug resistant strain was classified glycopeptid-intermediate *S. aureus* (GISA) by MIC (vancomycin MIC = 1 μgml^{-1} and teicoplanin MIC = 4 μgml^{-1}) and h-VISA by Macromethod Etest (vancomycin MIC = 12 μgml^{-1} and teicoplanin MIC = 24 μgml^{-1}). This strain was isolated from blood of a 26-years-old patient with nosocomial bacteremia acquired in an intensive care unit who failed teicoplanin and vancomycin therapy. Only one CA-MRSA strain was resistant to tigecycline (MIC = 1 μgml^{-1}). This strain caused skin and soft tissue infections (SSTI).

## Molecular characteristics and genetic relatedness of strains

Among the 135 strains studied, 131 (48 CA-MRSA and 83 HA-MRSA) were typeable by *smaI* macrorestriction. Employing a cut off similarity value of 80% in subsequent cluster analysis, we assigned the HA-MRSA strains to 61 different PFGE patterns. HA-MRSA strains belonged to the same pattern were isolated in different hospitals. In addition, there is no dominant clone suggesting that these strains were not closely related (Figure 1). However, twenty different pulsotypes were identified among the 48 CA-MRSA strains. Three patterns identified in different hospitals were dominant: pattern 9 (n = 7), pattern 10 (n = 8) and pattern 11 (n = 8) (Figure 2).

All MRSA strains carried *mecA* gene, which was detected by the PCR assay. The *pvl* gene was detected in 69 (51.1%) strains, 41 (83.7%) among CA-MRSA and 28 (32.6%) among HA-MRSA. Forty-five (69.6%) of *pvl*-positive MRSA strains were obtained from SSTI especially panaris (n = 17), phlegmon (n = 7), cutaneous abscesses (n = 5) and furuncle (n = 2).

The molecular characteristics (*spa* types, *agr* groups, SCC*mec*, presence of *pvl* gene, along with the drug resistance pattern of the most predominant types according to the origin of case (CA or HA) are summarized in Table 4.

Thirteen *spa* types were identified among CA-MRSA strains. The most common *spa* type was t044, 34 (69.4%). A combination of the molecular typing results revealed that thirty-two (78%) among *pvl*-positive CA-MRSA strains, belonged to *spa* type t044, have *agr3*, and most (n = 27) harbored SCC*mec* IV. These strains were often resistant to kanamycin, tetracycline and fusidic acid (Table 4). All of these characteristics were consistent with those of the European clone ST80. PFGE typing showed that among t044 strains, 41.7% were grouped into three dominant patterns (Figure 2).

Among HA-MRSA strains, 18 *spa* types were identified. The most prevalent were t037 (n = 25, 29.1%), followed by t311 (n = 7, 9.1%) and t052 (n =
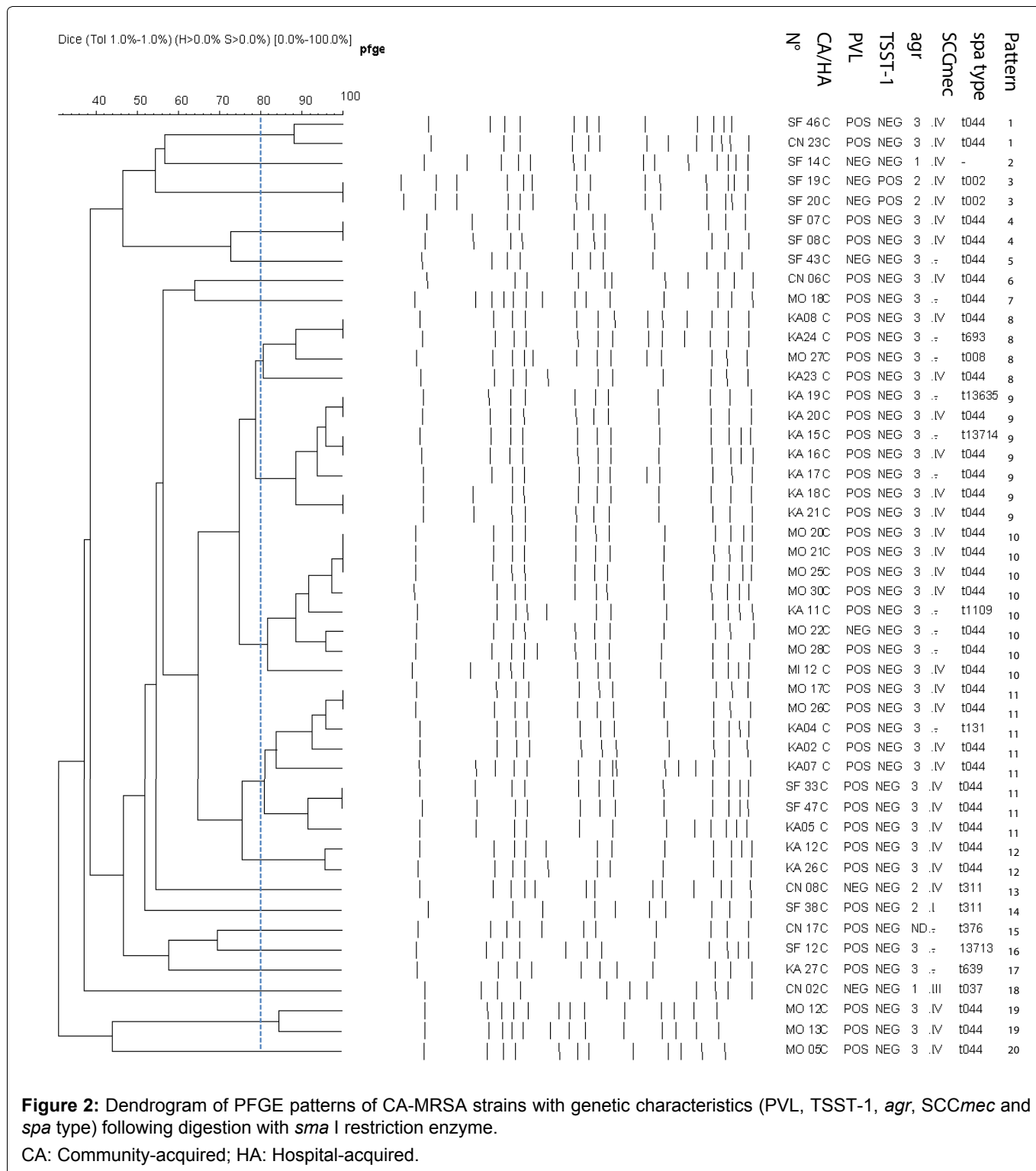
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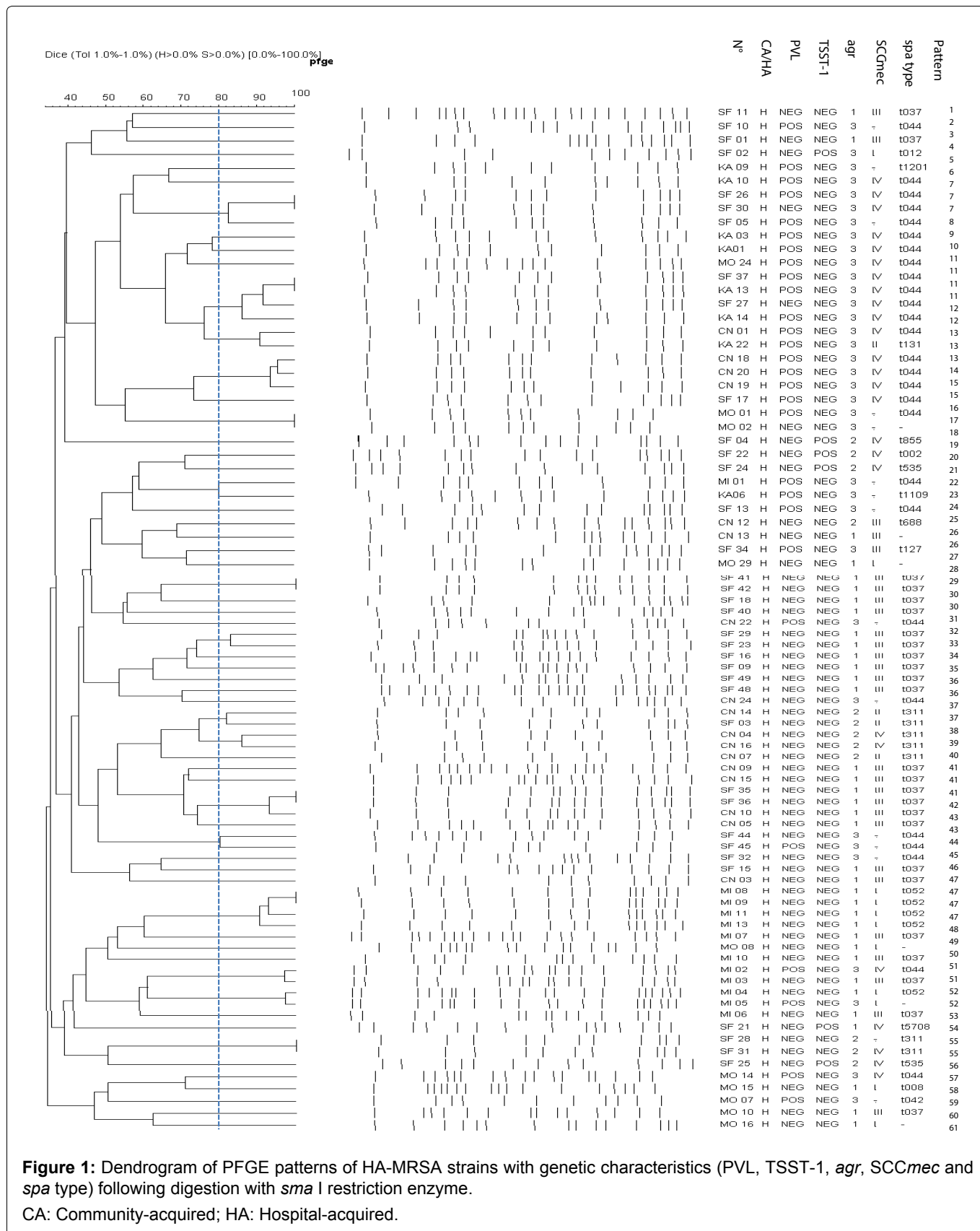
Figure 1: Dendrogram of PFGE patterns of HA-MRSA strains with genetic characteristics (PVL, TSST-1, *agr*, SCC*mec* and *spa* type) following digestion with *sma* I restriction enzyme.

CA: Community-acquired; HA: Hospital-acquired.

5, 5.8%) among *pvl*-negative strains and t044 (n = 22, 25.6%) among *pvl*-positive strains. Further molecular characterization showed that all of the strains belonging to *spa* type t037 had *agr1* and carried SCC*mec* III. In term of drug resistance, all of them exhibited resistance to kanamycin, tobramycin, gentamicin and tetracycline (Table 4). These characteristics were consistent with the ST239/247 clone.

Of the strains tested, only eight (5.9%) were *tst-1* positive. They caused a variety of clinical syndromes and were associated to both CA infection (two endocarditis) and HA infection (three SSTI, two prosthesis infections and one bacteremia). These strains were not multidrug resistant: resistant to fusidic acid (n = 5), tetracycline (n = 1) and trimethoprim + sulfamethoxazole (n = 1) and were not closely related after analysis by PFGE. Four *spa*

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Dice (Tol 1.0%-1.0%) (H>0.0% S>0.0%) [0.0%-100.0%] pfge

N° CA/HA	PVL	TSST-1	agr	SCCmec	spa type	Pattern
SF 46 C	POS	NEG	3	.IV	t044	1
CN 23 C	POS	NEG	3	.IV	t044	1
SF 14 C	NEG	NEG	1	.IV	-	2
SF 19 C	NEG	POS	2	.IV	t002	3
SF 20 C	NEG	POS	2	.IV	t002	3
SF 07 C	POS	NEG	3	.IV	t044	4
SF 08 C	POS	NEG	3	.IV	t044	4
SF 43 C	NEG	NEG	3	.=	t044	5
CN 06 C	POS	NEG	3	.IV	t044	6
MO 18 C	POS	NEG	3	.=	t044	7
KA08 C	POS	NEG	3	.IV	t044	8
KA24 C	POS	NEG	3	.=	t693	8
MO 27 C	POS	NEG	3	.=	t008	8
KA23 C	POS	NEG	3	.IV	t044	8
KA 19 C	POS	NEG	3	.=	t13635	9
KA 20 C	POS	NEG	3	.IV	t044	9
KA 15 C	POS	NEG	3	.=	t13714	9
KA 16 C	POS	NEG	3	.IV	t044	9
KA 17 C	POS	NEG	3	.=	t044	9
KA 18 C	POS	NEG	3	.IV	t044	9
KA 21 C	POS	NEG	3	.IV	t044	9
MO 20C	POS	NEG	3	.IV	t044	10
MO 21C	POS	NEG	3	.IV	t044	10
MO 25C	POS	NEG	3	.IV	t044	10
MO 30C	POS	NEG	3	.IV	t044	10
KA 11 C	POS	NEG	3	.=	t1109	10
MO 22C	NEG	NEG	3	.=	t044	10
MO 28C	POS	NEG	3	.=	t044	10
MI 12 C	POS	NEG	3	.IV	t044	10
MO 17C	POS	NEG	3	.IV	t044	11
MO 26C	POS	NEG	3	.IV	t044	11
KA04 C	POS	NEG	3	.=	t131	11
KA02 C	POS	NEG	3	.IV	t044	11
KA07 C	POS	NEG	3	.IV	t044	11
SF 33 C	POS	NEG	3	.IV	t044	11
SF 47 C	POS	NEG	3	.IV	t044	11
KA05 C	POS	NEG	3	.IV	t044	11
KA 12 C	POS	NEG	3	.IV	t044	12
KA 26 C	POS	NEG	3	.IV	t044	12
CN 08C	NEG	NEG	2	.IV	t311	13
SF 38C	POS	NEG	2	.I	t311	14
CN 17C	POS	NEG	ND	.=	t376	15
SF 12 C	POS	NEG	3	.=	13713	16
KA 27C	POS	NEG	3	.=	t639	17
CN 02C	NEG	NEG	1	.III	t037	18
MO 12C	POS	NEG	3	.IV	t044	19
MO 13C	POS	NEG	3	.IV	t044	19
MO 05C	POS	NEG	3	.IV	t044	20

Figure 2: Dendrogram of PFGE patterns of CA-MRSA strains with genetic characteristics (PVL, TSST-1, *agr*, SCC*mec* and *spa* type) following digestion with *sma* I restriction enzyme.

CA: Community-acquired; HA: Hospital-acquired.

types were identified, t002 possessed *agr2* and SCC*mec* IV (n = 3); t535, *agr2,* SCC*mec* IV (n = 2); t012, *agr3*, SCC*mec* I (n = 1) and t5708, *agr1*, SCC*mec* IV (n = 1).

Discussion

MRSA strains continue to be isolated from both healthcare-and community-associated infections in different parts of the world [3]. The increase in the number of MRSA infections reported worldwide has been accompanied by changes in the characteristics of MRSA strains emerging in different parts of the world. Consequently, epidemiological typing using a combination of phenotypic and genotypic typing methods has contributed for understanding the evolution and dissemination of MRSA clones [15,23]. To date, few studies have been focused on the phenotypic and genotypic characteristics of MRSA strains in Tunisia [24,25]. This is the first multicenter study of molecular epidemiology focusing on CA and HA MRSA in Tunisian hospitals. The rate of MRSA in Tunisia was relatively low: about 20%. However, a slightly increase was showed (18.4 in 2004; 22.7% in 2014) [26,27].

In our study, 36.3% of MRSA strains caused CA-infection. The prevalence of CA-MRSA varies geographically; with detection rate remain low in

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Table 4: Molecular characteristics of MRSA strains isolated in Tunisia.

	spa type	agr group	SCCmec type	Non-ß-lactam resistance
CA-MRSA (n = 49)				
PVL positive (n = 41)	t044(32)	3(32)	IV(27), NT(5)	KAN(29), TE(27), TIG (1), ERY(5), Lin(1), FUC(28), OFL(2), RA(1)
	t311(1), t376(1), t131(1), t693(1), t1109(1), t639(1), t13635(1), t13713(1), t13714(1)	3(6), 2(2), NT(1)	IV(4), I(1), NT(4)	KAN(9), TE(8), ERY(4), FU (7), OFL(1), C(2)
PVL negative (n = 8)	t002(2)	2(2)	IV(2)	FUC(1)
	t044 (2)	3(2)	NT(2)	KAN(2), ERY(1), FUC(1), OFL(1)
	t037(1)	1(1)	III(1)	KAN(1), TOB(1), GEN(1), TE (1), ERY(1), OFL(1), RA(1), SXT(1)
	t899(1), t311(1), ND(1)	1(2), 2(1)	IV(1)	TE(1), SXT(1)
HA-MRSA (n = 86)				
PVL positive (n = 28)	t044(22)	3(22)	IV(15), NT(7)	KAN(20), TOB(1), GEN(1), TE(16), ERY(9), LIN(2), PT(1), FUC(16), OFL(2), RA(3), CHL(2)
	t42(1), t127(1), t131(1), t1109(1), t1201(1), ND(1)	3(6)	I(1), II(1), III(1), IV(1), NT(2)	KAN(5), TOB(2), GEN(2), TE(5), ERY(4), LIN(1), FUC(4), OFL(2), R (2), FOS(1), SX (1)
PVL negative (n = 58)	t037(25)	1(25)	III(25)	KAN(25), TOB(25), GEN(25), TE(25), ERY(22), LIN(16),FUC(1), OFL(23), RA(8), FOS(1), SX (5), C(19)
	t311(7)	2(7)	II(4), IV(1), NT(1)	KAN(7), TOB(7), GE (3), TE(7), ERY(4), LIN(3), PT(3), FUC(3), OFL(3), RA(3)
	t052(5)	1(5)	I(5)	KAN(5), TOB(5), GEN(5), TE(5), ERY(5), LIN(5), OFL(5), RA(5)
	t044(5)	3(5)	IV(2)	KAN(5), TOB(2), GEN(2), TE(3), ERY(1), LIN(1), FU (3), OFL(1), RA(2)
	t535(2)	2(2)	IV(2)	TE(1), FUC(2)
	t002(1), t008(1), t012(1), t688(1), t855(1), t899(1), t4358(1), t5708(1), ND(6)	1(9), 2(3), 3(2)	I(6), III(2), IV(4), NT(2)	KAN(2), TOB(2), GEN(2), TE(5), ERY(4), LIN(3), FUC(2), OFL(6), R (3), FOS(1), SXT(4), C(1), TEC(1)

ND: Not Detected; NT: Non Typeable.

MRSA: Methicillin Resistant *Staphylococcus aureus*; PVL: Panton-Valentine Leukocidin; CA: Community Acquired; HA: Hospital Acquired; *spa* type: Staphylococcal Protein A Type; KAN: Kanamycin; TOB: Tobramycin; GEN: Gentamicin; TE: Tetracycline; TIG: Tigecycline; ERY: Erythromycin; LIN: Lincomycin; PT: Pristinamycin; FUC: Fusidic acid; OFL: Ofloxacin; RA: Rifampicin; FOS: Fosfomycine; SXT: Trimethoprim-Sulfamethoxazole; C: Chloramphenicol; TEC: Teicoplanin.

most European countries: ranges from less than 1% in Switzerland to less than 10% in Austria and 7% in Russia [28,29]. This rate is highest in other countries such as Colombia (12.6%) [30], Kuwait (17%) [31], Algeria (34.3%) [32], Saudi Arabia (59%) [31] and Argentina (62.9%) [7]. CA-MRSA strains predominantly infect young and previously healthy patients. They cause mainly SSTI and some strains are particularly virulent and induce life-threatening infections such as necrotizing pneumonia or invasive osteomyelitis [2,3,7,31]. In our study, CA-MRSA strains were more frequently isolated from younger patients than HA-MRSA strains (*p* < 0.001). SSTI were significantly predominated in CA-MRSA infections (77.6% versus 36%; *p* < 0.001). However, bacteremia, respiratory tract infections and catheter related infections were significantly related with HA-infections.

Antimicrobial susceptibility testing is a crucial step in selection of the appropriate antibiotic for treatment. Surprisingly, the rates of resistance to tobramycin, gentamicin, rifampicin and trimethoprim-sulfamethoxazole among MRSA strains found in the present study were lower than that reported in previously studies in Tunisia [26,27]. This event could be explained by the emergence of CA-MRSA strains in our hospitals.

Our Study showed a low rate of resistance to antibiotics in CA-MRSA strains except for kanamycin (83.7%), tetracycline (75.5%), and fusidic acid (75.5%). Only 2% of the CA-MRSA strains were resistant to lincomycin. Then, lincosamides (clindamycin and lincomycin) remains a useful option for outpatient treatment. HA-MRSA is generally multidrug resistant [33]. In our study, more than 45% of HA-MRSA strains were resistant to aminosides, erythromycin, ofloxacin, tetracycline and fusidic acid (Table 2). Four strains (2.9%) were resistant to pristinamycin. In Tunisia, this resistance was rare [26,27] and first emerged among *S. aureus* in 2007 in Sfax university hospital [34].

In our study, only one HA-MRSA and multidrug resistant strain (0.8%) isolated from blood was classified as GISA by MIC, and h-VISA by macroEtest method. In Tunisia, GISA strains were uncommon [26,27,35]. Previous studies have demonstrated that h-VISA/GISA are prevalent among bacteremic specimens, and that these strains can persist in the blood stream for a long time [14]. In the literature, the incidence of GISA is low [13,14,36]. However, the rate of h-VISA among MRSA strains varied from countries (13% in Australia, 32% in Turkey, 11% in France and 7% in China) [14,37] and was parallels with the increase of vancomycin MIC level [38].

Vancomycin resistant *S. aureus*, due to the acquisition of the *vanA* gene from enterococci are currently very low [36,39,40]. Although the prevalence of GISA is very low in our country (0.8% among MRSA), a continuous surveillance of susceptibility to glycopeptides is necessary particularly among MRSA strains.

Systematic review and meta-analysis of genetic backgrounds of h-VISA/GISA demonstrated that the majority of these strains harbored SCC*mec* II or III, rarely SCC*mec* I and IV and belonged to ST239 and ST5 [14,40,41]. In our study, the h-VISA strain contained SCC*mec* I and was not typeable by *spa* typing.

Linezolid and tigecycline have demonstrated excellent *in vitro* activity against *S. aureus* including MRSA. Only one strain was resistant to tigecyclin (MIC = 1 µgml^{-1}). Thus, they constitute a therapeutic alternative in severe infections especially due to GISA or h-VISA strains [33,42,43]. However, emergence of linezolid resistance in *S. aureus* has been reported in USA, in Europe and in Asia. This resistance was observed following exposure to linezolid [44-46].

A high rate of *pvl*-positive MRSA (50.4%) was found in our study especially in the community (83.7%). This finding has been reported in other studies [28,32,47-51]. *pvl* gene was strongly associated with SSTI especially panaris, phlegmon and cutaneous abscesses. In the literature, although the majority of MRSA-*pvl* positive infections are SSTI, sever life-threatening cases of necrotizing pneumonia and necrotizing fasciitis have been reported [1,3]. Fifty-four (78.3%) of the *pvl*-positive MRSA strains have characteristics of CA-MRSA European clone ST80-IV (*spa* type t044, *agr3*, SCC*mec* IV, *pvl* +). This clone accounted for 65.3% of CA-MRSA and 25.6% of HA-MRSA, thus proving its current emergence as an HA-pathogen.

CA-MRSA is particularly well established in the USA. Although USA400 (*pvl*-positive ST1 SCC*mec* IV) strains were responsible for initial cases, USA300 (*pvl*-positive ST8-IV) is now the predominant cause of North American infection and has begun to replace health-care-associated strains [5,23,28,31,52]. By contrast, USA300 is uncommon in Europe, where CA-MRSA is characterized by clonal diversity [48,50,53]. The most common European CA-MRSA clone is *pvl* positive ST80-IV, which have a characteristic antimicrobial susceptibility pattern of resistance to kanamycin, tetracycline and fusidic acid. European clone ST80 was reported in Greece since 2003, the country with the highest CA-MRSA incidence in Europe, where it is responsible for the majority of both CA-MRSA and HA-MRSA infections [53]. The European clone has been reported in many European countries [28,48,53], however, it is not very common in Slovenia [54]. CA-MRSA ST80 clone has been also identified in other countries from Africa, particularly in Algeria [32,55] and in the Middle East such as Kuwait, Lebanon, Jordan and Egypt [49,51].

Since their emergence, CA-MRSA ST80 strains were susceptible to several antibiotics and are usually resistant to kanamycin, tetracycline and fusidic acid [28]. However, new strains appeared to develop resistance to other antibiotics. In our study, among the CA-MRSA strains identified, we found multidrug resistant *pvl*-positive strains, showing resistance to other antibiotics such as rifampicin, gentamicin and ofloxacin as in the Algiers study [32]. These results prove that CA-MRSA are able to expand their resistance profiles in hospital setting where volumes of antimicrobial consumption are higher than in the community.

In recent years, several CA-MRSA clones have arisen in Europe, most notably the LA-MRSA, CC398, which was first detected in the Netherlands and Denmark in 2003 [8]. MRSA CC398 has been reported mostly in Europe and also in Asia and in the USA. It accounted for only a small proportion ranging from 0 to 25% of human MRSA isolates depending on regional differences in livestock density [5,8]. Methicillin-susceptible *S. aureus* belonging to CC398 have also been reported from animals and humans and have been associated with CA and HA infections in humans, many without livestock contacts [56-58]. Studies have revealed that LA-MRSA CC398 originated in humans as methicillin-susceptible *S. aureus* and then spread to livestock, where it acquired resistance to methicillin and tetracycline [57-58]. To data, in Tunisia, only one MRSA ST398-*spa* type t899 isolate in the nasal sample of a farmer patient was reported, representing the first report of ST398 in humans in North Africa in our knowledge [59].

Among HA-MRSA strains, twenty-five (29.1%) have the following characteristics: *pvl*-negative, *spa* type t037, SCC*mec* III and *agr1*. These strains were related to the Brazilian/Hungarian clone of HA-MRSA ST239 and ST like (ST239/240/241). This clone is an epidemic clone responsible for several HA-MRSA outbreaks. It has been found to be a cause of HA-infection in African countries including Algeria [55], Ghana, Morocco, South Africa, Nigeria and Kenya [23,60-62], and has been reported in the USA, Europe, Austria [63], Russia [29], Turkey [41], Asia, Argentina [7], China [64], and Romania [50]. This clone was also identified in the Middle East [65-68]. In fact, its broad distribution may be due to its advantageous properties with respect to other clones, such as an enhanced ability to form biofilm and a tendency to acquire genes that confer resistance to different classes of antimicrobial agents [29]. In this study, all of the HA-MRSA t037 strains were multidrug resistant, which is in agreement with previous reports [7,29,41,55,60-65].

Other *spa* types were also detected in our study such as t311 (n = 9) and t052 (n = 5) especially among HA-MRSA, *pvl* negative strains. t311 was also detected in CA and HA MRSA strains in previous study conducted in Military hospital of Tunis [59] and in some African towns

in Morocco, Cameroun and Senegal [61]. ST5-II/t311 is a major prevalent clone that is widespread in a tertiary hospital in China (45.8%) [64], and in Argentina [4,7]. In our study, the five t052 strains were isolated from the military hospital of Tunis. All of them harbored SCC*mec* I and *agr1* and showing the same profile (kanamycin, tobramycin, gentamicin, tetracycline, erythromycin, lincomycin, ofloxacin and rifampicin resistance). These strains were related to ST235/247 clone. This finding has also been reported in a previous study conducted in the military hospital of Tunis [59].

We also identified other minor *spa* types amongst CA-MRSA and HA-MRSA accounting for 2% each (one strain) such as t002, t008, t131, and t376 which have only one repetition of difference with *spa* type t044. These *spa* types were also described in many parts of World accounting for less than 10% among MRSA [4-7,30,52,64,69]. *spa* type t012, t127, t688 and t535 were rarely reported [6,33,64,66].

In our study, the *tst-1* gene was detected in only 8 strains (5.9%). There have been few reports of MRSA isolates producing *tst-1* in Japan, Germany and in France [70]. However, high prevalence of *tst-1* gene among MRSA was found in Gaza hospitals (27.4%) [66] and in Kuwait hospitals (62.2%) [71]. *tst*-1 positive MRSA strains appear to be highly virulent and to cause a variety of illness, ranging from toxic shock syndrome to various suppurative infections [72] such as SSTI, pneumonia, osteoarthritis [70-73]. Few cases of endocarditis have been reported [74,75]. In our study, three patients had SSTI, two had prosthesis infection, two had endocarditis and one bacteremia. The *tst-1* positive MRSA belonged to two clones: a major clone ST5 Geraldine clone, mainly *spa* type t002, and *agr2*, SCC*mec* IV, rarely SCC*mec* I, and one minor clone ST30, *agr3*, SCC*mec* IV [70]. However, in the middle East, other clone has been identified among *tst-1* positive strains, ST22, SCC*mec* IV, t233 (middle Eastern variant) in Kuwait and Gaza hospitals [66,71]. In our study, only three strains have the characteristics of the Geraldine clone. One strain t5708, SCC*mec*, *agr1* was also detected in Kuwait [71].

In conclusion, we reported that *pvl*-positive MRSA *spa* type t044 was spreading in both the community and hospital setting in different city of Tunisia. These strains have the characteristics of the European clone ST80 and were susceptible to most of the antistaphylococcal antibiotics and usually resistant to kanamycin, tetracycline and fusidic acid. However, the risk that multidrug resistant MRSA strains will spread to the community in Tunisia is a worrying prospect. *spa* type t037 clone which was multidrug resistant was found to be dominant in HA-infection. These findings support the need for measures designed to limit the spread of both CA and HA-MRSA, together with regular and systematic surveillance of MRSA infections in our country.

Conflicts of Interest

There is no conflict of interest.

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