AKADÉMIAI KIADÓ

Spa diversity and genetic characterization of t127 methicillin-resistant *Staphylococcus aureus* in a tertiary Greek hospital

Acta Microbiologica et Immunologica Hungarica

69 (2022) 3, 185–192

DOI:
10.1556/030.2022.01825

KATERINA TSERGOULI[1],
THEODOROS KARAMPATAKIS[1*],
KONSTANTINA KONTOPOULOU[2], STYLIANI PAPPA[1],
PARTHENA KAMPOURIDOU[3], GEORGIA KALLASIDOU[3],
KATERINA TSIOKA[1], SOPHIA ZOTOU[2],
ELEFTHERIA - EUGENIA FARMAKI[2],
CHARALAMPOS KOTZAMANIDIS[4] and ANNA PAPA[1]

[1] Department of Microbiology, Medical Faculty, School of Health Sciences, Aristotle University of Thessaloniki, Greece

[2] Department of Microbiology, "Georgios Gennimatas" General Hospital of Thessaloniki, Greece

[3] Department of Pediatrics, "Georgios Gennimatas" General Hospital of Thessaloniki, Greece

[4] Veterinary Research Institute of Thessaloniki, Greek Agricultural Organization-Dimitra, Thermi, Greece

RESEARCH ARTICLE

Received: July 11, 2022 • Accepted: July 26, 2022
Published online: August 29, 2022

ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) causes severe community and hospital acquired infections. Identification of staphylococcal cassette chromosome *mec* (SCC*mec*), multilocus-sequence typing, and sequencing of *S. aureus* protein A (spa) gene are used for MRSA typing. The aim was to investigate the *spa* types of MRSA isolates in a tertiary hospital in Greece and analyse the whole genome sequences of two t127 MRSA isolates. *Methods:* Totally, 39 MRSA isolates collected from July 2019 to June 2020 in "Georgios Gennimatas" General Hospital of Thessaloniki, Greece, were included in the study. Identification and antimicrobial susceptibility testing were performed using VITEK II automated system, and *spa* typing was performed. A minimum spanning tree was used to display the *spa* type frequencies and the genetic distances among them. Two t127-MRSA isolates (IM-MRSA and PD-MRSA) were selected for WGS. *Results:* Six isolates (15.4%) were resistant to mupirocin, 18 (46.2%) to fusidic acid, three (7.7%) to vancomycin and two (5.1%) to teicoplanin. Twenty-two different *spa* types were detected, with t002, t003, and t422 being the most frequent (5/39, 12.8% each), followed by t1994 (4/39, 10.3%). The isolates presented high genetic diversity and, taking into account the time between hospital admission and sampling, intrahospital spread did not occur. Even the two t127 isolates were assigned to different sequence types, ST9-XII-t127 and ST1-IVa-t127. Plasmids and genes conferring antimicrobial resistance and virulence were also identified. *Conclusions:* Various *spa* types were identified and together with the information about the time between hospital admission and sampling supports polyclonal MRSA spread in the hospital excluding a nosocomial infection. WGS provides a more detailed analysis distinguishing even the isolates belonging to the same *spa* type.

KEYWORDS

Methicillin-resistant, *Staphylococcus aureus*, whole-genome sequencing, *spa* typing

*Corresponding author. Department of Microbiology, Medical Faculty, School of Health Sciences, Aristotle University of Thessaloniki, GR-541 24, Thessaloniki, Greece.
E-mail: tkarampatakis@yahoo.com

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium causing community-acquired and nosocomial infections worldwide [1, 2]. According to the latest epidemiological report of the European Centre for Disease Prevention and Control (ECDC), the prevalence of MRSA in Greece is among the highest in Europe, reaching 37.6% in 2019 [3].

Various methods are used for MRSA typing, such as the identification of staphylococcal cassette chromosome *mec* (SCC*mec*), the multilocus-sequence typing (MLST), and the sequencing of the highly polymorphic repeat region of *S. aureus* protein A (*spa*) gene [4–6]. The detection of *spa*-clusters could be used as a rapid tool for the study of MRSA epidemiology in a hospital and for separation between relapse and re-infection [7].

Previous studies in Greece showed that *spa* types t044, t003 and t037 are the predominant types among MRSA isolated from clinical sources [8, 9]. In Europe, the most prevalent are the spa-types t032, t008 and t067, while t011, t108 and t034 predominate among livestock-associated (LA)-MRSA [10]. LA-MRSA were first detected in 2003 in pigs [11], while later, they have been isolated also in patients with no previous contact with livestock [12]. Knowledge of MRSA epidemiology is of great importance in organising and implementing infection control measures, especially in hospital settings [13]. Over the last decade, whole-genome sequencing (WGS) is an added value for the investigation of outbreaks caused by MRSA [14]. Although limited, there are reports in Greece based on whole genome MRSA sequences [15–18].

The aim of the present study was to evaluate the antimicrobial resistance patterns and the distribution and prevalence of *spa* types of MRSA isolated from patients hospitalized in various wards of a tertiary hospital in Greece, and to further characterize the whole genome sequences of two isolates belonging to *spa* type t127, which is often associated with LA-MRSA.

MATERIAL AND METHODS

Bacterial isolates

Thirty-nine MRSA isolates collected during one-year period (July 2019 to June 2020) from 39 patients (18 males, 46%) hospitalized in various wards of “Georgios Gennimatas” General Hospital of Thessaloniki in Greece, were included in the study. The median age of the patients was 59 years (range 0.25–91 years). The isolates were recovered from wound (18, 46.2%), ear swab (five, 12.8%), blood, throat and nasal swabs (four, 10.3% each), central intravenous catheter (two, 5.1%), urine and eye swab samples (one, 2.5%, each) (Table 1). Eight isolates (20.5%) were taken through testing for colonization, while 29 (79.5%) were collected from infection sites. The distinction between infection and colonization was performed using previously described criteria [19]. The time between patients’ admission to the hospital and sampling was estimated in order to associate or not with nosocomial infection (>48 or <48 h, respectively) (Table 1).

Microbiological methods

All samples were cultured in blood agar, and strain identification and antimicrobial susceptibility testing were performed using the GP ID and AST-P659 cards in VITEK II automated system, respectively (BioMérieux, Marcy-l’Étoile, France). The minimum inhibitory concentration (MIC) was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints reported in January 2019 [20]. MRSA isolates were defined as resistant to oxacillin [21]. Cefoxitin-screen test in VITEK 2 was used as a surrogate marker for the detection of methicillin resistance [22].

DNA extraction – Spa typing

DNA was extracted using the DNA extraction kit (Qiagen, Hilden, Germany). The *spa* gene was amplified and sequenced. Typing was performed through the Rindom Spa server (spaserver.ridom.de). A minimum spanning tree (MST) was generated using the *spa*-clustering method of the *spa*-typing plugin of BioNumerics v.7.1 software (Applied Maths, Sint-Martens-Latem, Belgium), which is connected to the SeqNet/Ridom *Spa* Server (https://www.spaserver.ridom.de/).

Whole genome sequencing - Bioinformatic analysis

Since t127 is often related with LA-MRSA, two t127 isolates [one collected from a patient hospitalized in internal medicine (IM-MRSA), and a second one from a patient hospitalized in the pediatric ward (PD-MRSA)] were selected for WGS analysis. WGS was performed on Ion Torrent PGM Platform (Life Technologies Corporation, Grand Island, NY, USA). All procedures were conducted according to manufacturer’s guidelines. PCR products were loaded on Ion-316^{TM} chip kit V2BC. The Ion PGM Hi-Q (200) chemistry (Ion PGM Hi-Q Sequencing kit, A25592) was applied.

The consensus sequence was taken using *S. aureus* strain WHC09 (GenBank accession number CP077755) as reference sequence. MLST analysis was performed using the web-based MLST-DTU tool [23]. Resfinder version 4.1 and the Comprehensive Antibiotic Resistance Database (CARD) were used for the detection of antimicrobial resistance genes [24, 25], while the virulence genes were detected using the Virulence Finder v. 2.0 software [26]. The SCC*mec* elements of the isolates were identified through the SCC*mec*Finder database version 1.2 [4]. PlasmidFinder version 2.1 was used for the identification of plasmids [27].

RESULTS

Antimicrobial resistance

The antimicrobial resistance patterns are seen in Table 1. Specifically, 18 isolates (46.2%) displayed resistance to fusidic acid, 16 (41%) to levofloxacin, seven (17.9%) to tetracycline, six (15.4%) to mupirocin, five (12.8%) to trimethoprim/sulfamethoxazole, three (7.7%) to vancomycin and two (5.1%) to teicoplanin. Thirty-seven isolates (94.9%) were resistant to cefoxitin (cefoxitin-screen positive); two isolates (5.1%) were resistant to cefoxitin and sensitive to oxacillin, while one isolate (2.6%) was sensitive to cefoxitin and resistant to oxacillin.

Table 1. Characteristics of the 39 MRSA strains included in the study

ID	Sex	Age (years)	Sample	Colonization/ Infection	Ward	Isolation Date	>48h	Spa type	Antimicrobial Resistance Profile
1	F	59	CVC	Infection	Cardiology	May 2020	YES	t653	Ox-Fox-Fu-E
2	F	45	urine	Infection	ER	Apr 2020	NO	t442	Ox-Fox-Fu-Le-Mu-Sxt-G
3	F	72	throat swab	Colonization	ICU	May 2020	NO	t2986	Ox-Fox
4	F	81	throat swab	Colonization	ICU	Jun 2020	NO	t008	Ox-Fox-Le-E
5	F	70	throat swab	Colonization	Internal medicine	Sept 2019	NO	t422	Ox-Fox-Fu-Le-E
6	M	78	blood	Infection	Internal medicine	Dec 2019	NO	t267	Ox-Fox-Fu
7	M	91	blood	Infection	Internal medicine	Jan 2020	NO	t328	Ox-Fox-Le
8	F	82	throat swab	Colonization	Internal medicine	Apr 2020	NO	t003	Ox-Fox-Fu-Le-Tet-E
9	M	84	wound	Infection	Internal medicine	Apr 2020	NO	t127	Ox-Fox-Tet-E
10	M	75	wound	Infection	Internal medicine	Jun 2020	NO	t002	Ox-Fox-Le-E
11	F	55	nasal swab	Colonization	Orthopaedic	Jul 2019	YES	t5452	Ox-Fox-E
12	F	86	wound	Infection	Orthopaedic	Nov 2019	YES	t003	Ox-Fox
13	M	62	wound	Infection	Orthopaedic	Nov 2019	YES	t002	Ox-Fox
14	M	84	wound	Infection	Orthopaedic	Dec 2019	NO	t003	Ox-Fox-Fu-Le
15	M	79	nasal swab	Colonization	Orthopaedic	Jan 2020	YES	t002	Ox-Fox
16	F	65	wound	Infection	Outpatient	Sept 2019	NO	t422	Ox-Fox-Fu-Le-Mu-Sxt-E-G
17	M	70	wound	Infection	Outpatient	Oct 2019	NO	t440	Ox-Fox-Va-Teic
18	F	47	ear swab	Infection	Outpatient	Oct 2019	NO	t084	Fox-Sxt
19	F	28	ear swab	Infection	Outpatient	Oct 2019	NO	t422	Ox-Fox-Fu-Le
20	F	0.5	wound	Infection	Outpatient	Jan 2020	NO	t328	Ox-Fox
21	M	0.42	wound	Infection	Outpatient	Jan 2020	NO	t1814	Ox-Fox-Sxt
22	M	0.25	ear swab	Infection	Outpatient	Apr 2020	NO	t131	Ox-Fox-Fu-Tet
23	F	73	ear swab	Infection	Outpatient	May 2020	NO	t422	Ox-Fox-Le-E
24	F	7	nasal swab	Colonization	Outpatient	Jun 2020	NO	t1994	Ox-Fox-Fu-Mu-E
25	M	11	ear swab	Infection	Outpatient	Jun 2020	NO	t012	Ox-Fox-E
26	F	0.83	wound	Infection	PD	Jul 2019	NO	t1994	Fox-Fu-Mu-E
27	F	0.42	wound	Infection	PD	Jul 2019	NO	t127	Ox-Fox-Tet-E
28	M	4	eye swab	Infection	PD	Oct 2019	NO	t1994	Ox-Fox-Fu-Mu-Va
29	F	0.58	nasal swab	Colonization	PD	Nov 2019	NO	t1994	Ox-Fox-Fu-Mu
30	F	59	wound	Infection	Surgery	Aug 2019	YES	t002	Ox-Fox-Fu-Le-E
31	M	40	blood	Infection	Surgery	Sept 2019	YES	t002	Ox-Fu
32	F	21	wound	Infection	Surgery	Oct 2019	NO	t091	Ox-Fox-Fu-Le-Tet-Sxt-Va-Teic
33	M	65	wound	Infection	Surgery	Nov 2019	YES	t726	Ox-Fox
34	M	22	wound	Infection	Surgery	Mar 2020	NO	t1309	Ox-Fox-Le
35	M	16	wound	Infection	Surgery	Mar 2020	NO	t044	Ox-Fox-Fu-Tet
36	F	28	CVC	Infection	Surgery	Mar 2020	NO	t034	Ox-Fox-Tet
37	F	59	wound	Infection	Surgery	Jun 2020	NO	t003	Ox-Fox-Le-E
38	M	81	blood	Infection	Urology	Aug 2019	NO	t422	Ox-Fox-Fu-Le-E
39	M	45	wound	Infection	Urology	Feb 2020	NO	t003	Ox-Fox-E

M: Male, F: Female, CVC: Central Venous Catheter, ER: Emergency Room, ICU: Intensive Care Unit, PD: Paediatric ward.
Ox: Oxacillin, Fox: Cefoxitin, Fu: Fusidic acid, Le: Levofloxacin, Mu: Mupirocin, Teic: Teicoplanin.
Te: Tetracycline, SXT: Trimethoprim/Sulfamethoxazole, G: Gentamicin, Va: Vancomycin.
*Erythromycin susceptibility results for strains with ID 6,7,12–15.17–21,28,29, and 31–33 are not available.

Spa typing

Twenty-two different *spa* types were detected, with t002, t003, and t422 being the most frequent (5/39, 12.8% each), followed by t1994 (4/39, 10.3%), t127 and t328 (2/39, 5.1% each) (Table 1). Different *spa* types were detected in the various wards, except the paediatric ward where t1994 (3/4, 75%) predominated. Most of the samples were taken <48h after admission; this was the case also for the t1994 isolates, suggesting that they were not associated with nosocomial infection (Table 1, Fig. 1).

Analysis of whole genome sequences

IM-MRSA and PD-MRSA isolates were assigned to ST9 and ST1, respectively. They were carrying SCC*mec* elements belonging to XII and IVa types, respectively.

The antimicrobial resistance and virulence genes, and the plasmids carried by the two isolates are seen in Table 2. Specifically, both had antimicrobial resistance genes for β-lactams (*mec*A and *bla*Z), aminoglycosides [*aph*(3′)-IIIa], and macrolide, lincosamide and streptogramin B (*erm*C); IM-MRSA carried three additional aminoglycoside resistance genes [*ant*(6)-Ia, *aad*D and *aac*(6′)-*aph*(2″)], while PD-MRSA harbored one additional aminoglycoside resistance gene [*aad*(6)]; *dfr*G gene conferring resistance to trimethoprim was detected only in IM-MRSA. Both isolates carried several efflux pump protein genes conferring resistance to streptogramin A, tetracycline, fluoroquinolones, cephalosporins and lincosamides.

Regarding plasmid content, IM-MRSA carried a plasmid replicon type *rep*22 (of the pGSA11 group), while PD-MRSA transferred plasmid replicon types *rep*7a (of the pGSA2 group, which includes the *rep*C cassette), *rep*7c, *rep*10 (of the pGSA3 group which includes the *erm*C gene) and *rep*5 along with *rep*16 (of the pGSA22 group which includes the *bla*Z gene) (Table 2).

DISCUSSION

The present study provides an insight into the distribution of MRSA isolates of various *spa* types in a tertiary hospital

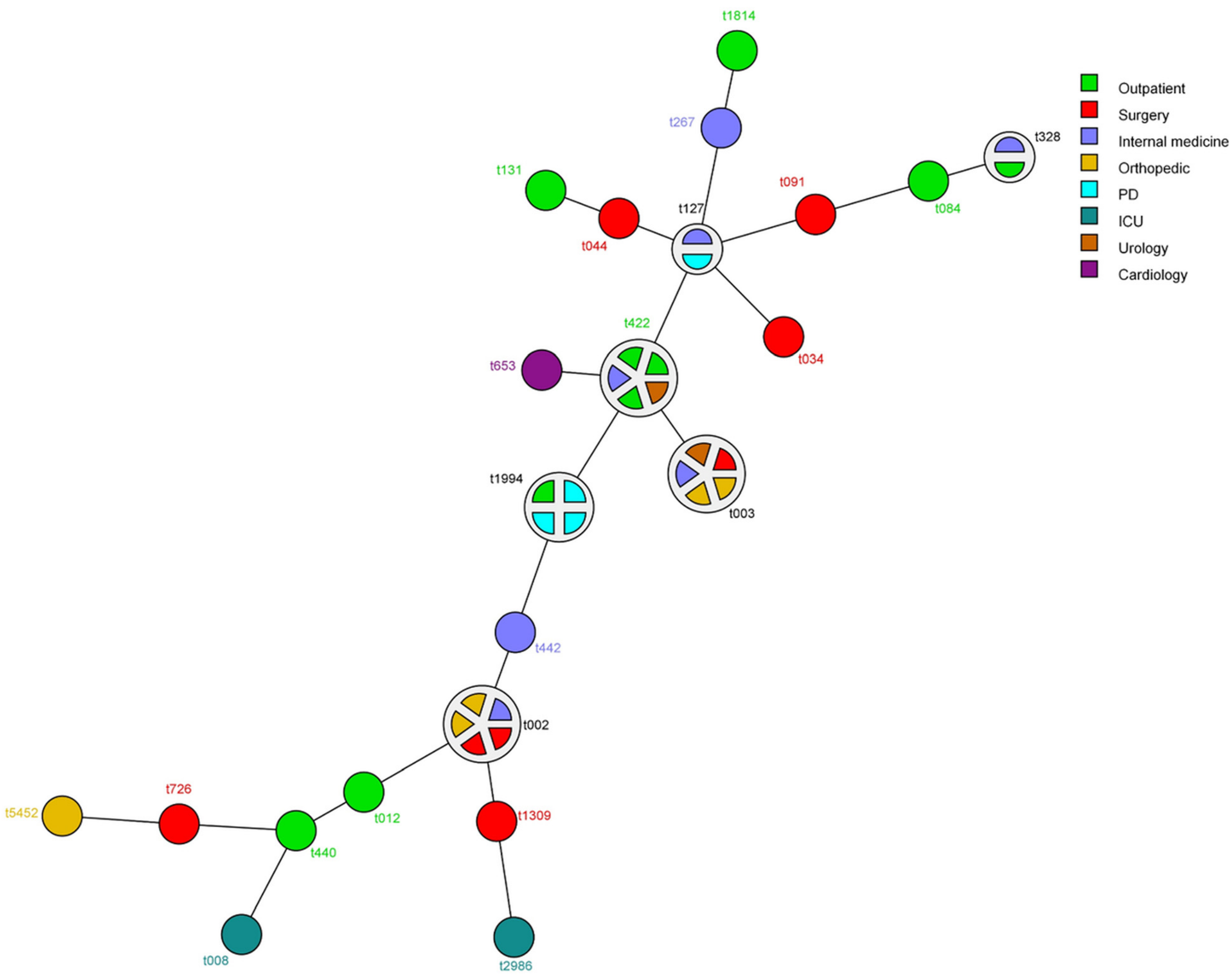

Fig. 1. Minimum spanning tree based on *spa*-typing results of MRSA strains depending on the department of isolation. Each *spa* type is represented by a single node. The size of the node depends proportionally on the number of strains within the *spa* type. The colored sections represent a different ward. The distance between nodes represents the genetic diversity of the isolates

Table 2. Genetic characteristics of two t127 MRSA strains of the study

	PD-MRSA	IM-MRSA
Size (bp)	2,971,258	2,849,316
GC content (%)	34.2	31.0
Number of contigs (with PEGs)	530	5,636
MLST	ST1	ST9
SCC*mec* element	IVa	XII
Antimicrobial resistance genes	*mec*A, *blaZ*,	*mec*A, *blaZ*,
Efflux pump genes	*aad(6)*, *aph(3′)-IIIa*, *erm*C, *mep*R, *nor*A, *mgr*A, *arl*R, *arl*S, *Lmr*S, *tet(45)*	*ant(6)-Ia*, *aph(3′)-IIIa*, *aad*D, *aac(6′)-aph(2′′)*, *dfr*G, *ermC* *tet*L, *fex*A, *Isa*E, *Inu*B
Plasmid group (rep family)	$pGSA_2$ (rep7) $pGSA_3$ (rep10) $pGSA_{22}$ (rep5, rep16)	$pGSA_{11}$ (rep22)
Virulence factor (*gene*)		
Chemotaxis inhibitory protein (*chp*)	−	−
Aureolysin (*aur*)	+	+
Serine protease (*spl*A, *spl*B)	+	−
Staphylococcal complement inhibitor (*scn*)	+	−
Staphylokinase (*sak*)	+	−
γ-hemolysin (*hlgA, hlgB, hlg*C)	+	+
Other leukocidin components (*luk*AB, *luk*ED)	+ (*luk*ED)	−
Staphylococcal enterotoxin	+ (*seh*)	+ (*sei, sem, seo, seu*)
Antiseptic resistance genes (*qac*A, *qac*B, *qac*C, *qac*D)	−	+ (*qac*C, *qac*D)

in Greece. Most of the isolates were collected from infection sites and few from colonization sites; however, colonization usually precedes infection [28]. It was shown that most isolates were resistant to several antimicrobial categories. The high resistance to fusidic acid (46.2%) exceeds by far that observed among MRSA globally (2.6%) [29]. The resistance rate to mupirocin (15.4%) was higher than the 3.1% revealed in a previous multicenter surveillance study [30]; this is of crucial importance since resistance to mupirocin could potentially diminish the efficacy of MRSA decolonizing strategies [31]. For both antimicrobials (fusidic acid and mupirocin) further studies are needed to elucidate the genetic background of the increased resistance (whether it is due to mutation(s) and/or acquired resistance genes). In contrast, resistance to levofloxacin was lower than that reported in a recently published study (17.9% versus 87.9%) [32]; further analysis is needed. The resistance rates of vancomycin and teicoplanin were low; although infections caused by vancomycin-resistant MRSA have been described [33], resistance to vancomycin and teicoplanin are rarely identified in MRSA strains, and they remain active against MRSA causing severe infections [34, 35]. It seems that there was no difference in the antimicrobial resistance profile between MRSA isolates that were considered hospital acquired vs community acquired ones (Table 1).

In total, 22 different *spa* types were identified, and most of them (16/22, 72.7%) were represented as singletons, suggesting a non-clonal MRSA distribution (Table 1, Fig. 1). An exception was the t1994 predominance in the paediatric ward during a four-months' time period; however, it seems that it was not associated with intra-hospital infection since the time of sampling was <48h from the admission to the hospital. The high prevalence of t002 and t003 *spa* types seen in the present study, has been reported in recent studies [36, 37], while the rare t422 type has been previously reported also in another hospital in Thessaloniki [9].

Since t127 type was initially associated with LA-MRSA, and it has been recently detected in Greek workers in the dairy production chain [17], two t127 MRSA isolates (IM-MRSA and PD-MRSA) of the present study were selected for analysis of their whole genome. The two isolates differed each other, as IM-MRSA was assigned to ST9-XII type, while PD-MRSA to ST1-IVa type as was the case in a previous study [17]. The two t127 MRSA isolates transferred the γ-hemolysin genes *hlg*A, *hlg*B, *hlg*C and aureolysin *aur* gene, which have been previously described in both ST1-IVa and ST9-XII MRSA [38, 39]. PD-MRSA carried the enterotoxin *seh* gene and the gene encoding leukocidin *luk*ED, as previously described in ST1-IVa MRSA isolates [38, 40]. IM-MRSA lacked leucocidin components, however it carried the enterotoxin *sei*, *sem*, *seo*, *seu* genes, although enterotoxin genes are usually absent in ST9-XII MRSA isolates [39].

LA-MRSA lack the human evasion genes *scn* (staphylococcal complement inhibitor), *chp* (chemotaxis inhibitory protein) and *sak* (staphylokinase), due to loss of the related φSa3 phage [41]. IM-MRSA lacked these three genes, while PD-MRSA lacked only the *chp* gene. IM-MRSA was isolated from wound infection and was assigned to ST9-XII, which, due to loss of *scn*, *chp* and *sak* genes, is considered to have been evolved from human to animal host [39]. ST9-XII is rarely reported as cause of infection [42]. *Spa*-type t127 has not been reported for *S. aureus* ST9 so far. Since generation of this *spa*-type from the repeat spa sequences observed in this clonal lineage would need several genetic events (mutations, deletion(s), insertion), the most probable mechanism seems to be the acquisition of the chromosomal sequence which contains *spa* from a ST9 donor; there are several examples for nontypical *spa* types for particular STs based on this genetic event, e.g. *spa* type t030 in MRSA ST239 [43]. Comparative studies are needed to confirm which mechanism(s) took place. The second t127 isolate, PD-MRSA, was isolated from an axillary abscess and belonged to ST1-IVa-t127, which is originally considered a human-associated lineage. ST1-t127 isolates have been reported as one of the most frequent types isolated from human samples [44].

CONCLUSIONS

The current study provides an insight into the *spa* types of MRSA in a tertiary hospital in Greece and together with the information of time interval between admission and sampling suggests polyclonal introductions in the various wards. The extensive resistance to most antimicrobial classes needs further attention since it is associated with high antimicrobial consumption. A more detailed genetic characterization is gained when WGS is applied, as shown by the results of the analysis of the two t127 MRSA isolates, which showed that although they belonged to the same *spa* type, they were different strains. Molecular surveillance studies are of high priority in the hospitals since they can lead the design of guidelines and infection control measures that must be applied to reduce and prevent the spread of MRSA.

GENOME SEQUENCES

The whole genome sequences of IM-MRSA and PD-MRSA were submitted to European Nucleotide Archive (ENA) under the study PRJEB47007 and received the Accession numbers ERS7262952 and ERS7262953, respectively.

DECLARATIONS

Funding: This work was financially supported by the European Union's Horizon 2020 project VEO (grant number 874735).

Conflicts of interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material: Available upon request.

Code availability: Not applicable.

Ethics approval: Not applicable.

Authors' contribution: All authors have equally contributed to the study and preparation of the manuscript.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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