A 12-year survey of methicillin-resistant Staphylococcus aureus infections in Greece: ST80-IV epidemic?

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A 12-year survey of methicillin-resistant *Staphylococcus aureus* infections in Greece: ST80-IV epidemic?

E. Drougka[1,2], A. Foka[1,2], A. Liakopoulos[3], A. Doudoulakakis[4], E. Jelastopulu[5], V. Chini[1,*], A. Spiliopoulou[6], S. Levidiotou[7], T. Panagea[8,†], A. Vogiatzi[8], E. Lebessi[4], E. Petinaki[3] and I. Spiliopoulou[1,2]

1) *Department of Microbiology, School of Medicine, University of Patras,* 2) *National Reference Laboratory for Staphylococci,* 3) *Department of Microbiology, School of Medicine, University of Thessaly, Larissa,* 4) *Department of Microbiology, 'P&A Kyriakou' Children's Hospital, Athens,* 5) *Department of Public Health, School of Medicine, University of Patras,* 6) *Laboratory of Microbiology, Karamandaneion Children's Hospital, Patras,* 7) *Department of Microbiology, School of Medicine, University of Ioannina, Ioannina and* 8) *Laboratory of Microbiology, General Children's Hospital Pentelis, Athens, Greece*

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of both healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) infections. Severe MRSA infections have been associated with the virulence factor Panton–Valentine leukocidin (PVL). The aim of this study was to investigate susceptibility patterns, the presence of toxin genes, including that encoding PVL, and clonality among MRSA isolates collected from patients in Greece over a 12-year period. MRSA isolates were collected from January 2001 to December 2012 from six different hospitals. Antibiotic susceptibility was determined with the disk diffusion method and the Etest. The presence of the toxic shock syndrome toxin-1 gene (*tst*), the enterotoxin gene cluster (*egc*) and the PVL gene was tested with PCR. The genotypic characteristics of the strains were analysed by SCC*mec* and *agr* typing, and clonality was determined with pulsed-field gel electrophoresis and multilocus sequence typing. An increasing rate of MRSA among *S. aureus* infections was detected up to 2008. The majority of PVL-positive MRSA isolates belonged to a single clone, sequence type (ST)80-IV, which was disseminated both in the community and in hospitals, especially during the warmest months of the year. Carriage of *tst* was associated with ST30-IV, whereas *egc* was distributed in different clones. CA-MRSA isolates were recovered mainly from skin and soft tissue infections, whereas HA-MRSA isolates were associated with surgical and wound infections. During the period 2001–2012, ST80-IV predominated in the community and infiltrated the hospital settings in Greece, successfully replacing other PVL-positive clones. The predominance of ST239-III in HA-MRSA infections was constant, whereas new clones have also emerged. Polyclonality was statistically significantly higher among CA-MRSA isolates and isolates from adult patients.

Keywords: Clones, epidemiology, Greece, methicillin-resistant *Staphylococcus aureus*, ST80-IV, toxins
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Corresponding author: I. Spiliopoulou, Department of Microbiology, School of Medicine, University of Patras, Rion 26504, Patras, Greece
E-mail: spiliopl@upatras.gr
*Present address: Shafallah Medical Genetics Centreer, Doha, Qatar
†Present address: Department of Microbiology, A. Fleming Hospital, Athens, Greece

Introduction

Staphylococcus aureus is the main cause of purulent infections [1]. The classification of *S. aureus* as one of the most important human pathogens is largely based on its virulence potential and ubiquitous occurrence as a colonizer in humans, domestic animals, and livestock [2].

In the modern era, it is estimated that 25–35% of healthy human individuals carry *S. aureus* on the skin or mucous membranes [2]. This means that up to 2 billion individuals carry *S. aureus* worldwide, and conservative estimates based on Dutch and US prevalence data predict that *c.* 2–53 million people carry methicillin-resistant *S. aureus* (MRSA) [3]. MRSA is one of the most significant healthcare-associated pathogens, causing a wide range of infections. The first MRSA isolate was reported in 1960, and since then various clones have disseminated worldwide [2]. The prevalence of MRSA bacteraemia

varies from 1% in Norway to 67% in Japan [2,4]. Factors contributing to the occurrence of MRSA infections are cross-transmission via the hands of healthcare workers and high selective pressure exerted by broad-spectrum antibiotic therapy [5]. As a result of clonal spread, outbreaks of MRSA have been described in hospital settings worldwide, and MRSA has become endemic in many international healthcare settings (healthcare-associated MRSA (HA-MRSA)) [5]. Besides healthcare-associated infections, MRSA causes community-associated infections (community-associated MRSA (CA-MRSA)) among patients without predisposing risk factors, especially skin and soft tissue infections (SSTIs) and necrotizing pneumonia [2,3]. Genotyping of MRSA is important, in order to detect outbreaks, assess the dissemination of virulent strains, and understand its epidemiology. This is achieved by identifying the presence of important mobile genetic elements, which include the methicillin resistance gene *mecA* on the various SCC*mec* elements, the bacteriophage-encoded Panton–Valentine leukocidin (PVL) toxin, and many resistance determinants conveyed by plasmids or transposons [6–8]. PVL-positive MRSA isolates are strongly associated with SSTIs and severe community-onset pneumonia [9,10]. The first PVL-positive MRSA isolate in Greece was identified at the University General Hospital of Patras from an intravenous catheter culture of a premature baby in 1998, suggesting that it was acquired from a healthcare worker [11]. During the year 2000, another ten PVL-positive MRSA isolates were obtained from patients with underlying diseases, and it was obvious that there had been an increase in the incidence of CA-MRSA and HA-MRSA infections caused by PVL-positive strains [11]. A parallel increase was also observed in the overall percentage of total MRSA infections during the period of the above studies, probably caused by the spread of PVL-positive strains. It has been reported that CA-MRSA has a faster growth rate than HA-MRSA, resulting in successful colonization [12]. In another Greek study performed from 2001 to 2003, a significant increase in the number of PVL-positive *S. aureus* isolates, most of which were MRSA, was found [13]. Among CA-MRSA isolates, 72% carried the PVL genes, and 23% of HA-MRSA isolates were also PVL-positive [13]. The prevalence of CA-MRSA infections varies widely from one country to another [14]. For example, many differences exist between the epidemiology of CA-MRSA in the USA and that in Europe [2]. In the USA, CA-MRSA is one of the most common causes of SSTIs in patients from emergency departments, and one clone, USA300 (sequence type (ST)8-SCC*mec* IV), predominates [2]. In Europe, the prevalence of CA-MRSA is much lower, but it is increasing, especially in countries where the incidence of HA-MRSA is low, such as Denmark and The Netherlands [15]. Many different CA-MRSA clones have been identified, of which the ST80-SCC*mec* IV European clone is the most widely disseminated [15]. Recently, however, several European countries have been confronted with a rise in the prevalence of USA300 strains [14].

The aim of the present study was to estimate the prevalence and clonal distribution of PVL-positive MRSA among healthcare-associated and community patients with staphylococcal infections in a wide geographical area of Greece during a 12-year period.

Materials and Methods

Bacterial isolates and hospitals

Four thousand six hundred and fourteen MRSA isolates from six *S. aureus* collections were studied. These collections originated from six different Greek hospitals serving three-fifths of the total Greek population: University General Hospital of Patras (2259 isolates) and Karamandaneion Children's Hospital of Patras (291 isolates), both located in south-western Greece; 'P. & A. Kyriakou' Children's Hospital (139 isolates) and General Children's Hospital Pentelis (46 isolates), both located in Athens; University Hospital of Ioannina (80 isolates), located in north-western Greece; and University Hospital of Larissa (1799 isolates), located in central Greece. All isolates were recovered from different inpatients and outpatients with *S. aureus* infection (one isolate per patient) during a 12-year period from January 2001 to December 2012. Each isolate was sent to the National Reference Laboratory for Staphylococci, with a report describing demographics and clinical data: age and sex of the patients, clinical specimen and date of sampling, underlying disease, hospital ward, and whether the isolate was epidemiologically representative of a cluster. Children's hospitals admit patients up to the age of 14 years.

Isolates obtained within 48 h of hospital admission were defined as CA-MRSA, as were isolates from patients with infection diagnosed in an outpatient department, provided that there was no history in the past year of hospitalization, admission to a nursing home, skilled nursing facility, or hospice, or association with dialysis, surgery, permanent indwelling catheters, or medical devices. Isolates obtained >48 h following admission were defined as HA-MRSA [16].

S. aureus identification

Isolates were identified on the basis of colony morphology, Gram stain, catalase production, and the coagulase test (Slidex Staph Plus; bioMérieux, Marcy l'Etoile, France), and identification was verified with molecular methods (PCR for 16S rRNA and *nuc* genes) [17].

Antimicrobial susceptibility testing

Susceptibility testing was performed with the disk diffusion method, according to CLSI guidelines for cefoxitin, tobramycin, gentamicin, rifampicin, kanamycin, erythromycin, clindamycin, ciprofloxacin, and sulphamethoxazole–trimethoprim, and according to the EUCAST breakpoints for fusidic acid (BBL disks; Becton Dickinson, Le Pont de Claix, France) [18,19]. Oxacillin, vancomycin, teicoplanin, daptomycin and linezolid MICs were determined with a gradient method (Etest; bioMérieux) [18]. Final determination of vancomycin MICs was achieved after testing, with the agar dilution method (antibiotic concentrations: 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 mg/L), those isolates showing MIC levels of >2 mg/L in the Etest [18]. All isolates with a cefoxitin zone diameter of ≤21 mm were tested with a latex agglutination test (Slidex MRSA Detection; bioMérieux) for the presence of penicillin-binding protein 2a, and with PCR for *mecA* [17]. Multiresistant isolates were those showing resistance to three or more classes of antimicrobial.

Toxin gene detection

Genes encoding PVL (*lukS*/*lukF*-PV) and toxic shock syndrome toxin-1 (*tst*), and the *egc* operon of the enterotoxin gene cluster (*sem* and *seg*), were investigated with PCR [20]. PCR products were analysed by electrophoresis on agarose 1% w/v gels. *S. aureus* strains ATCC49775 (PVL-positive, *agr*3), Fri 913 (*tst*-positive, *agr*1), Fri 137 (*egc* operon-positive, *agr*2) and HT 20000195 (*agr*4) were used as positive controls for PCRs [20].

Molecular typing

DNA extraction and pulsed-field gel electrophoresis (PFGE) of *Sma*I DNA digests were performed in a CHEF DR III apparatus (Bio-Rad Laboratories, Richmond, CA, USA), as described elsewhere [21]. Visual interpretation of the PFGE banding patterns and the assignments of types (pulsotypes) were performed for all MRSA isolates, according to the criteria of Tenover *et al.* [21]. PFGE types were named with capital letters. *agr* groups and SCC*mec* types were defined by PCR [20,22]. Multilocus sequence typing was performed on 946 selected representative isolates from all MRSA collections according to *agr*, PFGE type, SCC*mec* type, toxin gene profile and antibiotic resistance pattern (www.mlst.net). Clones were defined according to ST-SCC*mec* type.

Statistical analysis

Statistical analysis was performed with SPSS version 19.0 (SPSS, Chicago, IL, USA). The prevalence estimates for MRSA by hospital, sex, age, year, type, origin (healthcare-associated or community-associated), clone, toxin gene carriage and MIC_{90} value were compared by the use of Pearson's chi-square test or Fisher's exact test. Results were considered to be statistically significant at $p < 0.05$, and values are expressed as percentages.

Results

All isolates with a cefoxitin zone diameter of ≤21 mm were penicillin-binding protein 2a-positive, carried *mecA*, and were characterized as MRSA. The percentage of MRSA isolates in *S. aureus* infections in all six hospitals showed a gradual increase from 2001 to 2008, and slightly lower rates up to 2012 (Fig. 1).

The majority of MRSA isolates were recovered from adults (3378, 73.2%) and male patients (2811, 60.9%). In total, 3345 isolates were CA-MRSA, and the remaining 1269 were HA-MRSA, as defined according to epidemiological criteria. An increasing incidence of CA-MRSA in total during the study period was observed, reaching 68.6% of MRSA isolates (from 44.9% to 68.6%), showing a statistically significant difference from HA-MRSA in 2012 ($p < 0.001$). There was a greater proportion of multiresistant MRSA isolates in adults (533/3378, 15.8%) than in children (63/1236, 5.1%). HA-MRSA isolates showed significantly higher rates of resistance to the majority of the antimicrobials tested with the disk diffusion method than CA-MRSA isolates (Fig. 2). Moreover, CA-MRSA isolates showed high rates of resistance to kanamycin (89.5%), fusidic acid (88.4%), erythromycin (19.8%), and tobramycin (21.0%). Three hundred and ninety-seven isolates (11.9%) were resistant to macrolides and lincosamides (318 isolates showing inducible resistance, and the remaining 79 showing constitutive resistance) (Fig. 2).

Four hundred and sixty-nine *mecA*-positive isolates (10.2%) showed oxacillin MICs of <4 mg/L. The MIC_{90} of vancomycin was 2 mg/L (0.25–2 mg/L), that of linezolid was 1.5 mg/L (0.094–2 mg/L), and that of daptomycin was 0.5 mg/L (0.016–1 mg/L). No statistically significant difference was observed in MIC_{90} values between CA-MRSA and HA-MRSA, or among the six participating hospitals.

Among the 3345 CA-MRSA isolates, 2736 (81.8%) were recovered from SSTIs, 399 (11.9%) from abscesses, and the remainder from bacteraemias (79, 2.4%), wound infections (52, 1.6%), respiratory tract infections (47, 1.4%), and urinary tract infections (32, 0.9%).

Among the 1269 HA-MRSA isolates, 462 (36.4%) were recovered from bacteraemias, 447 (35.2%) from SSTIs, 168 (13.2%) from abscesses, and the remainder from surgical wound infections (92, 7.2%), especially when prosthetic devices were present, respiratory tract infections (87, 6.9%), and urinary tract infections (13, 1.1%).

The frequency of ST80-IV increased from 28.6% in 2001 to 73.3% in 2012 (Table 1). Isolates belonging to this clone were

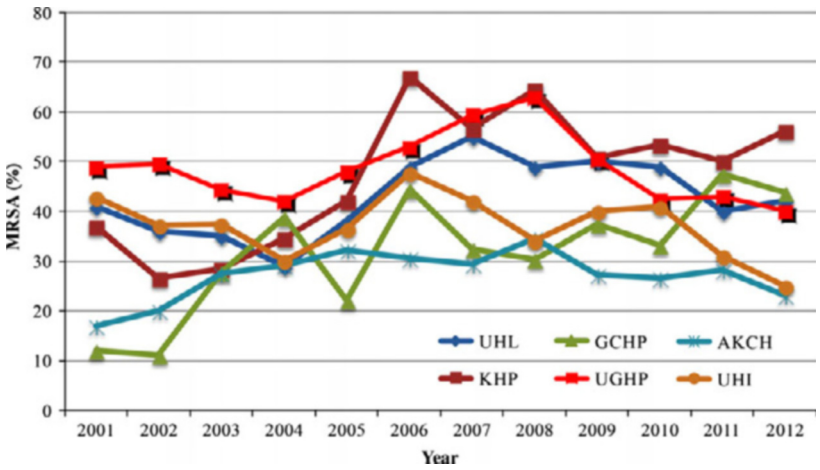

FIG. 1. Annual percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) among *S. aureus* infections in all participating hospitals. AKCH, 'P. & A. Kyriakou' Kyriakou Children's Hospital; GCHP, General Children's Hospital Pentelis; KHP, Karamandaneion Children's Hospital of Patras; UGHP, University General Hospital of Patras; UHI, University Hospital of Ioannina; UHL, University Hospital of Larissa.

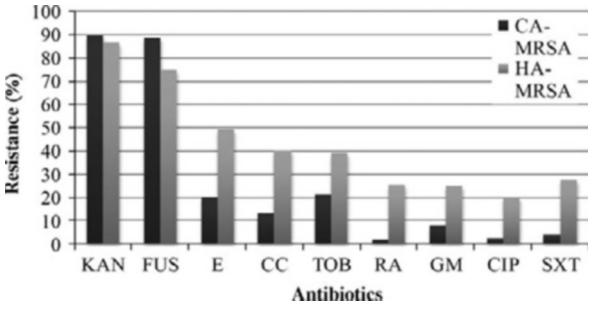

FIG. 2. Antibiotic resistance patterns among community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and healthcare-associated methicillin-resistant *S. aureus* (HA-MRSA), as determined with the disk diffusion method. KAN, kanamycin; FUS, fusidic acid; E, erythromycin; CC, clindamycin; TOB, tobramycin; RA, rifampicin; GM, gentamicin; CIP, ciprofloxacin; SXT, sulphamethoxazole–trimethoprim.

characterized as SCC*mec* IV; the majority were C/*agr*3, whereas ten were classified as A/*agr*3, B/*agr*3, H/*agr*3, and L/*agr*3. In 2004, we detected, for the first time, one ST80-IV isolate, classified as C/*agr*1. Since then, 14 more isolates have been identified with that profile. In 2007, 2008, and 2011, four strains were classified as ST80-IV and C/*agr*2.

ST80-IV was significantly more frequent in the summer season (June to September) than in winter (October to January) (1447 vs. 610 isolates, $p < 0.001$), whereas the remaining clones were distributed equally throughout the year. Eight hundred and sixty-eight ST80-IV isolates were recovered from 1236 children examined, and 1970 from 3378 adults (70.2% vs. 58.3%, $p < 0.001$).

The other main clones characterized in this study were: ST239-III (1051 isolates), ST30-IV (453 isolates), ST377-V (60 isolates), and ST225-II (52 isolates). ST239 isolates carrying SCC*mec* III belonged to two main PFGE types (E and B), whereas 12 isolates were classified as C, G, K, L, and N, combined with *agr*1,2,3. ST30-IV isolates were classified as A and O, combined with *agr*1,3, whereas ST377 isolates carried SCC*mec* V and were G/*agr*1,3. This clone was detected for the first time in 2004, and reached its highest prevalence in 2008, with 26 isolates in total. ST225 isolates carrying SCC*mec* II were classified as H (38 isolates), A, B, C, D, E, and G, combined with *agr*1,2,3. Minor clones in this collection were: ST8-IVc (H/*agr*1), ST72-IV (E/*agr*1 and B/*agr*1), ST121-V (R/*agr*3,4), ST461-NT (A/*agr*3), ST770-NT (E/*agr*1), ST22-IV (G/*agr*1,2,3 and T/*agr*1,2,3), ST97-V (A/*agr*1,2 and E/*agr*1,2), ST1659-II (B/*agr*1), ST5-IV (B/*agr*1,2 and C/*agr*1,2), ST7-IV (C/*agr*1), ST45-IV (H/*agr*1), ST217-IV (F/*agr*1), ST935-V (H/*agr*2), ST1-IV (B/*agr*1,3, C/*agr*1,3, and H/*agr*1,3), ST585-III (C/*agr*1), ST714-IV (A/*agr*3), ST842-NT (C/*agr*1), ST2486-IV (C/*agr*3), and ST2487-IV (C/*agr*1) (Fig. 3).

Polyclonality was observed to a lesser extent among children than among adults (13 vs. 21 clones, p 0.172).

From 2001 to 2012, ST80-IV was found in five hospital units, which included both adult and paediatric services. The adult services included the outpatient department, the trauma unit, the intensive-care unit, the internal medicine unit, the surgery unit, the nephrology unit, and the cardiopulmonary unit. The paediatric services included the outpatient department, the newborn and paediatric intensive-care unit, and the surgery and orthopaedic wards. ST80-IV was significantly more frequent in adults and paediatric outpatients than in patients of other wards (59.6% vs. 40.4%, $p < 0.001$).

Genes encoding PVL were detected in 2946 isolates: 2825 for ST80-IV, 54 for ST377-V, and one to eight each for ST8-IVc,

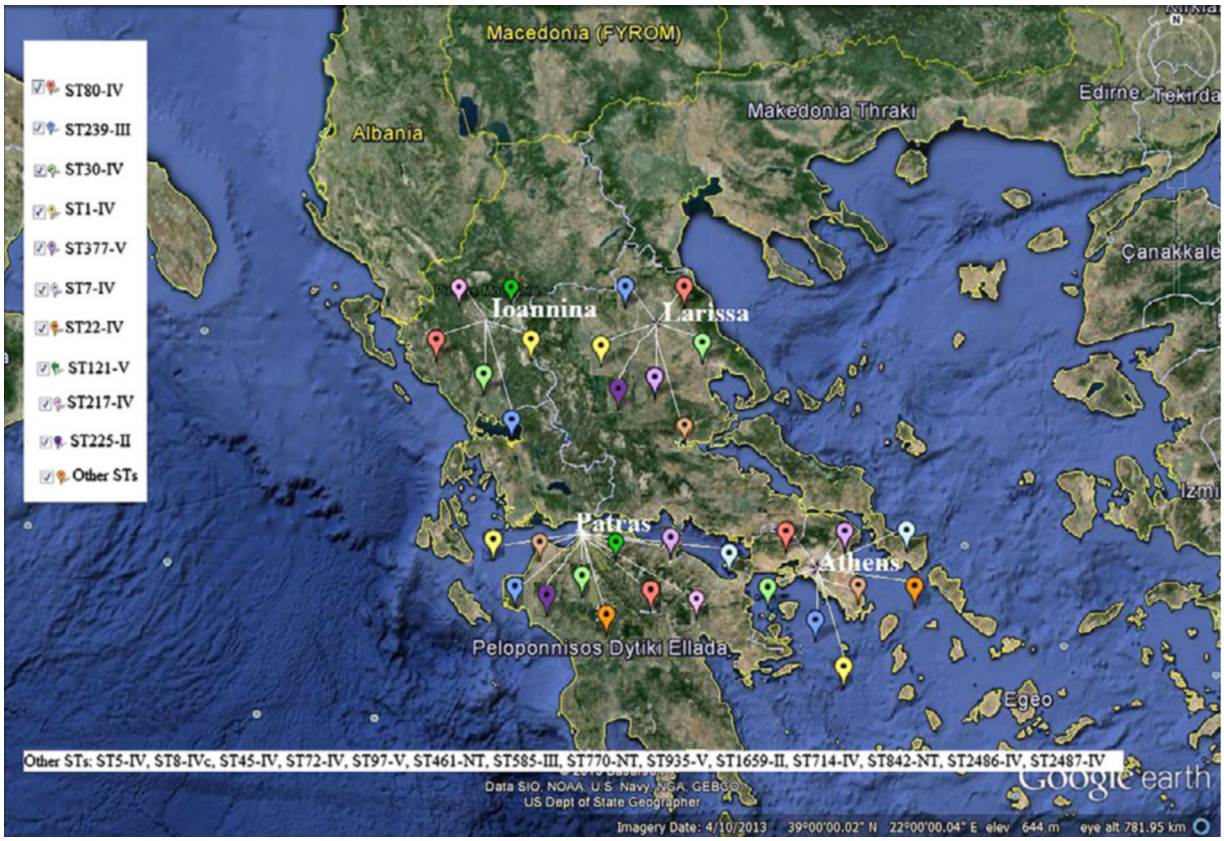

FIG. 3. Clonal distribution of methicillin-resistant *Staphylococcus aureus* isolates recovered from patients admitted to the participating tertiary-care hospitals in Greece, from 2001 to 2012.

TABLE 1. Frequencies of methicillin-resistant *Staphylococcus aureus* (MRSA) by clone during the 12-year study period (2001–2012); the percentage of clones was calculated within each year

	MRSA total	ST80-IV *N* (%)	ST239-III *N* (%)	ST30-IV *N* (%)	ST377-V *N* (%)	Other STs *N* (%)
2001	84	24 (28.6)	41 (48.8)	18 (21.4)	–	1 (1.2)
2002	155	54 (34.8)	79 (51.0)	19 (12.3)	–	3 (1.9)
2003	167	75 (45.0)	64 (38.3)	27 (16.2)	–	1 (0.5)
2004	315	157 (49.8)	117 (37.2)	36 (11.4)	4 (1.3)	1 (0.3)
2005	377	197 (52.3)	131 (34.7)	43 (11.4)	4 (1.1)	2 (0.5)
2006	496	280 (56.5)	144 (29.0)	58 (11.7)	5 (1.0)	9 (1.8)
2007	610	404 (66.2)	113 (18.5)	65 (10.7)	5 (0.8)	23 (3.8)
2008	847	589 (69.5)	120 (14.2)	67 (7.9)	26 (3.1)	45 (5.3)
2009	553	378 (68.4)	89 (16.1)	45 (8.1)	7 (1.3)	34 (6.1)
2010	561	368 (65.6)	72 (12.8)	39 (7.0)	7 (1.2)	75 (13.4)
2011	198	128 (64.6)	38 (19.2)	16 (8.1)	–	16 (8.1)
2012	251	184 (73.3)	43 (17.1)	20 (8.0)	2 (0.8)	2 (0.8)
Total	4614	2838 (61.6)	1051 (22.5)	453 (9.8)	60 (1.4)	212 (4.7)

ST, sequence type.

ST30-IV, ST72-IV, ST121-V, ST225-II, ST239-III, ST461-NT, ST714-IV, and ST770-NT (Table 2). One hundred and eleven isolates (2.4%) carried *tst*. The majority of these isolates were ST30-IV (76 isolates) and ST239-III (18 isolates), and the remaining 17 were ST225-II, ST22-IV, ST97-V, and ST1659-II. No isolate simultaneously carried both a PVL gene and *tst*. Isolates carrying the *egc* operon were classified into 16 clones: ST80-IV (222 isolates), ST30-IV (102 isolates), ST239-III (52 isolates), and ST5-IV, ST7-IV, ST22-IV, ST45-IV, ST72-IV, ST97-V, ST121-V, ST217-IV, ST225-II, ST377-V, ST714-IV, ST935-V, and ST2486-IV (1–13 isolates each) (Table 2). Among the 495 *egc*-positive isolates, 89 simultaneously carried *tst*, the majority being ST30-IV (71 isolates), and 248 simultaneously carried PVL genes, the majority of them being ST80-IV (210 isolates). Two of 13 PVL-negative ST80-IV isolates carried the *egc* operon.

PFGE and multilocus sequence typing analysis of the PVL-positive MRSA isolates in the present study revealed the

TABLE 2. Prevalence rates of methicillin-resistant *Staphylococcus aureus* (MRSA) and comparison between community-asociated and hospital-associated infections, identified according to epidemiological criteria (community-associated MRSA (CA-MRSA) and healthcare-associated MRSA (HA-MRSA)), clone, and toxin gene carriage (*lukS*/*lukF*-PV, *tst*, and *egc* operon)

	Total *N*	CA-MRSA *N* (%)	HA-MRSA *N* (%)	p
ST80-IV	2838	2520 (88.8)	318 (11.2)	<0.001
ST30-IV	453	319 (70.4)	134 (29.6)	<0.001
ST377-V	60	46 (76.7)	14 (23.3)	<0.001
ST239-III	1051	346 (32.9)	705 (60.8)	<0.001
ST225-II	52	8 (15.4)	44 (84.6)	<0.001
Other clones	160	106 (66.3)	54 (33.7)	<0.001
PVL in total	2946	2602 (88.3)	344 (11.7)	<0.001
PVL in ST80-IV	2825	2515 (96.7)	310 (90.1)	<0.001
PVL in ST30-IV	8	6 (0.2)	2 (0.6)	<0.001
PVL in ST377-V	54	42 (1.6)	12 (3.5)	0.453
PVL in ST239-III	7	5 (0.2)	2 (0.6)	<0.001
PVL in ST225-II	4	–	4 (1.2)	–
PVL in other clones	48	34 (1.3)	14 (4.0)	0.606
tst in total	111	43 (38.74)	68 (61.26)	0.034
tst in ST30-IV	76	35 (81.40)	41 (60.29)	0.080
tst in ST239-III	18	6 (13.95)	12 (17.65)	0.635
tst in ST225-II	10	–	10 (14.71)	–
tst in other clones	7	2 (4.65)	5 (7.35)	0.119
egc in total	495	322 (65.1)	173 (34.9)	<0.001
egc in ST80-IV	222	148 (46.0)	74 (42.8)	0.758
egc in ST30-IV	102	64 (19.9)	38 (22.0)	1
egc in ST239-III	52	41 (12.7)	8 (4.5)	0.960
egc in ST225-II	13	5 (1.5)	8 (4.5)	0.196
egc in other clones	106	64 (19.9)	42 (24.3)	0.767

PVL, Panton–Valentine leukocidin; ST, sequence type.

spread of a main clone among MRSA isolates in the community and in the geographically distinct participating hospitals. In total, 2825 (99.5%) of 2838 ST80-IV isolates were positive for PVL genes by PCR. Only 13 ST80-IV isolates were PVL-negative, and the first of them was detected in 2004. None of the ST80-IV isolates carried *tst*.

The main healthcare-associated clone in our collection was ST239-III. This clone showed a gradual decrease (Table 1). ST80-IV, ST30-IV and ST377-V were also found among HA-MRSA isolates. The presence of ST225-II was also significant in this group (44 isolates), and it was characterized by a low percentage of PVL gene carriage (4/44, 9.1%) and higher frequencies of *tst* and *egc* (10/44 and 8/44, respectively) (Table 2).

Discussion

The present study revealed a predominance of ST80-IV (61.6% in total and 88.8% in CA-MRSA) in the community, and of ST239-III in hospitals (22.5% in total and 60.8% in HA-MRSA). Furthermore, the MRSA clonal diversity that has been observed during recent years, mainly among adults, may reflect frequent travel and the importation of new MRSA clones into Greece, as these are present in other countries as well. Specifically, even though ST8-IV (USA300) is the most successful in the USA, in Greece it was identified for the first time in 2008, and it is still being identified in sporadic cases without being spread [2].

The multidrug-resistant phenotype is a particular characteristic of MRSA, related to the global presence and spread of multidrug-resistant clones [23]. The homogeneous lack of susceptibility to all β-lactams, which is characteristic of methicillin-resistant strains, together with the continuous accumulation and organization of many resistance genes have made infections with this species particularly difficult to treat [24]. This extensive antibiotic resistance is seen for many classes of antimicrobial, such as aminoglycosides, macrolides, lincosamides, and fluoroquinolones, which, as demonstrated in this study, appeared to maintain a low level of activity against HA-MRSA. Moreover, no resistance to vancomycin, daptomycin or linezolid was observed during this period. Nevertheless, a slight but continuous increase in the level of non-susceptibility to vancomycin has been found by many studies all around the world [23]. Seasonal variation in *S. aureus* infections is controversial. The present study confirmed that the majority of infections caused by ST80-IV (1447/2838) occurred during the warmest months of the year, probably because of the increased time that people spend outdoors and closer contact with the environment, especially among children, predisposing to skin damage and infection [24]. In a more recent study, Mermel *et al.* [25] found evidence for a seasonal effect on the frequency of CA-MRSA isolates per emergency department visit in both paediatric and adult patients. They found a two-fold to three-fold increased incidence of MRSA infections in paediatric patients during the second two quarters of the last decade. A lower degree of seasonal variation was observed for adult CA-MRSA infections [25].

None of the PVL-positive isolates in the present study carried *tst*. In contrast, Rossney *et al.* [26] found six of 25 ST30 isolates that harboured *tst* along with PVL genes, and five of them were CA-MRSA strains. In our collection, the ST30-IV isolates had a very low percentage of PVL gene carriage (1.8%) and a relatively high percentage of *tst* carriage (16.8%).

The predominant CA-MRSA clones differ between continents. USA300 (ST8) predominates in the USA, the European clone (ST80) predominates in Europe, and the Southwest Pacific clone (ST30) predominates in Australia, Asia, and South America [27], but, owing to increased travel, these strains are spreading throughout the world. It is very interesting, however, that, in the northern countries of Europe, such as Denmark, both the USA300 clone and the European clone are present [14]. Spain and Portugal have high rates of HA-MRSA but low, albeit increasing, rates of CA-MRSA. In two Spanish studies, ST8-IV

(USA300) was the most frequent clone, and these PVL-positive isolates were associated with immigrants from South America, mainly Ecuador and Bolivia [14]. Portugal has a very high rate of HA-MRSA in hospitals, but a very low rate of CA-MRSA [14]. A recent report from the German Reference Laboratory for Staphylococci suggests an increase in prevalence of the European clone, and also the emergence of a USA300-like ST8-IV PVL-positive clone, most likely imported from the USA [14].

In the present study, we identified the presence of the European clone ST80-IV in both CA-MRSA (88.8%) and HA-MRSA (11.2%). The vast majority, 99.5% (2825/2838), of the above isolates carried *lukS*/*lukF*-PV genes encoding PVL. In another Greek study conducted at one hospital in Athens during 2009, 30.7% of *S. aureus* isolates recovered from outpatients were characterized as MRSA; all were PVL-positive and classified as ST80-IV [28]. Moreover, in 2008, Dailiana *et al.* [29] showed that, in a 4-year *S. aureus* collection in central Greece, the majority of PVL-positive MRSA isolates (54/58) were ST80 and the remainder were ST377. We can conclude that ST80-IV is dominant in Greece, especially concerning CA-MRSA infections, and it has also been reported that this particular clone is expanding in other European countries, such as France, The Netherlands, and Switzerland [14]. The most common healthcare-associated clone in Greece remains ST239-III, followed by ST80-IV, which is successfully spreading in hospitals. Hospital invasion has also been reported for clones in other countries [2]. We assume that this hospital invasion is a result of the extensive use of β-lactam antibiotics in combination with a very low rate of routine MRSA colonization screening in the staff [14]. Moreover, Vandenesch *et al.* [30] reported that isolates carriying PVL genes and SCC*mec* IV may have a selective advantage among community-based MRSA pathogens. Okuma *et al.* [12] have suggested that CA-MRSA should show enhanced ecological fitness, as it has a shorter doubling time than HA-MRSA.

The epidemiological characteristics of MRSA in the countries neighbouring Greece vary. Despite the large size of Turkey, a single predominant clone, ST239-III, circulates in hospitals in different regions, and only few new types of MRSA have been introduced over ten years (1997–2006) [31]. In contrast, studies have shown that, in Italy, the main HA-MRSA clone is ST228-I, and that ST239-III has been present for only a short time [8].

In conclusion, this study has shown the predominance of a single MRSA clone in Greece, ST80-IV, which is prevalent in other European countries as well. Moreover, it appears not only to cause community-associated infections, but also to be an emerging cause of healthcare-associated infections. Special attention is required in diagnosing and identifying MRSA, in order to enable the prescription of appropriate antibiotic therapeutic regimens to adequately control the possible severe complications, and to prevent the dissemination of this pathogen.

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Author Contributions

E. Drougka performed the phenotypic characterization of all of the isolates recovered in the Patras area from 2005 to 2012, and the molecular analysis of all of the isolates collected in Athens, Ioannina, Patras, and Thessaly. She recorded all of the demographic data of the patients, contributed to the analyses, and wrote the paper. A. Foka contributed to the molecular analysis applied in the characterization of all MRSA isolates, and critically revised the paper. A. Liakopoulos and E. Petinaki performed the phenotypic and molecular analyses of MRSA isolates collected in Thessaly, and sent representative isolates to the National Reference Laboratory for Staphylococci in Patras for analysis. Both contributed to the writing and editing of the manuscript. V. Chini performed the phenotypic and

molecular characterization of MRSA isolates in the Patras area from 2001 to 2005, and recorded the demographic data of patients during this period. A. Doudoulakakis, A. Spiliopoulou, S. Levidiotou, T. Panagea, A. Vogiatzi and E. Lebessi determined the phenotypes, collected the demographic data from patients admitted to the participating hospitals from Athens and Ioannina, and critically revised the paper. E. Jelastopulu performed the statistical analysis of the data, and critically revised the paper. I. Spiliopoulou designed the study, supervised all of the experiments and statistical analyses, reviewed all data, and wrote the manuscript.

Transparency Declaration

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