

Changing Patterns in Frequency of Recovery of Five Methicillin-Resistant *Staphylococcus aureus* Clones in Portuguese Hospitals: Surveillance over a 16-Year Period[∇]

Marta Aires-de-Sousa,^{1,2†} Bruno Correia,^{1†} Hermínia de Lencastre,^{1,3*}
and the Multilaboratory Project Collaborators[‡]

Laboratório de Genética Molecular, Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa, Oeiras, Portugal¹; Escola Superior de Saúde da Cruz Vermelha Portuguesa, Lisbon, Portugal²; and Laboratory of Microbiology, The Rockefeller University, New York, New York³

Received 11 April 2008/Returned for modification 26 May 2008/Accepted 27 June 2008

A total of 629 nonduplicate methicillin-resistant *Staphylococcus aureus* MRSA isolates were recovered between June and November 2006 from 11 hospitals located in different areas of Portugal. Selected isolates ($n = 271$, 43%) were typed by pulsed-field gel electrophoresis (PFGE), representatives of which were additionally characterized by *spa* typing, multilocus sequence typing, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and the presence of Panton-Valentine leukocidin (PVL). The 271 isolates were classified into 13 different clonal types. Three pandemic clones included the majority ($n = 241$, 88%) of the isolates and were observed in several hospitals: (i) EMRSA-15 (54%)—PFGE type A, ST22, *spa* type t022, SCC*mec* IV—was found in the 11 hospitals studied and was identified as the major clone in seven of them; (ii) the New York/Japan clone (17%)—PFGE B, ST5, *spa* type t067, SCC*mec* II—was identified in nine hospitals and represented the major clone in four; and (iii) the Brazilian MRSA (17%)—PFGE C, ST239, *spa* type t037, SCC*mec* IIIA—was also detected in nine hospitals but never as the main clone. All isolates tested were PVL negative. Clone EMRSA-15 is currently the predominant MRSA clonal type circulating in Portuguese hospitals, but a new wave of MRSA has emerged in the country with the recent introduction and spread of the New York/Japan clone. The Brazilian MRSA that was the leading clone in Portugal in the late 1990s is declining and being progressively replaced by the two former clones. We report the first isolate SCC*mec* type V (ST45) in Portugal.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem all over the world, both in hospitals and in the community. In hospitals in Europe, it continues to spread in countries with high, medium, and even low endemicity (16). Although the prevalence of MRSA has been stabilized in some European countries and even decreased in France, Slovenia, Cyprus, and Turkey, in Portugal it was close to 45%, remaining one of the highest prevalences in Europe—just behind Malta and Romania (16).

The clonal structure of the MRSA population in different Portuguese hospitals has been analyzed in several reports since the early 1990s, and a temporal scheme for the evolution of MRSA clones between 1990 and 2000 has been constructed (2). At least two epidemiologically important events were recorded: (i) in 1992 and 1993, the replacement of the Portuguese clone (PFGE J-ST239-*spa* type t421-SCC*mec* type III variant), widespread in Portuguese hospitals in the mid-1980s and early 1990s, by the Iberian clone (PFGE G-ST247-t051-IA); and (ii) in 1994 and 1995, the emergence of the Brazilian clone (PFGE C-ST239-t037-III/IIIA) and its rapid dissemina-

tion since then. In addition, the massive replacement of the multiresistant Brazilian clone by the epidemic EMRSA-15 (PFGE A-ST22-t032-IV) clone was recently reported in a tertiary-care hospital in Oporto, Portugal (8). However, the last global national MRSA surveillance studies included isolates collected no later than 2000 (2, 43).

The aim of the present study was to identify the MRSA clonal types currently circulating in the Portuguese healthcare setting and consequently update the information on the MRSA clonal evolution over time in the country. An additional interest was to assess the entry into the hospital setting of community-acquired MRSA (CA-MRSA) strains. For this purpose, a collection of MRSA isolates recently recovered from hospitals scattered over the country was characterized by a combination of state-of-the-art molecular typing techniques.

MATERIALS AND METHODS

Bacterial isolates. A total of 629 MRSA single patient isolates were recovered between June and November 2006 from 11 hospitals situated in different areas of Portugal: six hospitals were located in the area of Lisbon, two in the area of Oporto, one in Braga, one in Coimbra, and one in Portimão (Fig. 1). Some hospitals were not enrolled in the study during the whole 6-month period, and in August some institutions were unable to preserve all isolates due to lack of personnel. A total of 271 (43%) representative isolates, including 171 hospital-acquired isolates (recovered >48 h after admission), 44 potentially community-acquired isolates (recovered <48 h of admission), and 56 isolates with no information concerning the admission date, were selected for further molecular analysis. Efforts were made to maximize the chance that the selected isolates reflected the composition of the MRSA flora in the particular hospital during the

* Corresponding author. Mailing address: The Rockefeller University, 1230 York Ave., New York, NY 10021. Phone: (212) 327-8278. Fax: (212) 327-8688. E-mail: lencash@mail.rockefeller.edu.

† M.A.-D.-S. and B.C. contributed equally to this study.

‡ The Multilaboratory Project Collaborators are listed in the Acknowledgments.

[∇] Published ahead of print on 9 July 2008.

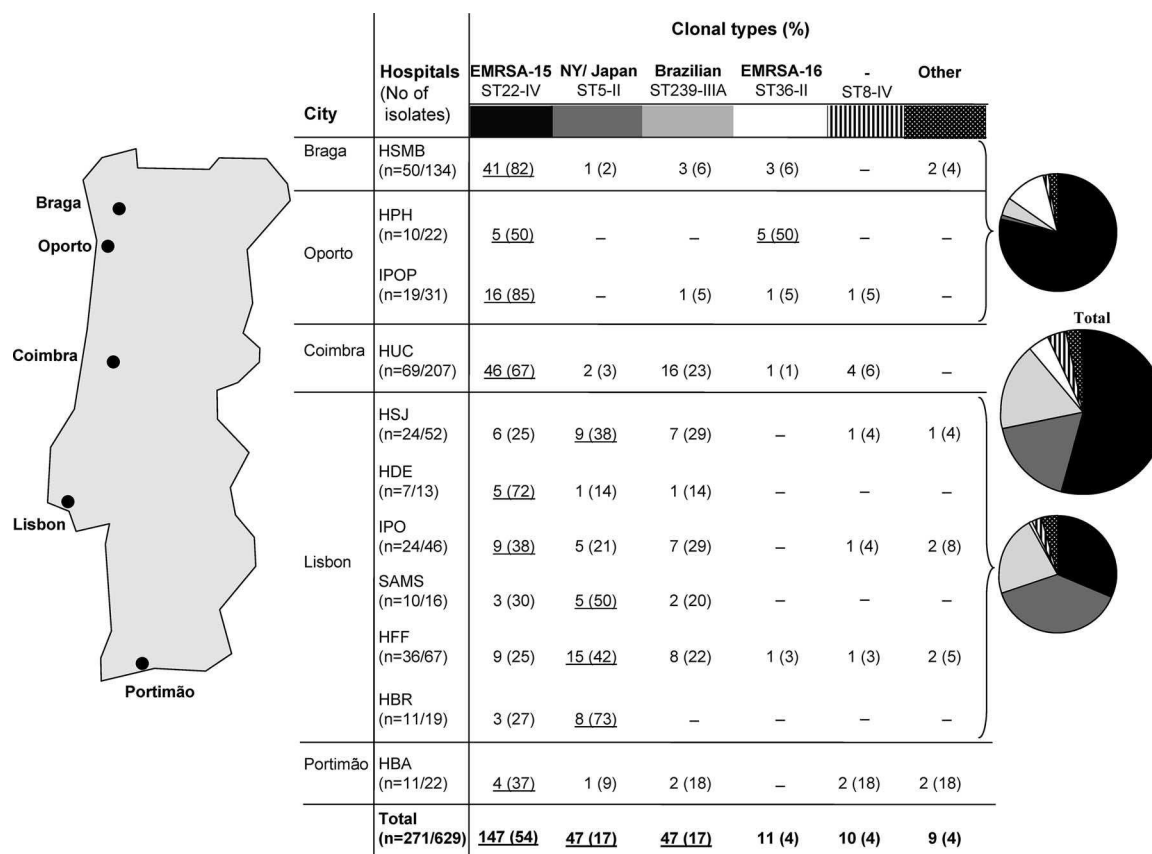


FIG. 1. Geographic location of the hospitals and clonal type distribution. In the "No. of isolates" column, the first number refers to the number of isolates studied, while the second number refers the total number of isolates recovered from the particular hospital. Abbreviations: HSMB, Hospital de São Marcos Braga, Braga; HPH, Hospital Pedro Hispano, Matosinhos; IPOP, Instituto Português de Oncologia do Porto Francisco Gentil, Oporto; HUC, Hospitais da Universidade de Coimbra, Coimbra; HSJ, Hospital de São José, Lisbon; HDE, Hospital D. Estefânia, Lisbon; IPO, Instituto Português de Oncologia de Francisco Gentil, Lisbon; SAMS, Hospital do SAMS, Lisbon; HFF, Hospital Dr. Fernando da Fonseca, Amadora; HBR, Hospital Nossa Senhora do Rosário, Barreiro; HBA, Centro Hospitalar do Barlavento Algarvio, Portimão. Underlined numbers represent the major clonal types in each hospital. Clones harboring a maximum of two isolates were grouped in "other" clonal types and include clones ST45-V variant, ST247-IA, ST5-IV, ST72-IV, ST34-II, ST228-I, ST88-IV, and ST1-IV.

surveillance period. For this reason, the first single patient isolate recovered in each ward and in each month during the study period was used in the molecular analysis. The isolates were recovered from a wide variety of infection or colonization sites: exudates from diverse origin (26%), blood (16%), lower respiratory tract (14%), sputum (9%), pus (9%), urine (9%), nasal swabs (7%), catheter (3%), and other sites (7%).

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed after SmaI digestion as described previously (12). The resulting SmaI patterns were analyzed by visual inspection using the BioNumerics software (version 4.0; Applied Maths, Gent, Belgium) as described previously (8, 10).

DNA extraction. DNA for PCR assays was extracted as previously described (31).

spa typing and MLST. *spa* typing was performed as described previously (20), and *spa* types were assigned through the Ridom web server (<http://www.ridom.de/spaserver/>). Multilocus sequence typing (MLST) was carried out as described previously (6), and MLST alleles and sequence types (STs) were identified by using the MLST database (<http://www.mlst.net>). *spa* typing and MLST were performed on a representative of the major subtype of each PFGE pattern (Table 1). The rationale for selecting representative strains and not the entire collection was the following: if any two strains share the same PFGE subtype, they have 97% probability of belonging to the same *spa* BURP group and 82% of belonging to the same ST (17).

SCCmec typing. SCCmec typing was determined by the multiplex PCR strategy developed by Oliveira and de Lencastre (40). In addition, all isolates that were found to be nontypeable or SCCmec type IV were subsequently studied using an updated version of the multiplex PCR scheme (34). Amplification of the cassette

chromosome recombinase genes (*ccrAB1*, *ccrAB2*, *ccrAB3*, *ccrAB4*, and *ccrC*) was additionally performed on these isolates, as previously described (24, 25, 41). *ccrB* sequencing was performed as described previously (42) for nontypeable isolates.

PVL detection. Pantone-Valentine leukocidin (PVL) detection was carried out as described previously (30) on representatives of each PFGE pattern.

RESULTS

SCCmec typing. The six major SCCmec types described in the literature were found in the present study (Table 1). SCCmec type I was found in association with three STs, ST247 (one isolate, SCCmec IA), ST8 (one isolate, SCCmec I variant), and ST228 (one isolate, SCCmec I). The SCCmec type I variant showed amplification of the *kdp* locus usually absent in SCCmec type I isolates. SCCmec type II was detected in isolates belonging to ST5 ($n = 47$), ST36 ($n = 11$), and ST34 ($n = 1$), whereas SCCmec type III was exclusively associated with ST239 isolates ($n = 47$). SCCmec type IV, the most frequent SCCmec type found in the present study, was linked to six STs: ST22 ($n = 147$), ST8 ($n = 8$), ST72 ($n = 2$), ST5 ($n = 1$), ST88 ($n = 1$), and ST1 ($n = 1$). A variant of SCCmec type V was

TABLE 1. Genotypic properties of the MRSA clones found in Portuguese hospitals

PFGE type	No. of isolates	No. of subtypes	Isolate tested (PFGE subtype)	<i>spa</i> ^a		ST ^b	SCCmec			Current name
				Profile	Type		Multiplex strategy ^c	<i>ccr</i> amplification	Final classification	
A	147	10	HDE461 (A1)	26-23-13-23-31-29-17-31-29-17-25-17-25-16-28	t022	(ST22)	IV	<i>ccrAB2</i>	IV	EMRSA-15
B	47	19	HBR73 (B1)	26-23-17-34-17-20-17-12-17	t067	(ST5)	II	ND ^e	II	NY/Japan
C	47	14	HUC343 (C1)	15-12-16-02-25-17-24	t037	(ST239)	IIIA	ND	IIIA	Brazilian
D	11	3	HPH2 (D1)	15-12-16-02-16-02-25-17-24-24-24	t018	(ST36)	II	ND	II	EMRSA-16
E	10	8	HSJ399 (E1)	11-19-12-21-17-34-24-34-22-25	t008	ST8	IV ^d	<i>ccrAB2</i> ^d	IV ^d	
F	2	2	HSMB103 (F1)	07-23-12-12-17-20-17-12-12-12-17	t2431	ST72	IV	<i>ccrAB2</i>	IV	
G	1	1	HSJ419 (G1)	11-02-12-21-17-34-24-34-22-25	t725	(ST247)	IA	ND	IA	Iberian
H	1	1	HBA3 (H1)	26-17-16	t535	ST5	NT	<i>ccrAB2</i>	IV	Pediatric
I	1	1	IPO516 (I1)	09-02-16-34-13-13-17-34-16-13	t2429	ST45	NT	<i>ccrC</i>	V variant	
M	1	1	HSMB35 (M1)	NT	NT	ST34	IV	<i>ccrAB2</i>	II ^f	
P	1	1	HFF335 (P1)	26-30-17-34-17-20-17-34-17-20-17-12-17-16	t041	ST228	I	ND	I	
S	1	1	IPO486 (S1)	07-12-21-17-13-13-34-34-33-34	t186	ST88	NT	<i>ccrAB2</i>	IV	
T	1	1	HBA17 (T1)	26-16-34-24-13-34-16-34-33-13	t2430	ST1	IV	<i>ccrAB2</i>	IV	

^a Nomenclature according to Harmsen et al. (20). NT, not typeable.

^b STs in parentheses were inferred according to a PFGE similarity of greater than 80% with the representatives of the corresponding pandemic clones, namely, strains JP1 (3), HU25 (50), 90/10685 (37), HPV107 (49), and 96/32010 (37) for the New York/Japan, Brazilian, EMRSA-15, Iberian, and EMRSA-16 clones, respectively;

^c Nomenclature according to Oliveira and de Lencastre (40).

^d Isolate IPO470 belonging to PFGE subtype E4 was classified as type IV by the multiplex strategy but did not amplify any of the *ccr* (*AB1*, *AB2*, *AB3*, *AB4*, and *C*) genes. Sequencing of *ccrB* showed 100% homology with strain HDE288 (42) indicating the isolate should be classified as SCCmec type VI; isolate HBA13 belonging to PFGE subtype E7 was nontypeable by the multiplex strategy but amplified *ccrAB1*. It was classified as SCCmec type I variant.

^e ND, not done.

^f The isolate is *ccrAB2*, *mecl1*, and *dcs* positive but *kdp* and *pUB110* negative.

found in one ST45 isolate. When performing the updated version of the multiplex strategy on this isolate, there was amplification of the *ccrC* locus but not of the specific region designed for SCCmec type V. One isolate belonging to ST8 was nontypeable by the multiplex strategies and did not amplify any of the *ccr* genes described thus far. However, when performing the *ccrB* typing for this isolate, we found 100% homology with the *ccrB* of SCCmec type VI reference strain, HDE288 (42).

Overall molecular typing. The characterization of the 271 MRSA strains by PFGE, *spa* typing, MLST, and SCCmec typing clustered the isolates into 13 clonal types (Table 1). PFGE analysis showed 63 subtypes and 13 types, out of which five types (A to E) were represented by at least 10 strains each and included 97% of the isolates ($n = 262$). PFGE type A, the major type ($n = 147$), was subdivided into 10 subtypes. Subtypes A1 ($n = 118$) and A6 ($n = 13$) represented 89% of the clone A isolates. PFGE A1 representative strain showed *spa* type t022 and was identical by MLST and SCCmec type analysis to the internationally disseminated EMRSA-15 clone (ST22-IV) (36). PFGE type B ($n = 47$) showed the highest number of subtypes ($n = 19$). Subtypes B1 ($n = 20$), B2 ($n = 7$) and B3 ($n = 8$) represented 74% of clone B strains. The PFGE B1 representative strain was classified as *spa* type t067 and belonged to ST5-SCCmec II clone, previously designated as the New York/Japan clone (3). PFGE type C strains ($n = 47$) were clustered into 14 subtypes. The major subtype, C1, represented 55% of the isolates ($n = 26$). Its representative strain was characterized by *spa* type t037, ST239, and SCCmec IIIA, characteristic of the pandemic Brazilian MRSA (50). PFGE type D, subdivided in three subtypes, was detected in 11 isolates. The PFGE D1 representative strain was *spa* type t018, ST36-II, characteristic of another international clone (EMRSA-16) (36). PFGE type E ($n = 10$) was associated with eight subtypes. The representative isolate E1 was *spa* type t008, ST8-IV, whereas subtype E4 belonged to SCCmec type VI, and

E7 was associated with a SCCmec type I variant. Two additional pandemic clones were also found in the present study represented by single isolates only, i.e., the Iberian (*spa* type t725, ST247-IA) and the Pediatric (*spa* type t535, ST5-IV) clones.

Hospital and geographic clonal distribution. The three clear major clones found in the present study were detected in several hospitals located in different cities (Fig. 1): (i) EMRSA-15/ST22-IV (54%) was found in the 11 hospitals studied and was identified as the major clone in seven mainly located out of Lisbon; (ii) the New York/Japan clone/ST5-II (17%) was identified in nine hospitals and represented the major clone in four all located in Lisbon; and (iii) the Brazilian MRSA/ST239-III (17%) was also detected in nine hospitals but never as the main clone and seems to be less prevalent in the northern part of the country. In addition, clone EMRSA-16/ST36-II was identified among 10 isolates recovered from four northern hospitals and a single isolate from a hospital in the area of Lisbon. Clone E (ST8) was found among 10 isolates distributed over six hospitals from four cities.

Detection of PVL. All isolates tested, representatives of the 13 PFGE types, were PVL negative.

Clonal evolution over time. Several major clones have chronologically dominated Portuguese hospitals during the last 16 years (Fig. 2): (i) the Portuguese clone (ST239-III variant) was the main clone until 1991; (ii) the Iberian clone was introduced in the country in the early 1990s and dominated between 1992 and 1998; (iii) the Brazilian clone was probably imported from Brazil in 1994–1995 and rapidly increased in prevalence since then becoming the major clone between 1998 and 2000; (iv) EMRSA-15 was most likely introduced in Portugal in 2001 (8) and replaced very soon the Brazilian clone becoming the predominant clone; and (v) the New York/Japan reported for the first time in a northern Portuguese hospital in 2005 (8) accounted for 17% of the isolates in 2006 and therefore seems to

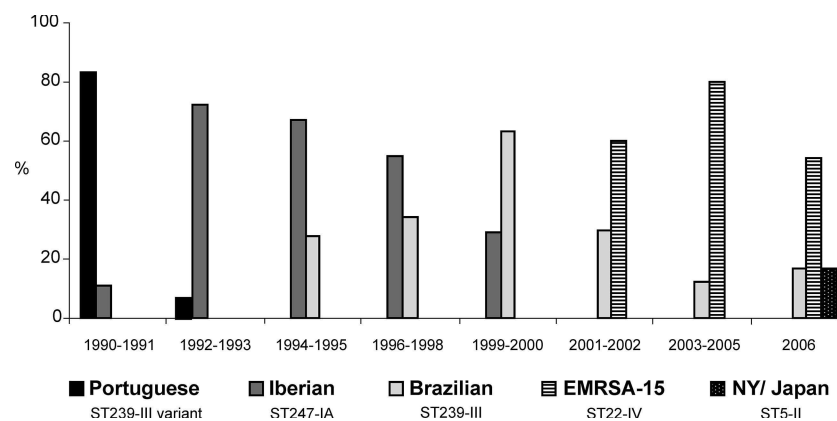


FIG. 2. Evolution of the clonal profile of MRSA in Portuguese hospitals between 1990 and 2006. The ST and SCC mec type are indicated under the clonal type current name. The data before 2000 were adapted from Aires de Sousa and de Lencastre (2).

be a probable candidate for the leading clone in Portugal in the near future. It should be mentioned that the values used for 2001 and 2002 (unpublished results) and 2003 to 2005 (8) were obtained in two hospitals only and therefore are simply indicators of the probable national trend. For that reason, the prevalence of the Brazilian clone is shown a little higher in 2003 to 2005 than in 2006 and does not reflect the national trend of the decline of the Brazilian clone.

DISCUSSION

The incidence of MRSA in Portuguese hospitals remains one of the highest in Europe (16). In order to obtain an update of the contemporary MRSA clonal types, we characterized isolates recovered during the second half of 2006 from 11 Portuguese hospitals scattered over the country and demonstrated the massive dominance of EMRSA-15 clone (ST22-IV) in coexistence with the New York/Japan (ST5-II) and the Brazilian (ST239-III) clones.

EMRSA-15, together with EMRSA-16, remains one of the two most dominant epidemic clones in the United Kingdom (26). Both clones emerged in the United Kingdom in 1991, spread widely, and account for 93 to 95% of MRSA isolates seen in recent years (27). Besides having been detected in several countries (<http://saureus.mlst.net>), EMRSA-15 is replacing previous established clones in different regions of the world. In Europe, it was found in a number of hospitals throughout Germany (53), and it is currently established as the major clone in the Czech Republic (33) and in the Majorcan islands (7). A recent study performed in a hospital in Oporto, Portugal, showed that clone EMRSA-15, which might have been introduced in the hospital in 2001, represents the overwhelming majority (79%) of the isolates collected between 2003 and 2005 (8). In Asia, EMRSA-15 represented 66.1% of all MRSA isolated in local hospitals in Singapore in the first half of 2006, replacing the ST239 clone island-wide (22).

The New York/Japan clone, initially described as the main clone in the United States (46) and Japan (3) and subsequently detected in other countries (<http://saureus.mlst.net>), has been recently reported for the first time as the main clone in a European country, in Hungary, in coexistence with the Southern German clone (ST228-I) (13). It was also found as

the main clone in Asia, in Korea (29). This clone was detected for the first time in a Portuguese hospital in 2005 as a single isolate (8).

The Brazilian MRSA was first shown to be widely disseminated in Brazilian hospitals (50) and to have spread to neighboring countries in South America (Argentina [14] and Uruguay and Chile [4]) and to Europe (Portugal [5, 39], the Czech Republic [32], and Greece [1]), where it displaced the local major clones. In addition, a recent study supports the view that at least 90% of MRSA isolates in mainland Asia correspond to ST239 or close relatives (18). Although the Brazilian clone is still well represented in Portuguese hospitals (17%) and has been found in nine out of 11 hospitals studied, it was never found as the dominant clone, which might indicate it is being progressively replaced in the Portuguese nosocomial setting by other clones.

The EMRSA-16 clone (ST36-II), which accounted for 4% of the isolates in the present study, has been reported to be currently the major clone in two hospitals in Vigo, northwest Spain (45), and in two hospitals of the Canary Islands (35, 44). Although EMRSA-16 was found as a minor clone, it has been detected in 5 of the 11 Portuguese hospitals studied, and it is possible that it will increase in prevalence in the future, as happened in some hospitals in the neighboring country.

The pandemic Iberian clone (ST247-IA), which was disseminated in the large majority of Portuguese hospitals between 1992 and 1996 (2, 43), was found in a single isolate only in the present study. This observation agrees with other studies that have demonstrated a decrease in the incidence of the Iberian clone in Europe (15, 31, 35, 44, 52). Similarly, the Pediatric clone (ST5-IV) described for the first time in a pediatric hospital in Lisbon (48) was not in the same hospital in 2006. The single isolate ST5-IV was recovered from an adult hospitalized in the southern region of the country.

Clonal type ST8 associated with different SCC mec types (IV, I variant, and VI), represented by 4% of the isolates in the present study, has been previously described mainly associated with CA-MRSA strains and in many cases coupled with PVL genes. In our study none of the isolates tested, which represented all clonal types, was PVL positive. An interesting observation was the detection of an isolate ST45-V variant.

Reports regarding strains carrying SCCmec type V are increasingly common. Strains harboring this SCCmec type were identified in CA-MRSA isolates from Australia (38), Taiwan (9, 23), Greece (19), Israel (11), Finland (28), and Hong Kong (21) mostly associated with STs 59 and 5 but also with ST45. ST398-V MRSA was identified in Dutch pigs and pig farmers and, although its natural host is probably porcine, the clone causes infections in humans (51). To our knowledge, this is the first report of a SCCmec type V strain in Portugal. Although the prevalence of CA-MRSA seems to be very low in Portugal (47), we cannot exclude the hypothesis that the clone ST45-V variant, as well as ST8, might have been imported from the community to the hospital.

Interestingly, the distribution of clones was not equivalent all over the country. Although EMRSA-15 is globally the major clone, it was found as the main clone mainly in hospitals out of Lisbon in opposition to the New York/Japan clone (Fig. 1). Hospitals located in the capital are probably more prone to international clone's exchange, and the observed results might be an indicator that EMRSA-15 is starting to be replaced by the New York/Japan clone.

Taking into consideration the present work combined with previous studies and the information recently reported by Amorim et al. (8), we could update the MRSA evolution in Portuguese hospitals (Fig. 2), showing there have been several waves of major MRSA clones during the last 16 years in Portugal. Interestingly, it seems the predominant MRSA clone in Portugal, a country with an increasing prevalence of MRSA, is replaced every few years in contrast to other countries with a similar prevalence of MRSA, such as the United Kingdom, where EMRSA-15 and EMRSA-16 have been stable for decades.

In summary, we describe here the clear dominance of EMRSA-15 in Portuguese hospitals and the significant coexistence of the recently introduced New York/Japan clone. These two clones are progressively replacing the previous dominant clone, the Brazilian MRSA. The global scheme for the MRSA clonal evolution in Portuguese hospitals was updated, including data between 1990 and 2006 showing unusual temporal shifts in the dominant MRSA clones. Moreover, this is the first report of an isolate ST45-V in Portugal, which might have been imported from the community.

ACKNOWLEDGMENTS

This study was supported by Project POCTI/SAU-ESP/57841/2004 from Fundação para a Ciência e Tecnologia, Portugal, awarded to H.D.L.

We thank Catarina Milheiro for performing SCCmec typing confirmation essays of isolates IPO516, IPO470, HSMB35, and HBA13.

The multilaboratory project collaborators included: Valquiria Alves (Hospital Pedro Hispano, Matosinhos, Portugal), Fernando Branca (Hospital de São Marcos Braga, Braga, Portugal), Luísa Cabral (Hospital do SAMS, Lisbon, Portugal), José Clemente (Hospital Nossa Senhora do Rosário, Barreiro, Portugal), Isabel Daniel (Hospital D. Estefânia, Lisbon, Portugal), Alberta Faustino (Hospital de São Marcos Braga, Braga, Portugal), Etelvina Ferreira (Hospital Nossa Senhora do Rosário, Barreiro, Portugal), Catarina Lameiras (Instituto Português de Oncologia do Porto Francisco Gentil, Oporto, Portugal), José Lopes (Hospital Nossa Senhora do Rosário, Barreiro, Portugal), João Marques (Hospital de São José, Lisbon, Portugal), Isabel Peres (Hospital D. Estefânia, Lisbon, Portugal), Graça Ribeiro (Hospitais da Universidade de Coimbra, Coimbra, Portugal), Luísa Sancho (Hospital Dr. Fernando da Fonseca, Amadora, Portugal), Odete Santos

(Hospital de São José, Lisbon, Portugal), Pedro Santos (Hospital Nossa Senhora do Rosário, Barreiro, Portugal), Maria Teresa Vaz (Centro Hospitalar do Barlavento Algarvio, Portimão, Portugal), and Zélia Videira (Instituto Português de Oncologia de Francisco Gentil, Lisbon, Portugal).

REFERENCES

- Aires de Sousa, M., C. Bartzavali, I. Spiliopoulou, I. Santos Sanches, M. I. Crisostomo, and H. de Lencastre. 2003. Two international methicillin-resistant *Staphylococcus aureus* clones endemic in a university hospital in Patras, Greece. *J. Clin. Microbiol.* **41**:2027–2031.
- Aires de Sousa, M., and H. de Lencastre. 2004. Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones. *FEMS Immunol. Med. Microbiol.* **40**:101–111.
- Aires de Sousa, M., H. de Lencastre, I. Santos Sanches, K. Kikuchi, K. Totsuka, and A. Tomasz. 2000. Similarity of antibiotic resistance patterns and molecular typing properties of methicillin-resistant *Staphylococcus aureus* isolates widely spread in hospitals in New York City and in a hospital in Tokyo, Japan. *Microb. Drug Resist.* **6**:253–258.
- Aires de Sousa, M., M. Miragaia, I. S. Sanches, S. Avila, I. Adamson, S. T. Casagrande, M. C. Brandileone, R. Palacio, L. Dell'Acqua, M. Hortal, Z. Camou, A. Rossi, M. E. Velazquez-Meza, G. Echaniz-Aviles, F. Solorzano-Santos, I. Heitmann, and H. de Lencastre. 2001. Three-year assessment of methicillin-resistant *Staphylococcus aureus* clones in Latin America from 1996 to 1998. *J. Clin. Microbiol.* **39**:2197–2205.
- Aires de Sousa, M., I. S. Sanches, M. L. Ferro, M. J. Vaz, Z. Saraiva, T. Tendeiro, J. Serra, and H. de Lencastre. 1998. Intercontinental spread of a multidrug-resistant methicillin-resistant *Staphylococcus aureus* clone. *J. Clin. Microbiol.* **36**:2590–2596.
- Aires-de-Sousa, M., C. E. Parente, O. Vieira-da-Motta, I. C. Bonna, D. A. Silva, and H. de Lencastre. 2007. Characterization of *Staphylococcus aureus* isolates from buffalo, bovine, ovine, and caprine milk samples collected in Rio de Janeiro State, Brazil. *Appl. Environ. Microbiol.* **73**:3845–3849.
- Alcoceba, E., A. Mena, M. Cruz Perez, E. Ruiz de Gopegui, E. Padilla, J. Gil, A. Ramirez, C. Gallegos, A. Serra, J. L. Perez, and A. Oliver. 2007. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Majorcan hospitals: high prevalence of the epidemic clone EMRSA-15. *Clin. Microbiol. Infect.* **13**:599–605.
- Amorim, M. L., N. A. Faria, D. C. Oliveira, C. Vasconcelos, J. C. Cabeda, A. C. Mendes, E. Calado, A. P. Castro, M. H. Ramos, J. M. Amorim, and H. de Lencastre. 2007. Changes in the clonal nature and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* isolates associated with spread of the EMRSA-15 clone in a tertiary care Portuguese hospital. *J. Clin. Microbiol.* **45**:2881–2888.
- Boyle-Vavra, S., B. Ereshesky, C. C. Wang, and R. S. Daum. 2005. Successful multiresistant community-associated methicillin-resistant *Staphylococcus aureus* lineage from Taipei, Taiwan, that carries either the novel Staphylococcal chromosome cassette *mec* (SCCmec) type VT or SCCmec type IV. *J. Clin. Microbiol.* **43**:4719–4730.
- Carrico, J. A., F. R. Pinto, C. Simas, S. Nunes, N. G. Sousa, N. Frazao, H. de Lencastre, and J. S. Almeida. 2005. Assessment of band-based similarity coefficients for automatic type and subtype classification of microbial isolates analyzed by pulsed-field gel electrophoresis. *J. Clin. Microbiol.* **43**:5483–5490.
- Chmelnitsky, I., S. Navon-Venezia, A. Leavit, E. Somekh, G. Regev-Yochai, M. Chowers, P. Shitrit, and Y. Carmeli. 2008. SCCmec and spa types of methicillin-resistant *Staphylococcus aureus* strains in Israel: detection of SCCmec type V. *Eur. J. Clin. Microbiol. Infect. Dis.* **27**:385–390.
- Chung, M., H. de Lencastre, P. Matthews, A. Tomasz, I. Adamsson, M. Aires de Sousa, T. Camou, C. Cocuzza, A. Corso, I. Couto, A. Dominguez, M. Gniadkowski, R. Goering, A. Gomes, K. Kikuchi, A. Marchese, R. Mato, O. Melter, D. Oliveira, R. Palacio, R. Sá-Leão, I. Santos Sanches, J. H. Song, P. T. Tassios, and P. Villari. 2000. Molecular typing of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis: comparison of results obtained in a multilaboratory effort using identical protocols and MRSA strains. *Microb. Drug Resist.* **6**:189–198.
- Conceicao, T., M. Aires-de-Sousa, M. Fuzi, A. Toth, J. Paszti, E. Ungvari, W. B. van Leeuwen, A. van Belkum, H. Grundmann, and H. de Lencastre. 2007. Replacement of methicillin-resistant *Staphylococcus aureus* clones in Hungary over time: a 10-year surveillance study. *Clin. Microbiol. Infect.* **13**:971–979.
- Corso, A., I. Santos Sanches, M. Aires de Sousa, A. Rossi, and H. de Lencastre. 1998. Spread of a methicillin-resistant and multiresistant epidemic clone of *Staphylococcus aureus* in Argentina. *Microb. Drug Resist.* **4**:277–288.
- Cuevas, O., E. Cercenado, E. Bouza, C. Castellares, P. Trincado, R. Cabrera, and A. Vindel. 2007. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Spain: a multicenter prevalence study (2002). *Clin. Microbiol. Infect.* **13**:250–256.
- European Antimicrobial Resistance Surveillance System. 2007. EARSS an-

- nual report 2006. EARSS, Bilthoven, The Netherlands. <http://www.earss.rivm.nl>.
17. Faria, N. A., J. A. Carrico, D. C. Oliveira, M. Ramirez, and H. de Lencastre. 2008. Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. *J. Clin. Microbiol.* **46**:136–144.
 18. Feil, E. J., E. K. Nickerson, N. Chantratita, V. Wuthiekanun, P. Srisomang, R. Cousins, W. Pan, G. Zhang, B. Xu, N. P. Day, and S. J. Peacock. 2008. Rapid detection of the pandemic methicillin-resistant *Staphylococcus aureus* clone ST 239 and its dominance in Asian hospitals. *J. Clin. Microbiol.*
 19. Gerogianni, I., G. Mpatavanis, K. Gourgoulialis, A. Maniatis, I. Spiliopoulou, and E. Petinaki. 2006. Combination of staphylococcal chromosome cassette SCCmec type V and Pantone-Valentine leukocidin genes in a methicillin-resistant *Staphylococcus aureus* that caused necrotizing pneumonia in Greece. *Diagn. Microbiol. Infect. Dis.* **56**:213–216.
 20. Harmsen, D., H. Claus, W. Witte, J. Rothganger, D. Turnwald, and U. Vogel. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J. Clin. Microbiol.* **41**:5442–5448.
 21. Ho, P. L., T. K. Wang, P. Ching, G. C. Mak, E. Lai, W. C. Yam, and W. H. Seto. 2007. Epidemiology and genetic diversity of methicillin-resistant *Staphylococcus aureus* strains in residential care homes for elderly persons in Hong Kong. *Infect. Control Hosp. Epidemiol.* **28**:671–678.
 22. Hsu, L. Y., N. Loomba-Chlebicka, Y. L. Koh, T. Y. Tan, P. Krishnan, R. T. Lin, N. W. Tee, D. A. Fisher, and T. H. Koh. 2007. Evolving EMRSA-15 epidemic in Singapore hospitals. *J. Med. Microbiol.* **56**:376–379.
 23. Huang, Y. C., K. P. Hwang, P. Y. Chen, C. J. Chen, and T. Y. Lin. 2007. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization among Taiwanese children in 2005 and 2006. *J. Clin. Microbiol.* **45**:3992–3995.
 24. Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu. 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **45**:1323–1336.
 25. Ito, T., X. X. Ma, F. Takeuchi, K. Okuma, H. Yuzawa, and K. Hiramatsu. 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. *Antimicrob. Agents Chemother.* **48**:2637–2651.
 26. Johnson, A. P., H. M. Aucken, S. Cavendish, M. Ganner, M. C. Wale, M. Warner, D. M. Livermore, and B. D. Cookson. 2001. Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *J. Antimicrob. Chemother.* **48**:143–144.
 27. Johnson, A. P., A. Pearson, and G. Duckworth. 2005. Surveillance and epidemiology of MRSA bacteraemia in the UK. *J. Antimicrob. Chemother.* **56**:455–462.
 28. Kerttula, A. M., O. Lyytikäinen, M. Karden-Lilja, S. Ibrahim, S. Salmenlinna, A. Virolainen, and J. Vuopio-Varkila. 2007. Nationwide trends in molecular epidemiology of methicillin-resistant *Staphylococcus aureus*, Finland, 1997–2004. *BMC Infect. Dis.* **7**:94.
 29. Ko, K. S., J. Y. Lee, J. Y. Suh, W. S. Oh, K. R. Peck, N. Y. Lee, and J. H. Song. 2005. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. *J. Clin. Microbiol.* **43**:421–426.
 30. Lina, G., Y. Piemont, F. Godail-Gamot, M. Bes, M. O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne. 1999. Involvement of Pantone-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin. Infect. Dis.* **29**:1128–1132.
 31. Melter, O., M. Aires de Sousa, P. Urbaskova, V. Jakubu, H. Zemlickova, and H. de Lencastre. 2003. Update of the major clonal types of methicillin-resistant *Staphylococcus aureus* in the Czech Republic. *J. Clin. Microbiol.* **41**:4998–5005.
 32. Melter, O., I. Santos Sanches, J. Schindler, M. Aires de Sousa, R. Mato, V. Kovarova, H. Zemlickova, and H. de Lencastre. 1999. Methicillin-resistant *Staphylococcus aureus* clonal types in the Czech Republic. *J. Clin. Microbiol.* **37**:2798–2803.
 33. Melter, O., P. Urbaskova, V. Jakubu, B. Mackova, and H. Zemlickova. 2006. Emergence of EMRSA-15 clone in hospitals throughout the Czech Republic. *Eur. Surveill.* **11**:E060803–E060806.
 34. Milheirico, C., D. C. Oliveira, and H. de Lencastre. 2007. Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **51**:3374–3377.
 35. Montesinos, I., T. Delgado, D. Riverol, E. Salido, M. A. Miguel, A. Jimenez, and A. Sierra. 2006. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* associated with the emergence of EMRSA-16 at a university hospital. *J. Hosp. Infect.* **64**:257–263.
 36. Moore, P. C., and J. A. Lindsay. 2002. Molecular characterization of the dominant UK methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and EMRSA-16. *J. Med. Microbiol.* **51**:516–521.
 37. Murchan, S., M. E. Kaufmann, A. Deplano, R. de Ryck, M. Struelens, C. E. Zinn, V. Fussing, S. Salmenlinna, J. Vuopio-Varkila, N. El Solh, C. Cuny, W. Witte, P. T. Tassios, N. Legakis, W. van Leeuwen, A. van belkum, A. Vindel, I. Laconcha, J. Garaizar, G. Coombes, and B. Cookson. 2003. Harmonization of pulsed-field gel electrophoresis for epidemiological typing of methicillin-resistant *Staphylococcus aureus* by consensus in 10 European centers and its use to plot the spread of related strains. *J. Clin. Microbiol.* **41**:1574–1585.
 38. O'Brien, F. G., G. W. Coombs, J. C. Pearson, K. J. Christiansen, and W. B. Grubb. 2005. Type V staphylococcal cassette chromosome *mec* in community staphylococci from Australia. *Antimicrob. Agents Chemother.* **49**:5129–5132.
 39. Oliveira, D., I. Santos-Sanches, R. Mato, M. Tamayo, G. Ribeiro, D. Costa, and H. de Lencastre. 1998. Virtually all methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the largest Portuguese teaching hospital are caused by two internationally spread multiresistant strains: the 'Iberian' and the 'Brazilian' clones of MRSA. *Clin. Microbiol. Infect.* **4**:373–384.
 40. Oliveira, D. C., and H. de Lencastre. 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **46**:2155–2161.
 41. Oliveira, D. C., C. Milheirico, S. Vinga, and H. de Lencastre. 2006. Assessment of allelic variation in the *ccrAB* locus in methicillin-resistant *Staphylococcus aureus* clones. *J. Antimicrob. Chemother.* **58**:23–30.
 42. Oliveira, D. C., M. Santos, C. Milheirico, J. A. Carrico, S. Vinga, A. L. Oliveira, and H. de Lencastre. 2008. CcrB typing tool: an online resource for staphylococci *ccrB* sequence typing. *J. Antimicrob. Chemother.* **61**:959–960.
 43. Oliveira, D. C., A. Tomasz, and H. de Lencastre. 2002. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect. Dis.* **2**:180–189.
 44. Perez-Roth, E., F. Lorenzo-Diaz, N. Batista, A. Moreno, and S. Mendez-Alvarez. 2004. Tracking methicillin-resistant *Staphylococcus aureus* clones during a 5-year period (1998 to 2002) in a Spanish hospital. *J. Clin. Microbiol.* **42**:4649–4656.
 45. Potel, C., M. Alvarez, P. Alvarez, I. Otero, and E. Fluiters. 2007. Evolution, antimicrobial susceptibility, and assignment to international clones of methicillin-resistant *Staphylococcus aureus* isolated over a 9-year period in two Spanish hospitals. *Clin. Microbiol. Infect.* **13**:728–730.
 46. Roberts, R. B., A. de Lencastre, W. Eisner, E. P. Severina, B. Shopsin, B. N. Kreiswirth, A. Tomasz, et al. 1998. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in 12 New York hospitals. *J. Infect. Dis.* **178**:164–171.
 47. Sa-Leão, R., I. S. Sanches, I. Couto, C. R. Alves, and H. de Lencastre. 2001. Low prevalence of methicillin-resistant strains among *Staphylococcus aureus* colonizing young and healthy members of the community in Portugal. *Microb. Drug Resist.* **7**:237–245.
 48. Sa-Leão, R., I. Santos Sanches, D. Dias, I. Peres, R. M. Barros, and H. de Lencastre. 1999. Detection of an archaic clone of *Staphylococcus aureus* with low-level resistance to methicillin in a pediatric hospital in Portugal and in international samples: relics of a formerly widely disseminated strain? *J. Clin. Microbiol.* **37**:1913–1920.
 49. Sanches, I. S., M. Ramirez, H. Troni, M. Abecassis, M. Padua, A. Tomasz, and H. de Lencastre. 1995. Evidence for the geographic spread of a methicillin-resistant *Staphylococcus aureus* clone between Portugal and Spain. *J. Clin. Microbiol.* **33**:1243–1246.
 50. Teixeira, L. A., C. A. Resende, L. R. Ormonde, R. Rosenbaum, A. M. Figueiredo, H. de Lencastre, and A. Tomasz. 1995. Geographic spread of epidemic multiresistant *Staphylococcus aureus* clone in Brazil. *J. Clin. Microbiol.* **33**:2400–2404.
 51. van Duijkere, E., R. Ikawaty, M. J. Broekhuizen-Stins, M. D. Jansen, E. C. Spalburg, A. J. de Neeling, J. G. Allaart, A. van Nes, J. A. Wagenaar, and A. C. Fluit. 2008. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. *Vet. Microbiol.* **126**:383–389.
 52. Vindel, A., P. Trincado, E. Gomez, R. Cabrera, T. Boquete, C. Sola, S. Valdezate, and J. A. Saez-Nieto. 2006. Prevalence and evolution of methicillin-resistant *Staphylococcus aureus* in Spanish hospitals between 1996 and 2002. *J. Clin. Microbiol.* **44**:266–270.
 53. Witte, W., M. Kresken, C. Bralke, and C. Cuny. 1997. Increasing incidence and widespread dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals in central Europe, with special reference to German hospitals. *Clin. Microbiol. Infect.* **3**:414–422.