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Intercontinental Spread of a Multidrug-Resistant Methicillin-Resistant *Staphylococcus aureus* Clone

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Two hundred ten methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered between 1990 and 1997 from three Portuguese hospitals located in Lisbon and Oporto were analyzed by molecular fingerprinting techniques. The hybridization of *Cla*I restriction digests with the *mecA*- and Tn*554*-specific DNA probes combined with pulsed-field gel electrophoresis documented the abrupt appearance and extensive intrahospital spread of the Brazilian epidemic MRSA clone in the 1995 samples of each one of the three hospitals analyzed—suggesting the intercontinental transfer of this strain from Brazil to Portugal. The appearance of this clone may challenge the dominance of another highly epidemic imported clone—the Iberian MRSA, currently the most widely spread MRSA clone in Portuguese hospitals.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the highest-ranking nosocomial pathogens throughout the world, capable of causing a wide range of hospital infections. MRSA remains a particularly serious problem in Portugal because of the alarmingly high incidence and multidrug resistance of the strains. The percentage of MRSA strains accounting for *S. aureus* infections in Portugal according to the European Prevalence of Infection in Intensive Care study carried out in 1992 was estimated as close to 65% (26). The incidence of MRSA according to two national multicenter studies was estimated as 49% in 1993 (10) and 47% in 1994 (9). These high values are presumably related to the inappropriate use of antimicrobial drugs and to insufficient infection control measures in Portuguese hospitals (9). In addition, many of the contemporary MRSA strains were multiresistant and were often untypeable with the international set of phages. For such strains, molecular typing techniques have begun to replace conventional typing methods in epidemiological investigations (2, 3, 19, 20, 22, 25).

By some of these methods, previous studies have documented the emergence of two particularly widely disseminated multiresistant clones of MRSA. One of these, the Iberian MRSA, first identified as the dominant clone in a major outbreak of MRSA disease in the Bellvitge Hospital in Barcelona, Spain, in 1989 (5), was subsequently detected in at least eight Portuguese hospitals (1, 13, 15–18) as well as in hospitals in western Scotland, United Kingdom; Rome, Italy; Brussels, Belgium; and Hanover, Germany (7), and in one hospital in New York City (14). A second and distinct multiresistant clone (Brazilian MRSA) was shown to be widely spread in Brazilian hospitals, separated by several thousand kilometers from one another (21).

Monitoring the geographic expansion of such epidemic clones is important for understanding why certain MRSA clones spread over considerable distances whereas others are limited to a single country. While a continuously operating nosocomial surveillance system is not yet functioning in Portugal, a substantial number of MRSA isolates became available to us from strain collections recovered between 1990 and 1997 in three Portuguese hospitals located in the two largest cities (Lisbon and Oporto) of Portugal.

In this paper, we describe the characterization of these strains by molecular typing techniques. While the size of samples available from these sources at the different collection periods was not uniform, our molecular data clearly document the arrival of the Brazilian MRSA clone in Portugal and also suggest a rapid increase in the representation of this clone during the surveillance period. The finding suggests transfer of this clone from Brazil to Portugal.

MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility. A collection of 210 isolates of MRSA was obtained from three hospitals located in the two largest Portuguese cities: the first group of isolates came from Hospital de São João in Oporto and was characterized at the Faculdade de Farmácia do Porto (FFP) (96 FFP isolates); the second and third groups of isolates were from two hospitals in Lisbon: Hospital de São José (HSJ) (91 HSJ isolates) and Instituto Português de Oncologia de Francisco Gentil (IPO) (23 IPO isolates), respectively. Of the 96 FFP isolates, 9 were isolated in 1990, 9 were isolated in 1991, 14 were isolated in 1992, and 64 were isolated in 1996. The percentage of MRSA strains was estimated in this hospital as 32% during 1990 to 1992 and as 65% in 1996. Among the 91 HSJ isolates, 36 isolates were recovered in 1995, 36 were recovered in 1996, and 19 were recovered in 1997. The percentage of MRSA strains was estimated in this hospital as 71% in 1995 and 1996 and as 72% in 1997. The 23 IPO isolates were all collected in 1997 when the percentage of MRSA strains in this hospital was estimated as close to 25%. Efforts were made to maximize the chance that the collection of isolates reflected the composition of the MRSA flora in the particular hospital during the surveillance period. For this reason, single patient isolates (with one exception) recovered from a wide variety of infection sites (sputum, pus, blood, bronchial secretions, and urine) and from several hospital wards (medicine, surgery, intensive care unit [ICU], dermatology, and burn unit) were used in the molecular analysis.

Antibiotic susceptibilities were tested by disk diffusion methods (12) or Vitek or ATB systems (BioMérieux, Marcy l'Etoile, France). A rapid screening for the methicillin resistance phenotype (homogeneous or heterogeneous [24]) was performed by the methicillin 1-mg disk method (4), and the presence of the *mecA* gene was confirmed in all strains by DNA hybridization (3) or PCR analysis (11). Resistance to 500 μg of spectinomycin per ml (6) was evaluated by spotting 5-μl

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TABLE 1. Evolution of the clonal types found in three different Portuguese hospitals between 1990 to 1992 and 1995 to 1997

Clonal type	Genotypic characteristics (*mecA*::Tn*554*::PFGE/RAPD)	Phenotypic characteristics (SXT/SPC)[c]	Hospital	% of isolates belonging to clone in yr:					
				1990	1991	1992	1995	1996	1997
Portuguese	III::α::C/A′D	S/R	FFP	78	89	36		0	
			IPO						0
			HSJ				0	0	0
Iberian and related[a]	I::E::A/AA	S/R	FFP	22	0	57		55	
			IPO						70
			HSJ				53	65	47
Brazilian and related[b]	XI::B::B/A′D	R/S	FFP	0	0	0		43	
			IPO						30
			HSJ				47	36	53

[a] Related clones for the Iberian clone: FFP (I::J::A and I::NH::A), IPO (I::J::A), and HSJ (I::J::A, I::NH::A, I::DD::A, II::DD::AA, I::νν::A, and I::μμ::A).
[b] Related clones for the Brazilian clone: FFP (XI::B′::B) and HSJ (XI::κ::B).
[c] SPC, spectinomycin; R, resistant; S, susceptible.

drops of overnight-grown cultures (10^9 to 10^{10} CFU/ml) onto tryptic soy agar plates containing this concentration of the antibiotic.

Control strains. Isolates representing the clonal types of the Iberian (strains HPV34, HPV107 [17], and PER34 [5]), Brazilian (strain HU25 [21]), and Portuguese (strain CPS21 [3]) MRSA clones were obtained from strain collections at the Instituto de Tecnologia Química e Biológica and The Rockefeller University.

***mecA* and Tn*554* polymorphisms.** The purification of whole genomic DNA was performed according to methods already established for *S. aureus* (3). Chromosomal DNA was digested with the restriction endonuclease *Bsp*106 (isoschizomer of *Cla*I) (Stratagene, La Jolla, Calif.), and the membrane was hybridized with the *mecA*- and the Tn*554*-specific DNA probes (3). DNA fragments were transferred to Hybond N^+ (Amersham International, Little Chalfont, United Kingdom) nylon membranes (3) and hybridized with a nonradioactive probe labeled with the RPN 3040 ECL system (Amersham International). Hybridization patterns involving *mecA* or Tn*554* were identified by comparison with previously described types (3, 5, 6).

PFGE. Pulsed-field gel electrophoresis (PFGE) after digestion with *Sma*I enzyme (New England Biolabs, Beverly, Mass.) was carried out in an LKB Pharmacia 2015 Gene Navigator apparatus-Pulsaphor system (Pharmacia LKB, Stockholm, Sweden). The running conditions have already been described elsewhere (16). Interpretation was performed with previously determined criteria (23).

Random amplification of polymorphic DNA (RAPD). Forty-two isolates representing PFGE subtype variants, different *mecA* polymorphs, and variants of Tn*554* type were also examined by RAPD as a potential confirmatory method. DNA isolation and PCR were performed essentially as described before (1), and two different primers were included in the typing assays. The designations and sequences of the primers were as follows: ERIC1, 5′-ATGTAAGCTCCTGGGGATTCAC-3′; ERIC2, 5′-AAGTAAGTGACTGGGGTGACGC-3′ (27). The amplification was performed in a Perkin-Elmer 9600 PCR machine (Perkin-Elmer, Norwalk, Conn.) and consisted of the following steps: predenaturation at 94°C for 4 min and 40 cycles of 45 s at 94°C, 45 s at 25°C, and 90 s at 74°C. PCR products were separated by 1% agarose gel electrophoresis. After photography, DNA fingerprints were compared by visual inspection. New RAPD types were defined on the basis of two band differences and identified with capital letters; patterns that differed in a single band were indicated by a prime after the capital letter.

RESULTS

Antibiotic resistance phenotype. Analyzing the antibiotic susceptibilities to the common panel of six antibiotics tested with the 210 isolates showed 100% resistance to penicillin, methicillin, and oxacillin and 100% susceptibility to vancomycin. All isolates except one were resistant to erythromycin, and resistance to trimethoprim-sulfamethoxazole (SXT) showed strain-to-strain variation which paralleled clonal features of the isolates (Table 1).

Among the 210 isolates, 149 strains (71%) were resistant to 500 μg of spectinomycin per ml, 6 strains (3%) were intermediately resistant, and 55 strains (26%) were susceptible.

Clonal assignments. Strains were classified into clonal types on the basis of a combination of their particular *mecA* and Tn*554* polymorphisms and PFGE patterns (*mecA*::Tn*554*::PFGE types). Some of the *mecA* polymorphs (I, II, III, and XI) and Tn*554* insertion patterns (E, J, NH, B, and DD) found in this study were previously described (3, 5, 6). One new *Cla*I-*mecA* type, XIII, and five novel Tn*554* patterns named χ, B′, μμ, νν, and κ were found in this study.

Hospital de São João. Of the nine isolates recovered during 1990 from the Hospital de São João (FFP isolates), two isolates (22%) belonged to clonal type I::E::A while the other seven (78%) strains belonged to clonal type III::α::C (Table 2).

Eight of the nine FFP isolates from 1991 belonged to clonal type III::α::C (89%); the remaining single strain had a completely different and novel clonal type, XIII::χ::D (Table 2). The new *mecA* type, XIII, has two hybridization bands of approximately 7.7 and 2.2 kb. The new Tn*554* pattern χ corresponds probably to a single insertion of the transposon. Interestingly, in this strain the Tn*554* hybridization produced only one band, of approximately 4.2 kb, suggesting either the absence of a *Cla*I restriction site or deletion of part of Tn*554*.

The study of 14 isolates recovered during 1992 from the Hospital de São João allowed the identification of four clonal types (Table 2). Twelve strains belonged to previously identified clones: I::E::A ($n = 7$ [50%]) and III::α::C ($n = 5$ [36%]). A single isolate, I::J::A, might have evolved from the clone I::E::A, because it differs only in the number of insertions of Tn*554*. The prevalence of clone I::E::A and its related clone I::J::A was 57% in 1992 (Table 1). The remaining isolate belonged to a new clone, III::α::E. This clonal type was probably unrelated to III::α::C because it differs in more than six *Sma*I fragments (23).

The analysis of the 64 FFP isolates collected during 1996 allowed the identification of six different clones (Table 2). Clonal type I::E::A and the related clones I::J::A (duplication of Tn*554*) and I::NH::A (loss of Tn*554*) represented the majority of the isolates (55%). Most interesting was the appearance of two new clones, XI::B::B (33%) and XI::B′::B (10%), representing together 43% of the isolates, which had molecular features characteristic of MRSA from Brazil (21). The *mecA* polymorph of the Brazilian clone was originally assigned the number III (21); upon careful comparison with other isolates belonging to patterns III (strain RN7164 [6]), IX (strain PER168 [5]), and XI (strain PER222 [5]), it seems that a more correct assignment is *mecA* polymorph XI (Fig. 1). The new Tn*554* pattern B′ is probably related to pattern B (it differs only in a *Cla*I restriction site of one of the three insertions of the transposon). A single isolate was assigned the clonal type II::NH::F. Interestingly, two isolates collected with an 11-day interval from the same product (bronchial secretions) of the same patient hos-

TABLE 2. Clonality of the MRSA clinical isolates from the HSJ (Oporto, Portugal)

Yr (*n*) and isolate no.	*mecA*::Tn*554*:: PFGE	RAPD ERIC1/ERIC2	No. of isolates	Clonal type (*n* [%])	Resistance to drug[f]: SPC	SXT
1990 (9)						
117[a]	I::E::A1	AA	1	I::E::A (2 [22])	R	S
109[a]	I::E::A3	AA	1			
103[a]	III::α::C2	A′D	1			
100	III::α::C3		1			
114[a]	III::α::C6	A′D	1	III::α::C (7 [78])	R	S
110	III::α::C8		1			
115	III::α::C9		1			
112[a]	III::α::C7	A′D	1			
111[a]	III::α::C11	A′D	1			
1991 (9)						
122,[a] 128, 129, 132,[b] 133	III::α::C1	A′D	5			
120	III::α::C2		1	III::α::C (8 [89])	R	S
121[a]	III::α::C10	A′D	1			
124[a]	III::α::C12	A′D	1			
126[a]	XIII::χ::D	CE	1	XIII::χ::D (1 [11])	R	R
1992 (14)						
153, 154, 157, 160, 161	I::E::A1		5	I::E::A (7 [50])		
135	I::E::A4		1		R	S
146	I::E::A5		1			
152[a]	I::J::A2	AA	1	I::J::A (1 [7])	R	S
137, 142	III::α::C1		2			
138	III::α::C4		1	III::α::C (5 [36])	R	S
151	III::α::C5		1			
159	III::α::C6		1			
136[a]	III::α::E	A′D	1	III::α::E (1 [7])	R	S
1996 (64)						
202, 221, 223	I::E::A1		3			
204, 219, 222, 226, 227, 229, 232, 234, 261, 282, 309, 312	I::E::A6		12			
210, 233, 244, 259	I::E::A7		4	I::E::A (32 [50])	R	S
213,[a] 249, 250	I::E::A8	AA	3			
216, 236, 248, 257, 258	I::E::A9		5			
245,[a] 276[a]	I::E::A10	AA	2			
208,[a] 217[a]	I::E::A11	AA	2			
274	I::E::A12		1			
212[a]	I::J::A1	AA	1	I::J::A (2 [3])	R	S
262[a]	I::J::A6	AA	1			
265[a]	I::NH::A11	AA	1	I::NH::A (1 [2])	S	S
311[a]	II::NH::F	BC	1	II::NH::F (1 [2])	S	S
200,[a,c] 230,[c] 247, 264, 271, 291, 301,[d] 307, 315	XI::B::B1	A′D	9			
228[c]	XI::B::B3		1			
251,[a,c] 253, 255[c,d]	XI::B::B4	A′D	3	XI::B::B (21 [33])	S	R
246,[a] 252,[e] 254	XI::B::B5	A′D	3			
218,[a,c] 240	XI::B::B6	A′D	2			
272	XI::B::B7		1			
290,[a] 314[a]	XI::B::B10	A′D	2			
256,[a] 284, 285,[c,d] 288	XI::B′::B1		4			
211[a,c]	XI::B′::B2	A′D	1	XI::B′::B (7 [10])	S	R
295	XI::B′::B8		1			
297	XI::B′::B9		1			

[a] Isolate assayed by RAPD.
[b] Exception: intermediately resistant to SXT.
[c] Exception: intermediately resistant to spectinomycin.
[d] Exception: susceptible to SXT.
[e] Exception: resistant to spectinomycin.
[f] SPC, spectinomycin; R, resistant; S, susceptible.

pitalized in the thorax surgery unit belonged to clones I::E::A and XI::B::B. Furthermore, the cooccurrence of clonal types I::E::A and XI::B::B was found in several wards or units in this hospital: medicine (three wards), surgery (two wards), emergency, neurology, infectious disease unit, and ICU.

HSJ. The 36 HSJ isolates of 1995 were divided between two major clones, I::E::A (47%) and XI::B::B (47%) (Table 3). The two remaining isolates were I::NH::A (loss of Tn*554*) and I::J::A (duplication of Tn*554*), probably related to I::E::A. The prevalence of clone I::E::A and its relatives (I::J::A and I::NH::A) was 53% in 1995 (Table 1).

Among the 35 isolates recovered during 1996, clonal types I::E::A and XI::B::B remained the main clones (50 and 33% of the isolates, respectively) (Table 3). Sporadic clones related to

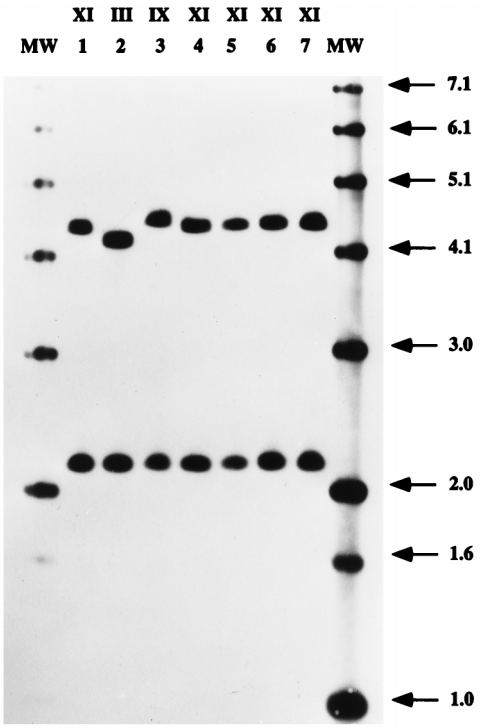

FIG. 1. *Cla*I-*mecA* hybridization profiles of some isolates belonging to the Brazilian clone. Chromosomal DNAs digested with *Cla*I were separated by conventional gel electrophoresis, transferred to a nylon membrane, and hybridized with a *mecA* DNA probe. Lanes MW, 1-kb ladder (BRL); lane 1, strain from Hospital Universitario Clementino Fraga Filho, Rio de Janeiro, Brazil (HU25; genuine representative of the Brazilian clone [21]); lanes 2 to 4, control strains (RN7164, type III [6], PER168, type IX [5], and PER222, type XI [5]); lane 5, strain from Hospital de São João (FFP200, type XI); lane 6, strain from HSJ (HSJ7, type XI); lane 7, strain from IPO (IPO118, type XI). Numbers at right show molecular size in kilobases.

I::E::A showing variation in the *mecA* polymorph or in the Tn*554* pattern but sharing the PFGE type A, represented by single isolates, were also abundant: I::J::A, I::DD::A, II::DD::A, I::μμ::A, and I::*νν*::A. Clonal type XI::κ::B, represented by a single isolate, may have evolved from XI::B::B, because it differs only in the number of insertions of the Tn*554* transposon. The new Tn*554* types μμ and *νν* contained probably four and five insertions, respectively, as suggested by the 8 to 10 *Cla*I hybridization fragments. Pattern κ contains two insertions (four hybridization fragments).

Clonal types I::E::A and XI::B::B and their related clones were represented in 1996 by 65 and 36% of isolates, respectively. In 1997 (a total of 19 isolates), the scenario of two major clones was maintained: I::E::A and its related clone I::DD::A (which included a single isolate) represented 47% of the isolates, while XI::B::B was found in 53% of the isolates (Table 1).

IPO. Clonal type I::E::A with its related clone I::J::A was found to be the dominant clone among the 23 IPO isolates recovered during 1997 (70%), while clone XI::B::B was represented by 30% (Table 4). Clonal types I::E::A and XI::B::B were found in distinct surgical services with the exception of one service (designated CI) where both clones were found. I::E::A was found in two other surgical services (pediatrics and CVII), while clonal type XI::B::B was found in the ICU.

RAPD analysis. A group of 42 strains representing different clonal types (defined by a combination of *mecA* and Tn*554* polymorphism and PFGE pattern) were also examined by RAPD with primers ERIC1 and ERIC2 as a potential confirmatory method (Table 2). RAPD type A′D was found in association with all the strains belonging to *mecA* polymorph III or XI independently of their Tn*554* pattern and PFGE type (III::α::C, III::α::E, XI::B::B, XI::B′::B, and XI::κ::B were RAPD type A′D). A second RAPD pattern, AA, was shared by all strains with *mecA* type I but also by the unique isolate II::DD::A.

DISCUSSION

Differential resolving powers of various genotypic methods. The resolving power of the RAPD analysis by the particular primers used was limited and found to be similar to the resolving power of Southern hybridization of *Cla*I digests with the *mecA* probe, as was observed in a previous work (1). The RAPD analysis could not distinguish strains belonging to the Portuguese clone (III::α::C) and the Brazilian clone; however, it was able to differentiate strains included in the two major clones (I::E::A [Iberian clone] and XI::B::B [Brazilian clone]). In fact, these two clones represented together the majority of the MRSA isolates recovered since 1995 in these three hospitals as well as in several other Portuguese hospitals, whereas the so-called Portuguese MRSA clone was seen as sporadic or absent since 1992. The single strain of this study belonging to clonal type II::NH::F (FFP 311) showed a unique RAPD pattern (BC) in confirmation of a previous finding with a different collection of MRSA isolates (1).

Of all the molecular fingerprinting methods used alone, PFGE with *Sma*I-digested DNA provided the highest degree of discrimination (FFP, 12 subtypes of A, 10 subtypes of B, and 12 subtypes of C; HSJ, 12 subtypes of A and 7 subtypes of B; IPO, 4 subtypes of A and 1 subtype of B). PFGE used in combination with *mecA* and Tn*554* typing was able to resolve the 210 MRSA isolates into 14 clonal types. Compared to RAPD, the PFGE method remains labor-intensive and time-consuming. Nevertheless, PFGE under carefully controlled conditions can yield highly reproducible results with a resolving power unmatched by any of the other currently used molecular typing methods.

The Iberian and Brazilian MRSA clones among the Portuguese MRSA isolates. Of the 210 MRSA strains recovered in the three Portuguese hospitals, the overwhelming majority of bacteria (182 of the 210, or 87%) belonged to one of two clonal types (as defined by *mecA*::Tn*554*::PFGE pattern): the Iberian clone (I::E::A) (together with a few [eight] isolates carrying the closely related clonal type I::NH::A or I::J::A) was represented by 107 isolates, and the Brazilian clone (XI::B::B) was represented by 75 isolates (together with eight isolates belonging to the closely related clone XI::B′::B or XI::κ::B).

Changes in MRSA clonal types—with time. Molecular typing studies performed in the period between 1985 and 1997 allowed construction of a tentative temporal scheme for the evolution of the clonal profile of MRSA in Portuguese hospitals. At least two epidemiologically important events were recorded: the replacement of clone III::α::C (the Portuguese MRSA), widespread in Portuguese hospitals in the mid-1980s and early 1990s, by the Iberian clone of MRSA (I::E::A) and the appearance and step increase in prevalence of the Brazilian clone of MRSA in the mid- to late 1990s.

Clonal type III::α::C was predominant in the Hospital de São João in the 1990 (78%) and 1991 (89%) samples (Table 1). The same clonal type was also dominant in the Hospital Dona Estefânia of Lisbon in 1985 (3), and this clone was detected widely in other Portuguese hospitals as well during surveillance in the early 1990s (15–17). By 1992, the prevalence of clone III::α::C declined to 36% in Hospital de São João and was no

TABLE 3. Clonality of the MRSA clinical isolates from HSJ (Lisbon, Portugal)

Yr (*n*) and isolate no.	*mecA*::Tn*554*:: PFGE	RAPD ERIC1/ERIC2	No. of isolates	Clonal type (*n* [%])	Resistance to drug[d]: SPC	SXT
1995 (36)						
93[a]	I::E::A1	AA	1			
13,[a] 18,[a] 20, 29, 33, 46,[a] 52, 84, 96,[a] 103, 110,[a] 153	I::E::A2	AA	12	I::E::A (17 [47])	R	S
73,[a] 115,[a] 118	I::E::A3	AA	3			
4[a]	I::E::A8	AA	1			
138[a]	I::NH::A4	AA	1	I::NH::A (1 [3])	S	S
99[a]	I::J::A2	AA	1	I::J::A (1 [3])	R	S
7,[a] 10, 11, 15, 17,[a] 56,[a] 60, 63,[a] 80,[a] 83, 114, 126,[a,b] 127, 131[a,b]	XI::B::B1	A′D	14			
24, 87[a]	XI::B::B2	A′D	2	XI::B::B (17 [47])	S	R
6[a]	XI::B::B3	A′D	1			
1996 (36)						
162,[a] 163,[a] 164, 169,[a] 175, 188, 192, 200, 205, 206, 207, 209, 210, 212	I::E::A2	AA	14			
171,[a] 203, 211	I::E::A5	AA	3	I::E::A (18 [50])	R	S
180[a]	I::E::A10	AA	1			
160[a]	I::J::A2	AA	1	I::J::A (1 [3])	R	S
159[a]	I::DD::A6	AA	1	I::DD::A (1 [3])	R	S
201[a]	II::DD::A7	AA	1	II::DD::A (1 [3])	S	S
173[a]	I::μμ::A12	AA	1	I::μμ::A (1 [3])	R	S
185[a]	I::νν::A11	AA	1	I::νν::A (1 [3])	R	S
161,[a,b] 170, 174,[a] 176, 178, 183, 187,[c] 191[a]	XI::B::B1	A′D	8	XI::B::B (12 [33])	S	R
167,[a,c] 199,[c] 213	XI::B::B5	A′D	3			
204[a,c]	XI::B::B6	A′D	1			
172[a]	XI::κ::B4	A′D	1	XI::κ::B (1 [3])	R	R
1997 (19)						
221, 224	I::E::A1		2			
220, 249, 251, 256	I::E::A2		4	I::E::A (8 [42])	R	S
225	I::E::A5		1			
255	I::E::A8		1			
219[a]	I::DD::A9	AA	1	I::DD::A (1 [5])	R	R
216, 217,[b] 218, 222,[a,c] 226,[c] 234,[c] 253[c]	XI::B::B1		7			
254	XI::B::B2		1	XI::B::B (10 [53])	S	R
230[b]	XI::B::B5		1			
227	XI::B::B7		1			

[a] Isolate assayed by RAPD.
[b] Exception: intermediately resistant to spectinomycin.
[c] Exception: resistant to spectinomycin.
[d] SPC, spectinomycin; R, resistant; S, susceptible.

longer detectable in collections from the HSJ (Lisbon) and the IPO (Lisbon) (Table 1).

Clonal type I::E::A was not detectable during the MRSA outbreak in Hospital Dona Estefânia in 1985 (3), and this clone was a minor one in the 1990 and 1991 samples from the Hospital de São João (Oporto). In 1992, the representation of clone I::E::A (the Iberian clone [17]) together with the related clone I::J::A increased to 57% in the Hospital de São João, and by 1995, this clone represented up to or above 50% of MRSA strains in the three hospitals studied (70% of isolates in the 1997 sample from the IPO) (Table 1).

The Iberian clone (Fig. 2) was found to be dominant in

TABLE 4. Clonality of the MRSA clinical isolates from IPO (Lisbon, Portugal)

Isolate no. (1997 [*n* = 23])	*mecA*::Tn*554*:: PFGE	RAPD ERIC1/ERIC2	No. of isolates	Clonal type (*n* [%])	Resistance to drug[e]: SPC	SXT
116,[a,b] 117, 119, 120, 122,[b] 124, 127, 137, 139	I::E::A1	AA	9			
114,[a] 129	I::E::A2	AA	2	I::E::A (15 [66])	R	S
131,[a] 135	I::E::A3	AA	2			
132,[a] 134	I::E::A4	AA	2			
115[a]	I::J::A2	AA	1	I::J::A (1 [4])	R	S
118,[a] 121,[c,d] 123, 125, 126, 128, 130	XI::B::B1	A′D	7	XI::B::B (7 [30])	S	R

[a] Isolate assayed by RAPD.
[b] Exception: resistant to SXT.
[c] Exception: resistant to spectinomycin.
[d] Exception: susceptible to SXT.
[e] SPC, spectinomycin; R, resistant; S, susceptible.

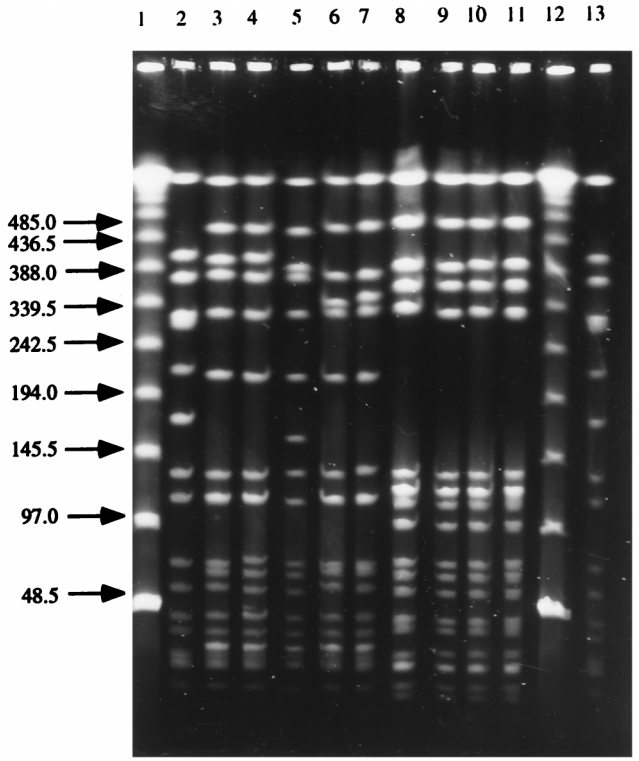

FIG. 2. PFGE of *Sma*I macrorestriction fragments of MRSA clinical isolates. Lanes 1 and 12, molecular size standards (lambda oligomers); lanes 2 and 13, NCTC8325; lanes 3 to 7, representatives of the Iberian clone; lane 3, strain from Hospital de Bellvitge Princeps d'Espanya, Barcelona, Spain (PER34; genuine representative of the Iberian clone; PFGE type A [5]); lane 4, strain from Hospital Pulido Valente, Lisbon, Portugal (HPV107, PFGE type A7 [17]); lane 5, strain from Hospital de São João (FFP202, PFGE type A1); lane 6, strain from HSJ (HSJ18, PFGE type A2); lane 7, strain from IPO (IPO116, PFGE type A1); lanes 8 to 11, representatives of the Brazilian clone; lane 8, strain from Hospital Universitario Clementino Fraga Filho, Rio de Janeiro, Brazil (HU25; genuine representative of the Brazilian clone [21]); lane 9, strain from Hospital de São João (FFP200, PFGE type B1); lane 10, strain from HSJ (HSJ7, PFGE type B1); lane 11, strain from IPO (IPO118, PFGE type B1). Numbers at left show molecular size in kilobases.

Spain already in 1989 (5), while in Portugal, this clone seems to have peaked only in 1992 to 1993 in several hospitals (13, 15–18). This observation suggests that during 1992 there was a clonal replacement of the Portuguese clone by the Iberian clone.

The molecular characterization of MRSA isolates recovered in the three Portuguese hospitals documents the occurrence of a second major epidemiological event since 1995. Since 1995, the dominance of the Iberian clone appears to have been challenged in the three hospitals studied in this work by the arrival of a new MRSA clone with the clonal type assignment XI::B::B. This MRSA clone, previously named the Brazilian MRSA (Fig. 2), was found to be widely spread over large distances in Brazil (21) in a survey of 1992 to 1994, and the clone appears to have retained high prevalence in that country in 1996 to 1998 (2a). MRSA clone XI::B::B was found in Portugal since 1995 with percentages that show a tendency to increase through the years. In the case of the HSJ in 1997, it may even have overtaken the Iberian clone (53 versus 47%) (Table 1). A similar situation was found in another Portuguese hospital situated between Lisbon and Oporto (13). A previous study in the IPO showed that the prevalence of clone XI::B::B was already significant (12%) in 1995 (18).

Distinct antibiotic resistance profiles of the Iberian and Brazilian MRSA clones. All strains belonging to the Iberian clone (I::E::A) were susceptible to SXT, while 95% of the strains belonging to the Brazilian clone (XI::B::B) were resistant to this antibiotic and one isolate showed intermediate resistance. A previous study of the Iberian clone in Spain showed the same result (5), and all strains belonging to the Brazilian clone isolated in Brazil showed resistance to SXT (21).

Interestingly, all MRSA isolates carrying the *mecA* polymorph I, irrespective of their Tn*554* or PFGE types (I::J::A, I::NH::A, I::DD::A, I::μμ::A, and I::νν::A), showed susceptibility to SXT. Similarly, MRSA isolates carrying the *mecA* polymorph XI but differing from the Brazilian clone in Tn*554* type (e.g., isolates XI::B′::B and XI::κ::B) showed in the majority of the cases resistance to SXT. The Portuguese clone (III::α::C) could not be distinguished from the Iberian clone by SXT resistance.

Another correlation between clonal type and drug resistance concerns susceptibility to spectinomycin. All strains belonging to clonal type I::E::A or related clones harboring the Tn*554* transposon showed high-level resistance to 500 μg of spectinomycin per ml, while the majority of the isolates belonging to the Brazilian clone (clonal type XI::B::B or the related clone XI::B′::B) (51 of 74 [69%]) were susceptible to this drug. It should be remembered that the Brazilian MRSA isolates carry three copies of Tn*554* (21). The mechanism responsible for the lack of resistance in these strains is not known. All strains belonging to the Portuguese clone (III::α::C) remained resistant to spectinomycin.

The observations described in this communication clearly establish the appearance of the Brazilian clone of MRSA in Portuguese hospitals. The most conservative interpretation of this finding is the transfer of this clone from South America to Portugal. A similar intercontinental transfer of a major epidemic clone of multiresistant *Streptococcus pneumoniae* was recently documented by molecular epidemiological techniques (8). The appearance of the Brazilian clone in Portugal may be linked to the increase in migration of human populations since 1992 to 1993 between these two Portuguese-speaking countries (primarily from Brazil to Portugal), particularly health care personnel, and to the absence of effective control barriers in Portuguese hospitals for colonized or infected patients prior to hospitalization.

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