JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2003, p. 4998–5005 Vol. 41, No. 11
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.11.4998–5005.2003

Update on the Major Clonal Types of Methicillin-Resistant *Staphylococcus aureus* in the Czech Republic

O. Melter,[1] M. Aires de Sousa,[1,2] P. Urbášková,[1] V. Jakubů,[1] H. Žemličková,[1] and H. de Lencastre[2,3]*

National Institute of Public Health, Prague, Czech Republic[1]; Laboratório de Genética Molecular, Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa (ITQB/UNL), Oeiras, Portugal[2]; and Laboratory of Microbiology, The Rockefeller University, New York, New York 10021[3]

Received 5 June 2003/Returned for modification 22 July 2003/Accepted 4 August 2003

The purpose of our study was the molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated in 21 hospitals in the Czech Republic in the period 2000-2002 and comparison with previous results from 1996-1997. Strains were analyzed by pulsed-field gel electrophoresis (PFGE) of *Sma*I digests and ribotyping of *Hin*dIII digests hybridized with a 16S-23S DNA probe. The prevalence of the most clinically important macrolide (*ermA*, *ermB*, *ermC*, and *msrA*) and aminoglycoside (*aph3′*, *ant4′*, and *aac6′-aph2″*) resistance genes was evaluated as well. Selected isolates representative of each clonal type were analyzed by multilocus sequence typing and by a multiplex PCR method capable of identifying the structural type of the staphylococcal cassette chromosome *mec* (SCC*mec*) carried by the bacteria. Our results document the displacement of the Brazilian clone (ST239, SCC*mec* type IIIA, PFGE type B, ribotype H1) by a new clone that we named "Czech clone" (ST239, SCC*mec* type IIIA, PFGE type F, ribotype H6) and the maintenance of the Iberian clone (ST247, SCC*mec* type IA, PFGE type A, ribotype H2) exclusively in one hospital in the Czech Republic. In addition, we found a correlation between the distribution of aminoglycoside resistance genes and MRSA clonal types.

In the first unique study dealing with the characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Czech Republic by molecular typing, the prevalence of two multiresistant clones of MRSA that were particularly widely disseminated in the country was documented for the years 1996-1997 (19). One of these clones was the pandemic Iberian MRSA (10, 18, 29, 31), which represented 12% of the Czech MRSA isolates. The other distinct multiresistant clone, the Brazilian MRSA, widely spread in South America (3, 8, 34) and Portugal (4, 25), represented 80% of the Czech isolates of 1996-1997.

MRSA strains have acquired multiple resistance to a wide range of antibiotics, including aminoglycosides and macrolides (11). Three genes (*aph3′*, *ant4′*, and *aac6′-aph2″*), encoding three types of aminoglycoside-modifying enzymes, are of particular significance because they modify aminoglycosides of therapeutic importance, including kanamycin, tobramycin, and gentamicin, respectively (14). Macrolide resistance can be triggered by several mechanisms (16), the predominant one being target modification mediated by one or more *erm* genes encoding a 23S rRNA methylase, rendering the strain resistant to most macrolides, lincosamides, and streptogramin B compounds (MLS_B) (33). Resistance to macrolide-streptogramin (MS) antibiotics resulting from the presence of macrolide efflux pumps in staphylococci (encoded by *msrA*) has also been documented (30).

In this paper, we focus on the characterization by different typing techniques of the following two collections of MRSA isolates from the Czech Republic: (i) 45 isolates collected during 2000-2001 exclusively from blood samples at 20 hospitals and (ii) 55 isolates collected during 2001-2002 from different clinical sources at three hospitals. In an attempt to determine the status of macrolide and aminoglycoside resistance among Czech MRSA isolates, we investigated the prevalence of the most clinically important macrolide (*ermA*, *ermB*, *ermC*, and *msrA*) and aminoglycoside (*aph3′*, *ant4′*, and *aac6′-aph2″*) resistance genes.

MATERIALS AND METHODS

Hospitals. Nine large (>1,000 beds), 6 medium (between 500 and 1,000 beds), and 6 small (<500 beds) hospitals in the Czech Republic, including 7 teaching hospitals and 14 general hospitals, participated in this study (Table 1). A map of the Czech Republic with the locations of the 21 participating hospitals is shown in Fig. 1.

Bacterial isolates. For this study, we analyzed 100 single-patient MRSA isolates from the following two different collections: (i) 45 invasive isolates from blood samples obtained from 20 hospitals in the period from April 2000 through November 2001 (collection 1) and (ii) 55 noninvasive isolates recovered from April 2001 through March 2002 from different clinical sources at three hospitals (two located in Prague and one located in the eastern part of the country) (collection 2). Collection 2 isolates were recovered from wounds (42%), sputum (20%), catheters (11%), and other diverse clinical sites (27%). Of the 100 isolates, 14 (31%), 17 (38%), and 13 (29%) from collection 1 and 16 (29%), 12 (22%), and 5 (9%) from collection 2 were recovered from patients interned in intensive care units, surgical wards, and internal medicine wards, respectively.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards guidelines (21) for oxacillin, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, tobramycin, kanamycin, streptomycin, mupirocin, tetracycline, trimethoprim-sulfamethoxazole (SXT), chloramphenicol, rifampin, vancomycin, and teicoplanin. Susceptibility to vancomycin and teicoplanin was

* 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.

TABLE 1. Hospital data and MRSA isolates from the Czech Republic collected during 2000–2002

Hospital code[a]	City	Hospital type	No. of beds	% MRSA isolates (July 2000–March 2002)	No. of MRSA in this study
Collection 1 (invasive MRSA isolates)					
3	Prague	University-teaching	≥1,000	16.7	9
4	Kladno	General	500–1,000	3.7	2
5	Prague	University-teaching	≥1,000	4.2	1
6	Plzeň	University-teaching	≥1,000	6.1	4
7	Liberec	General	500–1,000	4.3	1
A7	Brandys	General	<500	Not known	1
8	Most	General	≥1,000	6.3	1
13	Tábor	General	<500	5.0	1
15	České Budějovice	General	≥1,000	2.0	2
17	Ostrava	University-teaching	≥1,000	9.2	1
19	Příbram	General	500–1,000	6.7	1
20	Prague	University-teaching	<500	8.5	7
26	Pardubice	General	500–1,000	2.8	1
31	Prague	General	500–1,000	7.4	6
33	Ústí nad Labem	General	≥1,000	18.5	2
34	Brno	University-teaching	≥1,000	7.4	1
35	Prague	General	<500	18.0	1
36	Havličkuv Brod	General	<500	5.0	1
37	Trutnov	General	<500	7.7	1
42	Nový Jičín	General	<500	16.0	1
Collection 2 (noninvasive MRSA isolates)					
16	Prague	University-teaching	≥1,000	6.5	22
20	Prague	University-teaching	<500	8.5	10
42	Nový Jičín	General	<500	16.0	23
Total					100

[a] Underlined hospital codes indicate hospitals that are included in both collections. Collection 1, 45 invasive isolates (from blood samples) collected at 20 hospitals; collection 2, 55 noninvasive isolates collected at three hospitals.

also determined according to the EARSS protocol (22). Production of β-lactamase was tested by nitrocefin (Oxoid, Hampshire, United Kingdom).

Identification of MRSA isolates. *S. aureus* isolates were confirmed to be methicillin resistant by the MRSA-Screen slide latex agglutination kit for the rapid detection of PBP2a, according to the manufacturer's recommendations (Denka Seiken Co., Ltd., Tokyo, Japan).

Detection of enterotoxins A, B, C, D, and E, TSST-1, and exfoliative toxins A and B. Enterotoxins (A, B, C, D, and E), toxic shock syndrome toxin 1 (TSST-1), and exfoliative toxins A and B were detected by the SET-RPLA, TST-RPLA, and EXT-RPLA kits, respectively (Denka Seiken Co., Ltd.), following the manufacturer's recommendations.

Preparation of whole-cell DNA for PCR. One DNA disk prepared for pulsed-field gel electrophoresis (PFGE) as described by Chung et al. (7) was melted at 70°C. A volume of 180 μl of water was added, and the mixture was incubated at 95°C for 15 min and chilled on ice for 5 min. Two microliters of DNA was used as template.

PCR amplification of the *mecA*, aminoglycoside, and macrolide resistance genes. Amplification of the *mecA* (19), *aph3′*, *ant4′*, *aac6′-aph2″* (36), *ermA*, *ermB*, *ermC*, and *msrA* (17) genes was performed as previously described.

Ribotyping, PFGE, MLST, and SCC*mec* typing. Ribotyping of *Hin*dIII digests (19), PFGE of *Sma*I digests of chromosomal DNAs (7), and multilocus sequence typing (MLST) (12) were performed as previously described. The staphylococcal cassette chromosome *mec* (SCC*mec*) types were determined by a multiplex PCR strategy (24).

RESULTS

Antimicrobial susceptibility. All 100 strains were resistant to oxacillin, which was confirmed by the presence of the *mecA* gene and production of PBP2a. The majority of the isolates tested were resistant to ciprofloxacin (99%), erythromycin (98%), tetracycline (97%), streptomycin (95%), rifampin (94%), gentamicin, kanamycin, tobramycin (93%), and clindamycin (86%), and a few of them showed resistance to SXT (10%), chloramphenicol (9%), mupirocin (4%), and fusidic acid (1%). All isolates were susceptible to vancomycin and teicoplanin (Table 2). Constitutive production of β-lactamase was found in 95% of the isolates.

Production of enterotoxins, TSST-1, and exfoliative toxins A and B. Representatives of each clonal type detected were tested for the production of enterotoxins A, B, C, D, and E, TSST-1, and exfoliative toxins A and B. Enterotoxin A was found in 16 isolates. The other toxins were not detected in any isolate.

Prevalence of macrolide resistance genes. Among the 100 MRSA isolates screened for the presence of MLS_B resistance genes, 99% contained one or more of the *erm* genes, which is consistent with the erythromycin resistance phenotype. The most prevalent *erm* gene was *ermA*, which was detected in 94% of the isolates. The *msrA* gene was detected in only two isolates, in association with *ermC* (Table 3).

Prevalence of aminoglycoside resistance genes. Among the 100 isolates tested, the *aac6′-aph2″* gene was the most frequently encountered aminoglycoside resistance gene (94%), and 40% of the isolates carried this gene in combination with one of the other aminoglycoside resistance genes. The *ant4′* and *aph3′* genes were present in 29 and 12% of the isolates, respectively (Table 3). Isolates with none of the three genes ($n = 5$) were fully susceptible to gentamicin, tobramycin, and kanamycin.

Clonal assignments. (i) Ribotypes. The 100 strains were classified into ribotypes on the basis of fingerprints produced after hybridization of *Hin*dIII digests with the 16S-23S probe. All ribotypes found in this study are indicated in Table 2 and shown in Fig. 2. The majority of the isolates from collection 1 (41 of 45 isolates; 91%) were included in ribotype H6, whereas four other ribotypes (H1, H2, H7, and H8) were represented by single isolates. For collection 2, most isolates also belonged to ribotype H6 (28 of 55 isolates; 51%), followed by ribotype H2 (20 of 55 isolates; 36%). Ribotypes H1 and H7 included four and three isolates, respectively. However, the distribution of ribotypes for collection 2 varied from hospital to hospital (Table 2). Ribotype H6 was represented by 100% of the isolates from hospital 20, 82% of the isolates from hospital 16, and none of the isolates from hospital 42. Ribotype H2 was found exclusively in hospital 42 and was represented by 87% of those isolates. Ribotype H1 was only present in hospital 16 and included 18% of those isolates. Ribotype H7 was detected in two hospitals only (hospitals 42 and 17) and was represented by 3 of 24 isolates (13%) and 1 of 2 isolates, respectively.

(ii) PFGE types. The 100 MRSA isolates were distributed into five PFGE types (Table 2). A major PFGE type, F, appeared among collection 1 isolates (89%) and also among collection 2 isolates from hospitals 16 (82%) and 20 (100%). We provisionally named this clone the Czech clone. PFGE type A, characteristic of the Iberian clone, was represented by two isolates in collection 1 (4%) and was found as a major PFGE type (87%) among collection 2 isolates from hospital 42. PFGE type B, characteristic of the Brazilian clone, was represented by a single isolate in collection 1 and by four isolates from hospital 16 in collection 2. PFGE type H was found in both collection 1 (one isolate) and collection 2 (three isolates from hospital 42). Sporadic PFGE type I was represented by a single isolate in collection 1. Figure 3 shows the major PFGE types found in this study as well as representatives of some international MRSA clones.

(iii) MLST and SCC*mec* types. MLST and SCC*mec* typing were applied to representatives of each of the clonal types identified in this study by PFGE and ribotyping. ST239 and SCC*mec* type IIIA are characteristic of both the Brazilian (PFGE type B, ribotype H1) and the Czech (PFGE type F, ribotype H6) clones. A single strain that was ST239 and SCC*mec* type IIIA also was *Hin*dIII ribotype H6, typical of the Czech clone, and PFGE pattern B, typical of the Brazilian clone. A clone isolated in hospital 42 only, distinguished by *Hin*dIII ribotype H2 and PFGE type A, belonged to ST247 and SCC*mec* type IA, which are typical of the Iberian clone.

All four strains of a sporadic clone that were resistant to erythromycin, clindamycin, and ciprofloxacin only belonged to ribotype H, PFGE type H, ST22, and SCC*mec* type IV.

The single isolate of the clone characterized by ribotype H8 and PFGE type I displayed SCC*mec* type I and belonged to ST111, which has so far been only detected in one MRSA strain (AB-903627/02) from Norway (http://www.mlst.net).

DISCUSSION

The mean proportion of MRSA blood isolates in the Czech Republic over the years 1999-2001 was reported to be 3 to 10% (22). However, MRSA prevalence varies substantially among Czech hospitals (2 to 18.5%) (Table 1), which may be due to differences in the hospital hygiene guidelines applied and/or antibiotic use. In an attempt to determine the evolution of MRSA clonal types in the Czech Republic from 1996-1997 to 2000-2002, 100 recent isolates were characterized by different molecular typing methods. The most surprising observation of

FIG. 1. Map of the Czech Republic with locations of the 21 participating hospitals. Hospitals are designated with code numbers. The appearance of a particular clonal type is indicated by a symbol (+, Czech clone; ++, Iberian clone; +++, Brazilian clone) or by its molecular characteristics (ribotype:PFGE type).

TABLE 2. Phenotypic and genotypic properties of 100 MRSA isolates from the Czech Republic

Ribotype	PFGE type	No. of isolates	Clonal type	Antibiotic resistance[b] (>50% of the isolates)	Presence of aminoglycosides[c]			Presence of macrolides[c]				MLST type[d]	ST	SCC*mec* type
					aac6′-aph2″	*aph3′*	*ant4′*	*ermA*	*ermB*	*ermC*	*msrA*			
Collection 1[a]														
H1	A1	1	H1:A	O, E, G, K, S, TO, CLI, C, T, R	+	−	+	+	−	+	−	3-3-1-12-4-4-16	247	IA
H2	A2	1	H2:A Iberian	O, E, G, K, S, TO, CLI, C, T, R	+	−	+	+	−	+	−	3-3-1-12-4-4-16	247	
H6	F1	36	H6:F Czech	O, E, G, K, S, TO, CLI, C, T, R	+	−(7+)	−(1+)	+(1−)	−	+(3−)	−	2-3-1-1-4-4-3	239	IIIA
	F3	1		O, E, G, K, S, TO, CLI, C, T, R	+	−	+	+	−	+	−			
	F5	1		O, E, G, K, S, TO, CLI, C, T, R	+	−	+	+	−	+	−			
	F6	2		O, E, G, K, S, TO, CLI, C, T, R	+	−	+	+	−	+	−			
H6	B1	1	H6:B	O, E, G, K, S, TO, CLI, C, T, R	+	−	−	+	−	−	−	2-3-1-1-4-4-3	239	IIIA
H7	H2	1	H7:H	O, E, CLI, C	+	−	−	−	−	+	−			
H8	I	1	H8:I	O, E, G, K, S, TO, CLI, C	+	+	−	+	−	+	−	1-4-1-4-46-24-29	111	I
Collection 2														
Hospital 16														
H1	B1	3	H1:B Brazilian	O, E, G, K, S, TO, CLI, SXT, C, T, R	+	+	−	+	−	+	−	2-3-1-1-4-4-3	239	IIIA
	B5	1		O, E, G, K, S, TO, SXT, C, T, F, CHL, R	+	+	−	+	−	−	−			
H6	F1	14	H6:F Czech	O, E, G, K, S, TO, CLI, C, T, R	+(1−)	−	− (+1)	+	−	+(2−)	−	2-3-1-1-4-4-3	239	IIIA
	F2	2		O, E, G, K, S, TO, CLI, C, T, R[e]	±	−	−	+	−	+	−			
	F3	1		O, E, G, K, S, TO, CLI, C, T, R	+	−	−	+	−	+	−			
	F5	1		O, E, G, K, S, TO, CLI, C, T, R	+	−	−	+	−	+	−			
Hospital 20														
H6	F1	10	H6:F Czech	O, E, G, K, S, TO, CLI, C, T, R	+	−	−(4+)	+	−	+(4−)	−	2-3-1-1-4-4-3	239	IIIA
Hospital 42														
H2	A1	1	H2:A Iberian	O, E, G, K, S, TO, CLI, C, T, R	−	−	−	−	−	+	+(1−)	3-3-1-12-4-4-16	247	IA
	A2	19		O, E, G, K, S, TO, CLI, C, T, R	+(1−)	−	+	+(1−)	−	−	−			
H7	H1	3	H7:H	O, E, C	−	−	−	−	−	+	+(1−)	7-6-1-5-8-8-6	22	IV

[a] Collection 1, isolates collected exclusively from blood samples from 20 hospitals; collection 2, isolates collected from three different hospitals from a variety of clinical sources.

[b] O, oxacillin; C, ciprofloxacin; CLI, clindamycin; E, erythromycin; T, tetracycline; G, gentamicin; K, kanamycin; S, streptomycin; TO, tobramycin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; R, rifampin; F, fusidic acid.

[c] +, positive, −, negative; exceptions are indicated in parentheses.

[d] The strains characterized by MLST were the following: CCM 7109 (ST22, SCC*mec* type IV, PFGE *Sma*I type H, ribotype *Hin*dIII H7), CCM 7110 (ST111, SCC*mec* type, PFGE *Sma*I type I, ribotype *Hin*dIII H8), CCM 7111 (ST239, SCC*mec* type IIIA, PFGE *Sma*I type B, ribotype *Hin*dIII H1), CCM 7112 (ST239, SCC*mec* type IIIA, PFGE *Sma*I type F, ribotype *Hin*dIII H6), CCM 7113 (ST239, SCC*mec* type IIIA, PFGE *Sma*I type B, ribotype *Hin*dIII H6), CCM 7114 (ST247, SCC*mec* type IA, PFGE *Sma*I type A, ribotype *Hin*dIII H1), and CCM 7115 (ST247, SCC*mec* type IA, PFGE *Sma*I type A, ribotype *Hin*dIII H2). CCM refers to the Czech Culture Collection of Microorganisms.

[e] One isolate was susceptible to gentamicin, kanamycin, and tobramycin.

this study was the high frequency of a clonal group defined by ST239, SCC*mec* type IIIA, PFGE type F, and ribotype H6 which was dominant (89%) among the isolates from blood samples (collection 1) and also among the isolates from two hospitals of collection 2 (82 and 100%) (Table 2). This clonal type was detected in 17 of the 21 hospitals included in this study (Fig. 1).

The Brazilian clone (ST239, SCC*mec* type IIIA, PFGE type B, ribotype H1), which in 1996-1997 was the major clone spread in two Prague and one Brno hospital (19), was no longer the dominant one in 2000-2002, being represented only by four isolates from the same hospital in collection 2. Interestingly, three of these isolates were collected from outpatients, which indicates that the Brazilian clone may still be present in the community. The Iberian clone (ST247, SCC*mec* type IA, PFGE type A, ribotype H2), represented by 12% of the isolates in 1996-1997 and detected in two hospitals, one in Prague and the other in Plzeò (19), was found to be the dominant clone in one of the hospitals of collection 2 (hospital 42) and was detected in one isolate of collection 1 collected at the

TABLE 3. Distribution of macrolide and aminoglycoside resistance genes in MRSA isolates from the Czech Republic[a]

No. of isolates with macrolide profile	Macrolide profile				No. of isolates with aminoglycoside profile	Aminoglycoside profile		
	ermA	*ermB*	*ermC*	*msrA*		*aac6′-aph2″*	*aph3′*	*ant4′*
30	+	−	−	−	54	+	−	−
0	−	+	−	−	1	−	−	+
3	−	−	+	−	0	−	+	−
0	−	−	−	+	28	+	−	+
0	+	+	−	−	12	+	+	−
64	+	−	+	−	0	−	+	+
0	+	−	−	+	0	+	+	+
0	−	+	+	−	5	−	−	−
0	−	+	−	+				
2	−	−	+	+				
1	−	−	−	−				

[a] The numbers of isolates that carried each antibiotic resistance gene were as follows: macrolide genes, *ermA*, 94; *ermB*, 0; *ermC*, 69; *mrsA*, 2 (total, 100), aminoglycoside genes, *aac6′-aph2″*, 94; *aph3′*, 12; *ant4′*, 29 (total, 100).

same hospital. This hospital was located 350 km from Prague and 450 km from Plzeò. We can hypothesize that the blood isolates (collection 1), which are often unique isolates of the hospitals included in this study, might reflect the predominant clone of the hospital in which they were collected. What should be pointed out is the switch in the SXT resistance pattern from 90% SXT-resistant strains in 1996-1997 to only 10% in 2000-2002, which is consistent with the displacement of the SXT-resistant (Sxt^r) Brazilian clone by the new SXT-susceptible (Sxt^s) Czech MRSA clone. A similar situation had been observed in a Portuguese hospital in which the Sxt^s Iberian clone was replaced by the Sxt^r Brazilian clone (5). Although susceptible to SXT, the new Czech MRSA clone is multidrug resistant, showing resistance to penicillin, oxacillin, erythromycin, gentamicin, clindamycin, ciprofloxacin, and rifampin.

On the basis of identical MLST profiles (ST239) and SCC*mec* types (IIIA) between the Brazilian and the new Czech MRSA clones, we can hypothesize that the later clone might have evolved from the Brazilian clone, which was spread in large Czech cities in 1996-1997. The possible origin of the new Czech clone from the Brazilian one could be explained by rearrangement of chromosomal DNA. The precise mechanism of such rearrangement is still unclear, but some mutations must have taken place in, or close to, some of the ribosomal operons, because the *Hin*dIII ribotype of the Czech clone (H6) was different from that of the Brazilian one (H1). Interestingly, we found one isolate that showed *Hin*dIII profile H6, typical of the Czech clone, and PFGE profile B, characteristic of the Brazilian clone. This fact is also in agreement with our hypothesis that the Czech clone evolved from the Brazilian clone on Czech territory between 1996 and 2000. However, the biological properties that conferred a selective advantage to this new clone are still unknown, except for the production of enterotoxin A that seems to be associated with the new Czech clone (seven strains of nine strains tested expressed the toxin) and not with the Brazilian clone (all of four strains tested were negative). What is alarming is the production of enterotoxin A by most strains of the new Czech clone, to which the majority

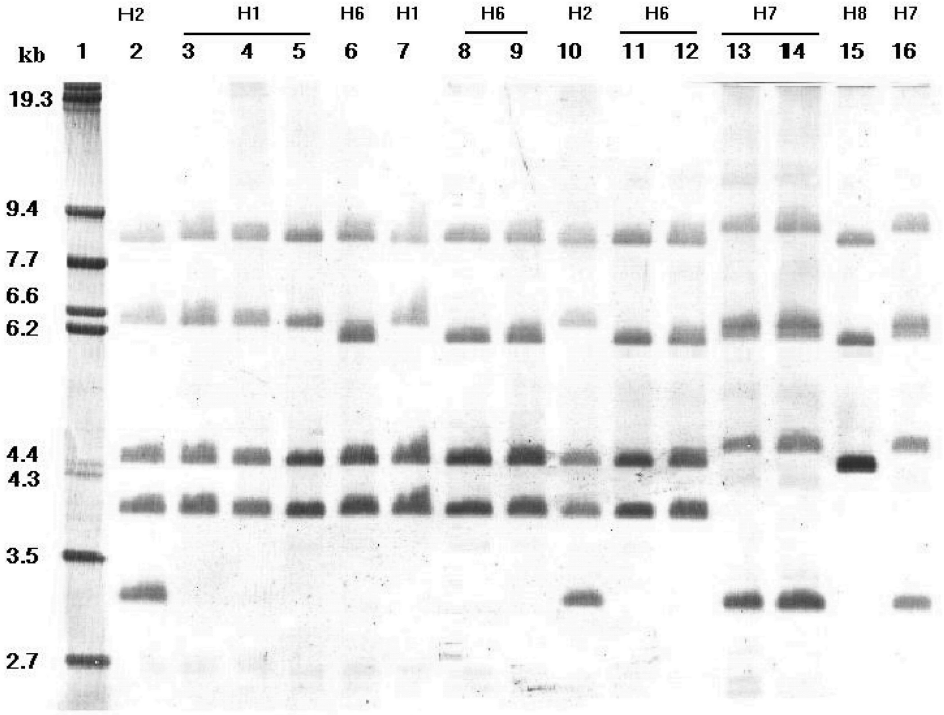

FIG. 2. Ribotypes found among 100 MRSA clinical isolates from the Czech Republic. Lane 1, molecular size standard (lambda digest with *Sty*I plus *Hin*dIII); lanes 3 to 5 and 7, ribotype H1; lanes 2 and 10, ribotype H2; lanes 6, 8, 9, 11, and 12, ribotype H6; lanes 13, 14, and 16, ribotype H7; and lane 15, ribotype H8.

of the Czech MRSA isolates from 2000-2002 belonged: this enterotoxin with its superantigen properties might cause development of toxic shock syndrome, with possibly lethal outcomes (6).

The Hungarian (9) and Taiwan clones, which belong to ST239 and ST241 (single locus variant of ST239) (1, 23, 26, 27) and ribotypes H1 and H2, respectively, may also be progenitors of the Czech clone. Representatives of ST239 were also reported from other countries all over the world, including the countries neighboring the Czech Republic (Hungary, Poland, and Germany) (13, 23).

Some hospitals in Prague and one hospital in Plzeò have specialized units (hospital 3, transplantation unit; hospital 5, transplantation and burn units; hospital 20, trauma and neurology diagnosis units; hospital 6, transplantation unit) that receive patients from all over the country who are further transferred to regional hospitals. This fact might have played an important role in the massive dissemination of the new Czech MRSA clone all over the country in a relatively short period of time. The spread of MRSA strains could be traced based on movements of patients and healthcare staff between hospitals. The precise mechanism of the displacement of the predominant (80%) Brazilian clone (1996-1997) by the Czech clone before the year 2000 still remains unknown.

All of the four strains of a sporadic clonal type that were resistant to erythromycin, clindamycin, and ciprofloxacin only and were characterized by *Hin*dIII ribotype H7 and PFGE profile H shared the group ST22. PFGE type H seems to be identical to the PFGE type characteristic of EMRSA-15, which, together with EMRSA-16, is the most prevalent MRSA clone in hospitals in the United Kingdom and was also detected in northern Berlin, Germany (20, 28, 38). The strains from the United Kingdom and Germany showed the same antibiogram as clone H7:H (28, 38) but were producers of enterotoxin C (28), whereas clone H7:H produced enterotoxin A. Both clone H7:H and the German strains possess the *ermC* determinant (38), and two strains of clone H7:H possess the *msrA* gene as well. Clone ST22, H7:H, could have spread to the Czech Republic from the United Kingdom or from Germany.

We have studied the distribution of MLS_B resistance determinants by PCR among the 100 MRSA isolates. Resistance to erythromycin was found for 98 isolates, and all of them contained one or more *erm* genes. When a single MLS_B resistance determinant was present, the *ermA* gene was the most common (94%), followed by *ermC* (69%), whereas *ermB* was not detected in any isolate. Macrolide resistance due to *msrA* was rare (2%) and seems to be more frequent in coagulase-negative staphylococci than in *S. aureus* (17). Analysis of *S. aureus* strains isolated from blood in Denmark indicated that the *ermA* and/or *ermC* genes were responsible for erythromycin resistance in 98% of 428 isolates (37). A study involving 851 *S. aureus* isolates collected in 1997-1998 from 24 European university hospitals (33) and another one involving 294 *S. aureus* strains isolated in 1995 in French hospitals (17) concordantly concluded that the *ermA* gene was more common in MRSA isolates than in methicillin-susceptible *S. aureus* isolates. *ermA* occurs on the transposon Tn*554* (35), which is present in the large majority of clinical MRSA isolates and absent from methicillin-susceptible *S. aureus* isolates (15), which may explain the high prevalence of the *ermA* gene among clinical MRSA isolates.

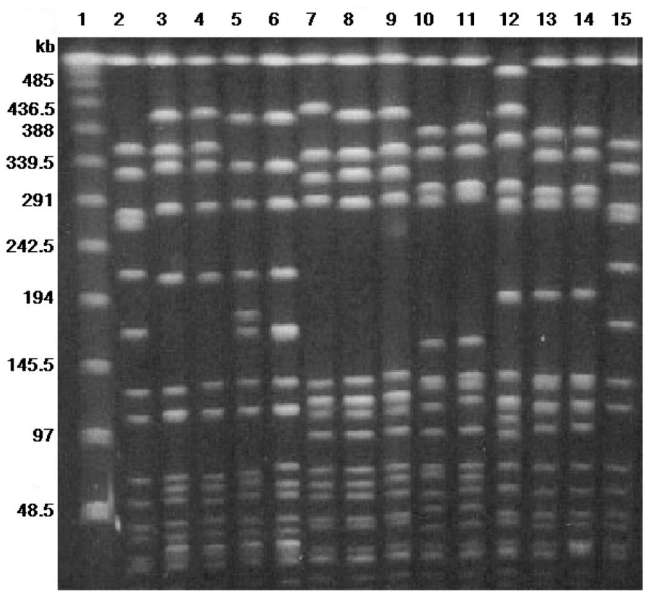

FIG. 3. PFGE of *Sma*I macrorestriction fragments of MRSA clinical isolates from the Czech Republic and representatives of some international MRSA clones. Lane 1, lambda marker; lanes 2 and 15, NCTC8325; lanes 3 to 6, representatives of the Iberian clone (lane 3, PER34 [10]; lane 4, PL21 [19]; lane 5, 1NJ [this study]; lane 6, 2NJ [this study]); lanes 7 to 9, representatives of the Brazilian clone (lane 7, HU25 [34]; lane 8, KV173 [19]; lane 9, 2A8 [this study]); lanes 10 and 11, representatives of the Hungarian clone (lane 10, HUSA 304 [9]; lane 11, HU101 [23]); lane 12, representative of the new Czech MRSA clone, 2HK (this study); lanes 13 and 14, representatives of the major clone of Taiwan (lane 13, TAW3; lane 14, TAW 10 [1]).

The frequency of genes encoding aminoglycoside-modifying enzymes was studied in the 100 MRSA isolates. The *aac6′-aph2″*, *ant4′*, and *aph3′* genes were present in 94, 29, and 12% of the isolates, respectively. These results confirmed those of Schmitz et al. (32), who documented that *aac6′-aph2″* has been the gene most frequently found in MRSA strains isolated in Europe. However, *ant4′* was more prevalent in Japanese isolates (14). The major MRSA clonal type found in Japan (2) is very distinct from the ones found in European countries, which might explain the predominance of the *ant4′* gene in Japanese isolates (14) compared with the higher prevalence of *aac6′-aph2″* in Europe. Among the 100 MRSA isolates included in this study, five were susceptible to gentamicin, kanamycin, and tobramycin and did not carry any of the three aminoglycoside determinants. However, two of these isolates were resistant to streptomycin, which could be explained by the presence of the resistance gene *str* or chromosomal mutations (*strA*) (32).

When we combined data of aminoglycoside and macrolide resistance genes with clonal types, we observed that the different clonal types found in this study could be distinguished by the presence or absence of these resistance genes (Table 2). The Iberian clone could be distinguished from the Brazilian clone by the presence of *ant4′* and the absence of *aph3′*, whereas the new Czech MRSA clone seldom carried even one of these two genes. None of the aminoglycoside resistance genes was detected in isolates belonging to the minor clone

characterized by PFGE type H, ribotype H7, ST22, and SCC*mec* type IV, which also had a different aminoglycoside resistance gene profile. Regarding macrolide resistance genes, the Iberian clone could be distinguished from the Brazilian clone and the new Czech clone by the absence of *ermC*. However, the Brazilian and the Czech clones have identical macrolide resistance gene profiles.

In summary, our results, based on two distinct collections of invasive and noninvasive MRSA isolates, clearly document the displacement of the Brazilian clone (ST239, SCC*mec* type IIIA, PFGE type B, ribotype H1) by the new Czech clone (ST239, SCC*mec* type IIIA, PFGE type F, ribotype H6) and the maintenance of the Iberian clone (ST247, SCC*mec* type IA, PFGE type A, ribotype H2) exclusively in one hospital in the Czech Republic. In addition, we found a correlation between the distribution of aminoglycoside resistance genes and the different MRSA clonal types.

ACKNOWLEDGMENTS

This study was supported by grant 310/01/1363 of the Grant Agency of the Czech Republic awarded to O. Melter. Support was also received from the Fundação para a Ciência e Tecnologia, Lisbon, Portugal, within project POCTI/1999/ESP/34872 awarded to H. de Lencastre. M. Aires de Sousa was supported by grant BD/13731/97, PRAXIS XXI, from the Fundação para a Ciência e Tecnologia, Lisbon, Portugal, and by grant 310/01/1363 of the Grant Agency of the Czech Republic.

We are grateful to the Czech EARSS participants for collecting staphylococcal isolates. We thank P. Petras for the detection of staphylococcal toxins, E. Kodytková and H. Leitnerová for excellent assistance, and A. Gabrielová for providing data and additional isolates. Some observations were made possible by the information available at the MLST database (www.mlst.net) hosted by The Imperial College and the University of Bath.

REFERENCES

1. **Aires de Sousa, M., M. I. Crisóstomo, I. Santos Sanches, J. S. Wu, J. Fuzhong, A. Tomasz, and H. de Lencastre.** 2002. Frequent recovery of a single clonal type of multidrug resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. J. Clin. Microbiol. **41:**159–163.
2. **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.
3. **Aires de Sousa, M., M. Miragaia, I. Santos Sanches, S. Ávila, I. Adamson, S. T. Casagrande, M. C. C. Brandileone, R. Palacio, L. Dell'Acqua, M. Hortal, T. 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, 1996–1998. J. Clin. Microbiol. **39:**2197–2205.
4. **Aires de Sousa, M., I. Santos 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.
5. **Amorim, M. L., M. Aires de Sousa, I. S. Sanches, R. Sá-Leão, J. M. Cabeda, J. M. Amorim, and H. de Lencastre.** 2002. Clonal and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) from a Portuguese hospital over time. Microb. Drug Resist. **8:**301–309.
6. **Bargs, N. L., and T. Harris.** 1997. Toxin-mediated syndromes, p. 527–545. *In* K. B Crossley and G. L. Archer (ed.), The staphylococci in human disease. Churchill Livingstone, New York, N.Y.
7. **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. Sa-Leao, 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.
8. **Corso, A., I. Santos Sanches, M. Aires de Sousa, A. Rossi, and H. de Lencastre.** 1998. Spread of a dominant methicillin-resistant multiresistant *Staphylococcus aureus* (MRSA) clone in Argentina. Microb. Drug Resist. **4:**277–288.
9. **de Lencastre, H., E. P. Severina, H. Milch, M. Konkoly Thege, and A. Tomasz.** 1997. Wide geographic distribution of a unique methicillin-resistant *Staphylococcus aureus* clone in Hungarian hospitals. Clin. Microbiol. Infect. **3:**289–296.
10. **Dominguez, M. A., H. de Lencastre, J. Liñares, and A. Tomasz.** 1994. Spread and maintenance of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone during an outbreak of MRSA disease in a Spanish hospital. J. Clin. Microbiol. **32:**2081–2087.
11. **Duval, J.** 1985. Evolution and epidemiology of MLS resistance. J. Antimicrob. Chemother. **16**(Suppl. A)**:**137–149.
12. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus.* J. Clin. Microbiol. **38:**1008–1015.
13. **Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99:**7687–7692.
14. **Ida, T., R. Okamoto, C. Shimauchi, T. Okubo, A. Kuga, and M. Inoue.** 2001. Identification of aminoglycoside-modifying enzymes by susceptibility testing: epidemiology of methicillin-resistant *Staphylococcus aureus* in Japan. J. Clin. Microbiol. **39:**3115–3121.
15. **Kreiswirth, B., J. Kornblum, R. D. Arbeit, W. Eisner, J. Maslow, A. McGeer, D. E. Low, and R. Novick.** 1993. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus.* Science **259:**227–230.
16. **Leclercq, R., and P. Courvalin.** 1991. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob. Agents Chemother. **35:**1267–1272.
17. **Lina, G., A. Quaglia, M. E. Reverdy, R. Leclercq, F. Vandenesch, and J. Etienne.** 1999. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob. Agents Chemother. **43:**1062–1066.
18. **Mato, R., I. Santos Sanches, M. Venditti, D. J. Platt, A. Brown, and H. de Lencastre.** 1998. Spread of the multiresistant Iberian clone of methicillin resistance *Staphylococcus aureus* (MRSA) to Italy and Scotland. Microb. Drug Resist. **4:**107–112.
19. **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.
20. **Moore, P. C. L., and J. A. Lindsay.** 2002. Molecular characterization of the dominant UK methicillin-resistant S. aureus strains, EMRSA-15 and EMRSA-16. J. Med. Microbiol. **51:**516–521.
21. **National Committee for Clinical Laboratory Standards.** 1995. Performance standards for antimicrobial disk susceptibility test. National Committee for Clinical Laboratory Standards, Wayne, Pa.
22. **National Institute of Public Health and the Environment.** 2001. European antimicrobial resistance surveillance system annual report. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.
23. **Oliveira, D. C., I. Crisóstomo, I. Santos Sanches, P. Major, C. R. Alves, M. Aires de Sousa, M. K. Thege, and H. de Lencastre.** 2001. Comparison of DNA sequencing of the protein A gene polymorphic region with other molecular typing techniques for typing two epidemiologically diverse collections of methicillin-resistant *Staphylococcus aureus.* J. Clin. Microbiol. **39:**574–580.
24. **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. **36:**2155–2161.
25. **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.
26. **Oliveira, D. C., A. Tomasz, and H. de Lencastre.** 2001. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb. Drug Resist. **7:**349–361.
27. **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.
28. **O'Neill, G. L., S. Murchan, A. Gil-Setas, and H. M. Aucken.** 2001. Identification and characterization of phage variants of a strain of epidemic methicillin-resistant *S. aureus* (EMRSA-15). J. Clin. Microbiol. **39:**1540–1548.
29. **Roberts, R. B., A. M. Tennenberg, W. Eisner, J. Hargrave, L. M. Drusin, R. Yurt, and B. N. Kreiswirth.** 1998. Outbreak in a New York city teaching hospital caused by the Iberian epidemic clone of MRSA. Microb. Drug Resist. **4:**175–183.
30. **Ross, J. I., E. A. Eady, J. H. Cove, W. J. Cunliffe, S. Baumberg, and J. C.**

Wootton. 1990. Inducible erythromycin resistance in staphylococci is encoded by a member of ATP-binding transport super-gene family. Mol. Microbiol. **4:**1207–1214.

31. **Sanches, I. S., M. Ramirez, H. Troni, M. Abecassis, M. Pádua, 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.
32. **Schmitz, F. J., A. C. Fluit, M. Gondolf, R. Beyrau, E. Lindenlauf, J. Verhoef, H. P. Heinz, and M. E. Jones.** 1999. The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. J. Antimicrob. Chemother. **43:**253–259.
33. **Schmitz, F. J., R. Sadurski, A. Kray, M. Boos, R. Geisel, K. Köhrer, J. Verhoef, and A. C. Fluit.** 2000. Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals. J. Antimicrob. Chemother. **45:**891–894.
34. **Teixeira, L., C. A. Resende, L. R. Ormonde, R. Rosenbaum, A. M. S. Figueiredo, H. de Lencastre, and A. Tomasz.** 1995. Geographic spread of epidemic multiresistant *Staphylococcus aureus* clone in Brazil. J. Clin. Microbiol. **33:** 2400–2404.
35. **Tillotson, L. E., W. D. Jenssen, L. Moon-McDermott, and D. T. Dublin.** 1989. Characterization of a novel insertion of the macrolide-lincosamide-streptogramin B resistance transposon Tn*554* in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis.* Antimicrob. Agents Chemother. **33:** 541–550.
36. **Vanhoof, R., C. Godard, J. Content, H. J. Nyssen, and E. Hannecart-Pokorni.** 1994. Detection by polymerase chain reaction of genes encoding aminoglycoside-modifying enzymes in methicillin-resistant *Staphylococcus aureus* isolates of epidemic phage types. J. Med. Microbiol. **41:**282–290.
37. **Westh, H., D. M. Hougaard, J. Vuust, and V. T. Rosdahl.** 1995. Prevalence of *erm* gene classes in erythromycin-resistant *Staphylococcus aureus* strains isolated between 1959 and 1988. Antimicrob. Agents Chemother. **39:**369–373.
38. **Witte, W., M. Enright, F. J. Schmitz, C. Cuny, C. Braulke, and D. Heuck.** 2001. Characteristics of a new epidemic MRSA in Germany ancestral to United Kingdom EMRSA15. Int. J. Med. Microbiol. **290:**677–682.