JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2003, p. 5113–5120 Vol. 41, No. 11
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.11.5113–5120.2003

Pulsed-Field Gel Electrophoresis Typing of Oxacillin-Resistant *Staphylococcus aureus* Isolates from the United States: Establishing a National Database

Linda K. McDougal,* Christine D. Steward, George E. Killgore, Jasmine M. Chaitram, Sigrid K. McAllister, and Fred C. Tenover

Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia 30333

Received 2 June 2003/Returned for modification 10 July 2003/Accepted 22 August 2003

Oxacillin-resistant *Staphylococcus aureus* (ORSA) is a virulent pathogen responsible for both health care-associated and community onset disease. We used *Sma*I-digested genomic DNA separated by pulsed-field gel electrophoresis (PFGE) to characterize 957 *S. aureus* isolates and establish a database of PFGE patterns. In addition to PFGE patterns of U.S. strains, the database contains patterns of representative epidemic-type strains from the United Kingdom, Canada, and Australia; previously described ORSA clonal-type isolates; 13 vancomycin-intermediate *S. aureus* (VISA) isolates, and two high-level vancomycin-resistant, *vanA*-positive strains (VRSA). Among the isolates from the United States, we identified eight lineages, designated as pulsed-field types (PFTs) USA100 through USA800, seven of which included both ORSA and oxacillin-susceptible *S. aureus* isolates. With the exception of the PFT pairs USA100 and USA800, and USA300 and USA500, each of the PFTs had a unique multilocus sequence type and *spa* type motif. The USA100 PFT, previously designated as the New York/Tokyo clone, was the most common PFT in the database, representing 44% of the ORSA isolates. USA100 isolates were typically multiresistant and included all but one of the U.S. VISA strains and both VRSA isolates. Multiresistant ORSA isolates from the USA200, -500, and -600 PFTs have PFGE patterns similar to those of previously described epidemic strains from Europe and Australia. The USA300 and -400 PFTs contained community isolates resistant only to β-lactam drugs and erythromycin. Noticeably absent from the U.S. database were isolates with the previously described Brazilian and EMRSA15 PFGE patterns. These data suggest that there are a limited number of ORSA genotypes present in the United States.

Oxacillin-resistant *Staphylococcus aureus* (ORSA), more commonly referred to as methicillin-resistant *S. aureus* (MRSA) (even though methicillin is rarely tested in U.S. laboratories), is a frequent cause of infections both in health care and community settings and is endemic in many U.S. hospitals (12, 26, 27, 32, 48, 56). While a variety of strain typing methods have been used over the years to track the spread of ORSA (64), most outbreaks of ORSA have been characterized by bacteriophage typing or pulsed-field gel electrophoresis (PFGE). Although public health institutions in several countries, such as Australia (68), Denmark (41, 53, 67), The Netherlands (41, 66, 67), Canada (59), and the United Kingdom (41), have tracked ORSA strain types over the years, this has not been true in the United States for the most part.

Recent reports of vancomycin-intermediate *S. aureus* (VISA) (vancomycin MICs, 8 to 16 μg/ml) (5, 22, 28, 33, 50, 60, 65), of *vanA*-positive ORSA showing high-level vancomycin resistance (8, 9, 11), and of ORSA causing severe disease and death in children (6, 27, 42) all argued that better tracking of ORSA strains nationwide was needed to monitor the spread of such organisms. Thus, the Centers for Disease Control and Prevention (CDC), in collaboration with state health departments, has undertaken the goal of assembling a national database of *S. aureus* PFGE profiles similar to the PulseNet program for *Escherichia coli* O157:H7 (62).

The overall goal of this study was to assemble a database of ORSA PFGE profiles and to identify major lineages of ORSA present in the United States. PFGE was chosen over other typing methods, such as multilocus sequence typing (MLST), staphylococcal protein A gene (*spa*) typing, and restriction fragment length polymorphism-based methods, because the infrastructure and expertise for PFGE typing already exist within the state health departments in the United States. However, a national PFGE-based typing system for *S. aureus* would have to be validated with MLST and *spa* typing data to maintain continuity with the nomenclature already established in the literature. This would make the national database information useful for global tracking of ORSA, an organism with a limited number of lineages that exists endemically in health care and non-health-care settings. Thus, we typed a large number of ORSA isolates and developed a PFGE nomenclature scheme that was consistent with MLST and *spa* data yet was also useful for microbiologists and epidemiologists who were specifically studying ORSA infections in the United States. Herein, we present a framework for describing the lineages of ORSA present in the United States and correlating *Sma*I PFGE profiles with the *spa* and MLST types already established in the literature (18, 19, 40, 46, 47, 58).

* Corresponding author. Mailing address: MS G-08, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333. Phone: (404) 639-2823. Fax: (404) 639-1381. E-mail: lkm1@cdc.gov.

MATERIALS AND METHODS

Bacterial isolates. A total of 957 *S. aureus* isolates were examined in the study; 722 were oxacillin resistant and 235 were oxacillin susceptible according to NCCLS criteria (43). Of the 957 isolates, 300 *S. aureus* isolates were chosen at

random from the CDC *S. aureus* strain collection. These strains were from outbreaks in hospitals, food-borne disease, and community-acquired infections and were submitted to CDC for typing between 1995 and 2000. They were selected mainly for geographical and source diversity. An additional 381 ORSA isolates were from health care and community outbreak investigations conducted by CDC and state health departments from 2001 to 2003. These included two *vanA*-positive VRSA strains from Michigan (8) and Pennsylvania (9), eight U.S. VISA isolates for which the vancomycin MICs were 8.0 μg/ml, and 22 isolates for which the vancomycin MICs were 4 μg/ml (22, 60). A further 221 isolates were from various CDC-sponsored surveillance studies. Also included in the database were one VISA isolate from Japan (28), one from France (50), one from Hong Kong, one from Korea (33), and two from Scotland; 15 clonal-type isolates (graciously provided from the collection of H. de Lencastre, Rockefeller University, New York, N.Y.) (13); 15 epidemic MRSA isolates (EMRSA; isolates obtained from H. Aucken, Public Health Laboratory Service, Colindale, United Kingdom); four representative epidemic Canadian MRSA isolates (CMRSA) (59) (courtesy of B. Willey, Mt. Sinai Hospital, Toronto, Ontario, Canada); 12 isolates from community outbreaks in Australia (39, 44) (courtesy of J. Bell, Women and Children's Hospital, Adelaide, Australia), and three staphylococcal SCC*mec*-type control isolates (31) (courtesy of K. Hiramatsu, Juntendo University, Tokyo, Japan).

Antimicrobial susceptibility testing. The antimicrobial susceptibility profiles of the isolates were determined by broth microdilution with cation-adjusted Mueller-Hinton broth (BD BioSciences, Sparks, Md.) as described in the NCCLS publication M7-A5 (43). The antimicrobial agents tested were chloramphenicol, clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, quinupristin-dalfopristin, rifampin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. Quality control strains included *S. aureus* ATCC 29213, *E. coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212. Multiresistance was defined as resistance to three or more classes of antimicrobial agents. Inducible clindamycin resistance was determined with a disk diffusion test in which an erythromycin disk was placed 12 mm from the edge of a clindamycin disk on a blood agar plate after inoculating the plate with a suspension of the test organism that was adjusted to the turbidity of a 0.5 McFarland standard. The presence of a D-shaped zone of inhibition indicated the induction of methylase production by erythromycin (69). Inducible clindamycin resistance was presumed to be mediated by an *erm* gene. If no clindamycin induction occurred, the resistance mechanism was assumed to be efflux of the macrolide via *msrA* (35). High-level resistance to spectinomycin (500 μg/ml), which is associated with the majority of the ORSA strains that carry the transposon Tn*554* (49, 55), was determined by disk diffusion with use of a 300-μg disk (made in-house). The absence of a measurable zone of inhibition after 24 h of incubation at 35°C was indicative of resistance. *S. aureus* strains HDE1 and HPV107, which are known to be susceptible and resistant to spectinomycin, respectively (55), were used as controls.

PFGE. A single colony of the test isolate was inoculated into 5 ml of brain heart infusion broth and incubated with vigorous shaking at 35 to 37°C for 24 h. The concentrations of the cell suspensions were adjusted with saline either by using a MicroScan Turbidity Meter (Dade Behring, Inc., Deerfield, Ill.) to a turbidity reading of 1.1 to 1.3 or by using a spectrophotometer to an absorbance of 0.9 to 1.1 at 610 nm. Two hundred microliters of the adjusted cell suspension was centrifuged at 12,000 × *g* for 2 to 4 min, and the supernatant was aspirated. The pellet was resuspended in 300 μl of Tris-EDTA (TE) buffer (10 mM Tris HCl, 1 mM EDTA [pH 8]) and equilibrated in a 37°C water bath for 10 min. Three microliters of recombinant (no. L-0761; Sigma, St. Louis, Mo.) or 4 μl of conventional (no. L-7386; Sigma) lysostaphin stock solution (1 mg/ml in 20 mM sodium acetate [pH 4.5]) and 300 μl of 1.8% (wt/vol) SeaKem Gold agarose (FMC, Rockland, Maine) in TE buffer (equilibrated to 55°C) were added to the cell suspension, gently mixed, and dispensed into the wells of either a large-plug mold (volume of each well, ~250 μl) or into the wells of a small disposable mold (~100 μl each). The plugs were allowed to solidify at room temperature for 10 to 15 min or in the refrigerator (4°C) for 5 min. The plugs were removed and placed into a tube containing at least 3 ml of EC lysis buffer (6 mM Tris HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, 0.5% sodium lauroylsarcosine) and incubated at 37°C for at least 4 h. The EC lysis buffer was poured off, and 4 ml of TE buffer was added. The TE washings were repeated at least three more times, and the plugs were stored at 4°C.

***Sma*I restriction enzyme digestion and gel electrophoresis conditions.** A plug slice was cut to the desired comb size (2 by 5 mm for a 10-, 20-, or 30-tooth comb or 2 by 10 mm for a 10- by 10-tooth comb) and equilibrated in 1× restriction buffer for at least 30 min. After removal of the 1× restriction buffer, 3 μl of *Sma*I restriction enzyme (Promega no. R6125, 10 U/μl; Promega Corp., Madison, Wis.) in 200 μl of 1× restriction buffer was added to each tube, and the tubes were incubated at 25°C for 2 to 3 h. A SeaKem Gold 1% (wt/vol) agarose gel was prepared in 0.5× TBE from 10× Tris-borate-EDTA buffer (Gibco BRL no. 15581044; Life Technologies, Inc., Gaithersburg, Md.). The plug slices were loaded directly on the end of the comb tooth before placing the comb into the comb holder, and the equilibrated agarose was poured carefully into the gel casting platform. PFGE was performed using a contour-clamped homogeneous electric field apparatus, i.e., DR-II, DR-III, or CHEF Mapper (Bio-Rad, Hercules, Calif.). Running parameters were as follows: 200 V (6 V/cm); temperature, 14°C; initial switch, 5 s; final switch, 40 s; and time, 21 h. After the electrophoresis run was completed, the gel was stained in a 1.5 μg/ml ethidium bromide solution (AMRESCO X328, 10 mg/ml; Amresco, Inc., Solon, Ohio) for 20 min in a covered container and destained in fresh distilled water for 45 min.

Data analysis. Gels were photographed and digitized with the FOTO/Analyst Archiver system (Fotodyne, Inc., Hartland, Wis.) and saved as a TIFF file for analysis with BioNumerics software (Applied Maths, Kortrijk, Belgium). The reference standard *S. aureus* NCTC 8325, which was included in the first, seventh, fourteenth, twentieth, and last lanes of each gel, was normalized to the global-standard *S. aureus* NCTC 8325. Percent similarities were identified on a dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Band position tolerance and optimization were set at 1.25 and 0.5%, respectively. A similarity coefficient of 80% was selected to define the pulsed-field type (PFT) clusters after reviewing the epidemiologic data associated with each of the clusters of isolates.

***spa* typing.** The typing of the polymorphic region of the protein A gene was performed according to previously described procedures (58).

SCC*mec* typing. SCC*mec* typing was performed as described by Okuma et al. (45).

MLST. MLST was performed on selected isolates as described by Enright et al. (18).

Nomenclature of ORSA clones. The nomenclature chosen is similar to that described by Enright et al. (20), Feil et al. (21), and Hiramatsu et al. (29), except that the genetic backgrounds of the strains in this study were determined by *Sma*I PFGE and designated as PFTs. The PFTs were further described as either ORSA or oxacillin-susceptible *S. aureus* (OSSA), and ORSA PFTs were subdivided into SCC*mec* types I, II, III, and IV.

RESULTS

The *Sma*I macrorestriction fragment profiles of 957 *S. aureus* isolates were determined by PFGE. A dendrogram of percent similarity, calculated with Dice coefficients from the PFGE data using a cutoff of 80%, revealed eight major clusters of isolates, designated as PFTs USA100 through USA800 (Fig. 1). Of the 667 U.S. ORSA isolates, 622 (93%) clustered within these eight PFTs. In addition, 134 of the 235 OSSA isolates (57%) had PFGE patterns that fell within the same eight PFTs. The results of MLST, *spa* typing, SCC*mec* typing, antimicrobial resistance profiles, and other relevant properties of ORSA strains are summarized in Table 1. All but four PFTs (USA300 and -500, and -100 and -800) had a unique MLST sequence type and *spa* type motif. Five of the eight PFTs (USA100, -200, -500, -600, and -800) contained isolates that were predominantly obtained from health care-associated infections, while the isolates from two PFTs (USA300 and -400) were from community infections. USA700 isolates were obtained from patients in both community and health care settings.

Of isolates from health care-associated infections, USA100 was the largest and most diverse of the PFTs, containing 292 ORSA isolates from throughout the United States. For the seven housekeeping genes, these isolates shared a common MLST allelic profile (1- 4- 1- 4- 12- 1- 10), which is designated as ST 5, and a common *spa* motif (MDMGMK). USA100 isolates were usually spectinomycin resistant (consistent with SCC*mec* II) and multiresistant to commonly used therapeutic agents. This group included seven of eight U.S. VISA isolates and VISA isolates from Japan (Mu50) and Korea. USA100 also included the two U.S. VRSA isolates from Michigan and Pennsylvania. All isolates were resistant to erythromycin: 72%

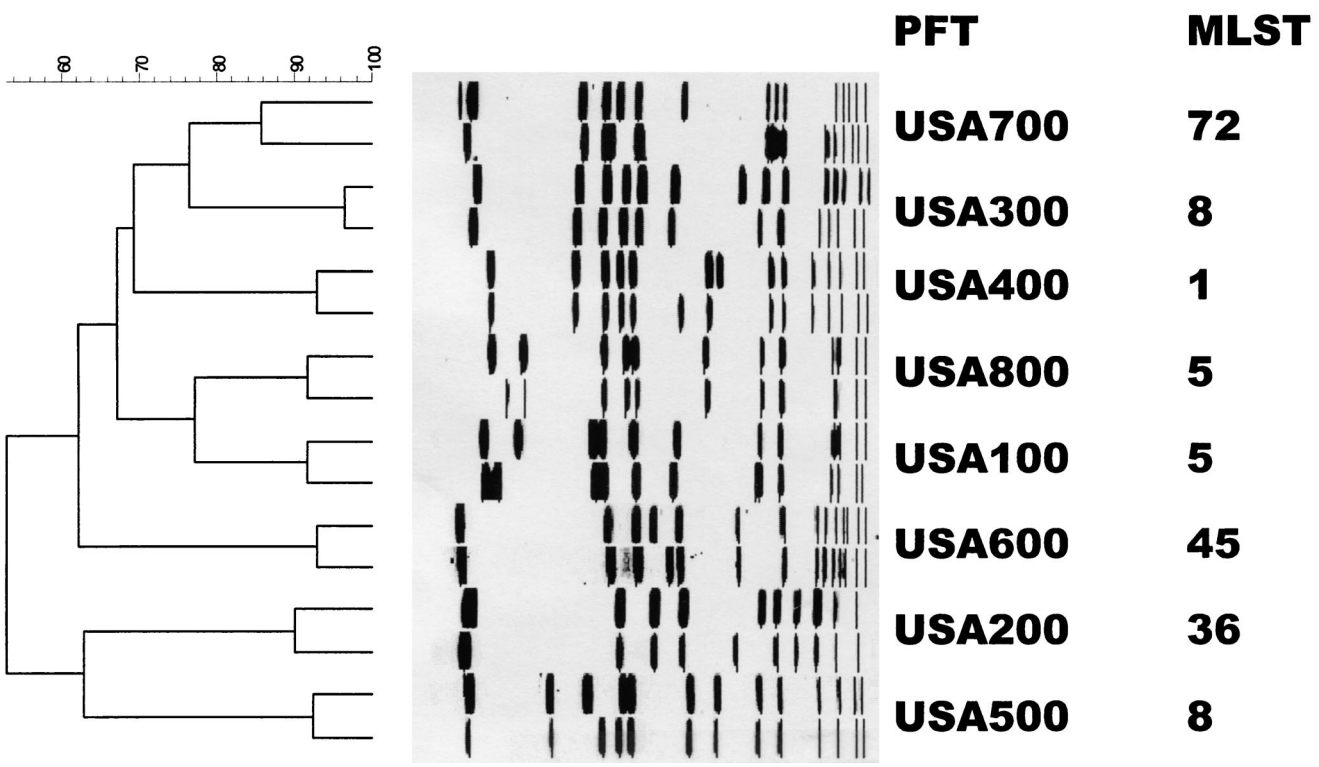

FIG. 1. Dendrogram of PFTs with type strain (most frequent pattern) and a variant strain. Also shown is the corresponding MLST for each PFT (18, 19, 20).

were constitutively clindamycin resistant, and 28% showed inducible clindamycin resistance. *S. aureus* isolates BK2464, PA237, and JA48, from the New York/Japan clone (1, 51, 52), belonged to this PFT.

USA800 isolates shared the same MLST sequence type and *spa* motif as the USA100 isolates. These isolates were primarily from community surveillance studies, were spectinomycin susceptible (suggesting the lack of Tn*554* [consistent with SCC*mec* type I or IV]) and were generally resistant only to β-lactam drugs. Twenty-three percent were erythromycin resistant. When we tested the isolates, SCC*mec* typing showed that most of them were SCC*mec* type IV. *S. aureus* isolates HDE1, HDE288, and COB94 from the Pediatric clone (24, 55) belonged to this group. Other isolates, such as EMRSA3, which carried SCC*mec* type I and fell just outside the USA100 and -800 clusters, shared the same MLST and *spa* motif.

USA200, the second most common health care-associated PFT among U.S. isolates, contained ORSA isolates that were spectinomycin resistant (consistent with SCC*mec* II) and were multiresistant to therapeutic agents. All ORSA isolates were erythromycin resistant, with 98% showing constitutive resistance to clindamycin. The isolates had the same MLST profile, i.e., ST 36 (2- 2- 2- 2- 3- 3- 2), and *spa* type motif (WGKAK AOMQQQ) as isolates from the EMRSA16 epidemic clone.

Isolates from community onset infections belonging to PFT USA400 had the MLST ST 1 profile (1- 1- 1- 1- 1- 1- 1) and *spa* type motif (UJJJFE). These ORSA isolates were spectinomycin susceptible, carried SCC*mec* IV, and were not multiresistant. *S. aureus* MW2, an isolate from a rapid fatal infection in a child from Minnesota (6), was included in this group.

Although representative isolates from USA300 and USA500 had the same MLST allelic profile (ST 8, 3- 3- 1- 1- 4- 4- 3) and *spa* type motif (MBQBLO), these isolates clustered into separate, but contiguous, groups by *Sma*I PFGE. USA300 isolates carried SCC*mec* IV, were resistant to β-lactam drugs, were frequently resistant to erythromycin, and were predominantly from community onset skin infections. Eighty-five percent of the erythromycin-resistant isolates were susceptible to clindamycin and were not inducible with erythromycin (probably due to *msrA*). On the other hand, USA500 isolates were generally from health care-related infections. The majority of USA500 isolates were spectinomycin susceptible, indicating the absence of Tn*554* (consistent with SCC*mec* type I or IV). Most of the isolates were resistant to clindamycin, erythromycin, gentamicin, levofloxacin, tetracycline, trimethoprim-sulfamethoxazole, and the β-lactams. The remaining U.S. VISA isolate and the three isolates for which the vancomycin MICs were 4 μg/ml had PFGE profiles belonging to this PFT. PFGE patterns of *S. aureus* isolates PER34, E2125, and HPV107 from the Archaic/Iberian clones (13, 14, 57); EMRSA isolates 2, 5, 6, 10, 12, 13, and 14; VISA isolates from Hong Kong and France; and the Iberian clonal-type isolate from Scotland clustered near the USA500 isolates. EMRSA isolates 2, 6, 12, 13, and 14 shared the same MLST profile (3- 3- 1- 1- 4- 4- 3) as the USA300 and USA500 isolates, which differed at a single locus from ST 250 (3- 3- 1-1- 4-4 -16) of isolate Per34. *S. aureus* E2125 and HPV107 (Archaic/Iberian clonal-type isolates) and the *S. aureus* EMRSA5 isolate have the MLST profile ST 247 (3- 3- 1- 12- 4- 4- 16), which differed at one locus from ST250 and two

TABLE 1. Characteristics of ORSA PFTs[a]

Name of PFT	Total no. of U.S. ORSA strains in PFT (%)	MLST and allelic profile[b]	*spa* type[c]	Clone name[d]
USA100	292 (44)	ST 5, 1- 4- 1- 4- 12- 1- 10	TJMB**MDMGMK**	USA100 ORSA II
USA800	45 (6.7)	ST 5, 1- 4- 1- 4- 12- 1- 10	TJMB**MDMGMK**	USA800 ORSA IV[e]
			TJMB**MDMGMK**	ST 5 ORSA I
USA200	57 (8.5)	ST 36, 2- 2- 2- 2- 3- 3- 2	WGKAKAOMQQQ	USA200 ORSA II
USA400	42 (6.3)	ST 1, 1- 1- 1- 1- 1- 1- 1	UJJJFE	USA400 ORSA IV[e]
USA300	91 (13.6)	ST 8, 3- 3- 1- 1- 4- 4- 3	YHGF **MBQBLO**	USA300 ORSA IV[e]
USA500	34 (5.1)	ST 8, 3- 3- 1- 1- 4- 4- 3	YHGC **MBQBLO**	USA500 ORSA IV,[e] USA500 ORSA II
		ST 8, 3- 3- 1- 1- 4- 4- 3	YHGC **MBQBLO**	ST8 ORSA I, ST8 ORSA IV, ST8 ORSA III
		ST 250, 3- 3- 1- 1- 4- 4- 16	YH GF **MBQBLO**	ST250 ORSA I
		ST 247, 3- 3- 1- 12- 4- 4- 16	YHFGF **MBQBLO**	ST247 ORSA I
USA600	23 (3.4)	ST 45, 10- 14- 8- 6- 10- 3- 2	A2AKEEMBKB	USA600 ORSA II
		ST 45, 10- 14- 8- 6- 10- 3- 2	A2AKEEMBKB	USA600 ORSA IV[e]
USA700	38 (5.7)	ST 72, 1- 4- 1- 8- 4- 4- 3	UJGFMGGM	USA700 ORSA IV[e]
Unnamed	14 (2)	Not tested	ZDMDMNKB	
Unnamed	3	ST 239, 2- 3- 1- 1- 4- 4- 3	WG**KAOMQ**	ST239 ORSA III
		ST 240, 2- 3- 1- 1- 2- 4- 3	WG**KAOMQ**	ST240 ORSA III
Unnamed	0	ST 22, 7- 6- 1- 5- 8- 8- 6	TJEJN12MN12MOM	ST 22 ORSA IV

[a] PFT is based on *Sma*I PFGE with ≥80% similarity identified on an UPGMA-derived dendrogram using Dice coefficients.
[b] Allelic profile is based on the DNA sequences of seven housekeeping genes: *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*.
[c] DNA sequence of polymorphic region of protein A gene. The *spa* type motif is shown in boldface.
[d] The clone name consists of the following three parts: PFT or MLST sequence type, the oxacillin phenotype (all are ORSA in this table), and the SCC*mec* type (when available).
[e] Isolates in this PFT subgroup were susceptible to spectinomycin.
[f] β-Lactams (oxacillin and penicillin); chl, chloramphenicol; cli, clindamycin; ery, erythromycin; gen, gentamicin; lvx, levofloxacin; tet, tetracycline; sxt, trimethoprim-sulfamethoxazole.
[g] CMRSA strains are epidemic type strains from Canada (59). Four strains are included in this study.
[h] EMRSA strains are epidemic type strains from the United Kingdom (16, 54). Fifteen strains are included in this study.
[i] J. Block, M. F. Orlando, L. K. McDougal, L. Jevitt, W. M. Dunne, S. Fitzsimmons, and J. Gerst, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol. 2003, abstr. C-088.

TABLE 1—*Continued*

Published name (references)	Antimicrobial resistance pattern[f]	Published isolates (reference[s])	Comments
New York/Japan (1, 51)	β-Lactams, ery, cli, lvx	7 U.S. VISAs (22, 60, 65), 2 U.S. VRSAs (8, 9, 11), Japan VISA Mu50 (28), Korea VISA (33), CMRSA-2,[g] BK2464 (1, 13, 51), PA237 (52), JP48 (1)	Predominate U.S health care-associated PFT; endemic in many U.S. hospitals
Pediatric (24, 55)	β-Lactams	HDE1 (55), HDE288 (13, 55), COB94 (24)	
	β-Lactams, ery, cli, gen	ERMSA3[h]	
	β-Lactams, ery, cli, lvx, gen	EMRSA16[h]	Second most common U.S. health care-associated PFT
	β-Lactams, ery	MW2 (6, 42)	Community onset; isolates from (i) Native Americans (25), (ii) children without risk factors (6, 27, 42), (iii) early Australian community onset (39, 44), and (iv) San Francisco urban poor (12)
	β-Lactams, ery		Community onset; isolates from (i) correctional facilities in Miss. (7), Ga., Tenn., Tex., and Calif. (10) and (ii) athletic teams in Pa. and Calif. (10)
	β-Lactams, ery, cli, tet, lvx, gen, sxt	U.S. VISA (Ohio)	
Archaic/Iberian (46, 47)	β-Lactams, ery, tet	EMRSA2, EMRSA6, EMRSA7, EMRSA12, EMRSA13, EMRSA14[h]	
Archaic/Iberian (46, 47, 57)	β-Lactams, ery, tet	EMRSA 8, PER34 (57), NCTC10442 (31)	SCC*mec* I identified (31)
Archaic/Iberian (46, 47, 57)	β-Lactams, ery, cli, tet, lvx, gen, sxt	EMRSA5,[h] E2125 (13), HPV 107 (13, 57) France VISA (50), Scotland 463	
	β-Lactams, ery, cli, lvx	CMRSA-1[g]	
	β-Lactams, ery	PLN49 (2)	Tenn. food outbreak (34)
	β-Lactams		Second genotype at Miss. correction facility outbreak (7); hospital-associated *mec*$^+$, susceptible phenotype[i]
	β-Lactams, ery		Alaska surveillance; Vt. wrestling team outbreak (37)
Brazilian/Hungarian (2, 46, 63)	β-Lactams, ery, cli, tet, chl, lvx, gen, sxt	EMRSA1, EMRSA11, EMRSA4,[h] HU25 (2), HSJ216, HUSA304 (13), 85/2082 (31), CMRSA-3,[g] Scotland 461	SCC*mec* III identified (31)
Brazilian/Hungarian (2, 46, 63)	β-Lactams, ery, cli, tet, chl, lvx, gen, sxt	EMRSA9[h]	
	β-Lactams	EMRSA15[h] (54)	Endemic to United Kingdom (54)

loci from ST 8. All of these isolates shared a similar *spa* type motif (MBQBLO).

USA600 contained 23 isolates. Representative strains were ST 45 (10- 14- 8- 6- 10- 3- 2) and *spa* type A2AKEEMBKB. All but four of the ORSA isolates in this PFT were spectinomycin resistant (SCC*mec* II) and multiresistant; the remaining four were spectinomycin susceptible. Isolates from USA700 shared a unique MLST type, i.e., ST 72 (1- 4- 1- 8- 4- 4- 3), and *spa* motif (UJGFMGGM) from community surveillance and a hospital-acquired outbreak.

There were two clusters within the remaining 45 U.S. ORSA isolates. The first cluster (14 isolates) included surveillance isolates, mainly from Alaska, and isolates from an outbreak of soft-skin infections among Vermont wrestlers (37). The second cluster included three isolates that clustered with *S. aureus* isolates HU25, HSJ216, and HUSA304 (Brazilian and Hun-

garian type strains [13, 63]); EMRSA isolates 1, 4, and 11; and MRSA and VISA isolates from Scotland. These isolates had the same MLST profile (ST 239) and *spa* type motif (WGKAOMQ). Twenty-eight isolates had either miscellaneous PFGE patterns or patterns that could not be identified within an 80% PFT cutoff.

Of the 235 U.S. OSSA isolates, 134 (57%) clustered within the eight PFTs. All of the PFTs, with the exception of USA500, contained OSSA. Twenty OSSA isolates were USA800, 1 isolate was USA100, 78 isolates were USA200, 9 isolates were USA300, 1 isolate was USA400, 22 isolates were USA600, and 3 isolates were USA700. Twenty-eight OSSA isolates clustered in a unique PFT. There were 10 OSSA clusters with 3 to 13 isolates, and 23 isolates with unique *Sma*I PFGE profiles.

DISCUSSION

Because of its high discriminatory power, PFGE is a valuable tool for investigating outbreaks of *S. aureus* infections, particularly in hospital settings (3, 4, 16, 27, 61, 64, 66). PFGE has also been used to discriminate among community- and health care-acquired ORSA strains (42). In previous work, de Lencastre and colleagues used PFGE to delineate lineages of ORSA that circulate in Europe, South America, and the United States, and they have given the lineages names, such as the Archaic clone, the Iberian clone, the Pediatric clone, and the New York/Tokyo clone (13). Similarly, Simor et al. have used PFGE to designate four lineages of ORSA in Canada, designated as CMRSA 1 through 4 (59). These and other studies have shown that PFGE can identify stable lineages of ORSA and can be used to track the spread of these lineages from continent to continent over extended periods of time.

In the past, the sharing of PFGE data among laboratories was difficult (15), and typing results for the same strains performed in different laboratories often lacked concordance (66). However, recent advances in gel analysis software programs allow the creation and storage of large databases of normalized fragment patterns in which similarity calculations and cluster analyses can be performed with relative ease. Normalization of the fragment patterns using established standards (such as *S. aureus* NCTC 8325) and the advent of new database sharing tools both serve to facilitate the exchange of PFGE strain typing data and epidemiologic information among reference laboratories, even in different countries. Thus, the traditional barriers to the sharing of PFGE patterns, even those run using different switching parameters, have to a large extent been overcome with powerful new software programs (62).

However, the appropriateness of using PFGE to study the long-term evolution of *S. aureus* lineages in general, and ORSA isolates in particular, remains a concern (19, 40). While MLST, which examines changes in the sequences of seven neutral loci on the *S. aureus* genome, may be more amenable to long-term population studies of ORSA, the higher discriminatory ability of PFGE profiles over that of MLST for *S. aureus* is advantageous. This holds true for epidemiologic studies of particular clusters of isolates, such as those isolates causing community onset disease (12, 27, 44) and for assessing the effectiveness of targeted-prevention programs. For example, USA300 and -500 isolates were assigned different PFTs because they separated into two distinct pulsed-field clusters and had different antimicrobial susceptibility patterns and epidemiology, even though they both shared ST 8 (Table 1). USA300 isolates are predominantly from community onset infections and are resistant only to β-lactam drugs and macrolides, while USA500 isolates differ from representatives of the Archaic and Iberian clones by only one or two locus variants (14, 20), tend to be from health care-associated infections, and are multidrug resistant. USA300 isolates have been identified in multiple community onset ORSA outbreaks associated with soft-skin infections in correctional facilities (7, 10), athletic teams (10), and nurseries (10). Isolates from prisons in Mississippi, Texas, Tennessee, and Georgia all shared this PFT, as did isolates from an outbreak of ORSA infections in football players. Thus, the epidemiology of USA300 isolates is quite distinct from the health care-associated USA500 isolates, even though they are indistinguishable by MLST typing. Similarly, although USA100 and -800 isolates shared a common MLST sequence type (ST 5) and *spa* motif (MDMGMK), the isolates carried different SCC*mec* structures and had different susceptibility profiles. USA100 isolates cluster with representatives of the multiresistant New York/Japan clone containing SSC*mec* II, while isolates from USA800 cluster with representatives of the Pediatric clone containing SSC*mec* IV. These critical epidemiologic differences are obscured by MLST. Thus, the PFGE typing system described here will likely be more useful than MLST, particularly for public health agencies, for targeted epidemiologic studies aimed at understanding the epidemiology of specific clonal groups, and for developing interventional studies to halt the transmission of the disease. Given the large data sets of PFGE profiles already established in the United States and around the world (15, 16, 41), the development of a surveillance system that can integrate these databases and make the information available to other reference centers is critical.

The other lineage associated with community onset disease was USA400. This PFT included ORSA isolates that caused severe, and in some cases fatal, disease in children from Minnesota and North Dakota, and was associated with skin disease in Native Americans from eastern Washington (state) (6, 25, 27, 42). Also in this group were isolates from Australian Aborigines (39, 44). Although not present in the U.S. database, isolates of this same MLST type (ST 1) were obtained in a community study in England from healthy carriers of *S. aureus* and those with health care-associated infections (21). This may be another instance in which MLST obscures epidemiologically relevant information regarding clusters of strains with different virulence characteristics. Many *S. aureus* strains responsible for primary skin infections and necrotizing pneumonia harbor the Panton-Valentine leukocidin determinant (17, 23, 36), and preliminary studies suggest that many USA300 and -400 isolates harbor this virulence determinant (CDC unpublished observations) in addition to the more recent SSC*mec* IV.

Among the five PFTs associated predominantly with health care-related infections (i.e., USA100, -200, -500, -600, and -800), USA100 was by far the most common (44% of all U.S. ORSA isolates examined). U.S. isolates of this PFT carried SCC*mec* II. This PFT was previously designated as the New York/Japan clone (1). Interestingly, several other previously described global lineages that are common as causes of health care-associated infections (e.g., the Brazilian clone and EMRSA15) were not found among U.S. isolates.

Most of the ORSA PFTs also contained OSSA isolates, suggesting that each of these lineages had independently acquired *mecA*. Five types of SCC*mec* have been identified. Type I (34 kb) was detected in the first ORSA strain isolated in 1961 in the United Kingdom (strain NCTC 10442) (31); type II (52 kb) was identified in an ORSA strain isolated in 1982 in Japan (strain N315) (30); type III (66 kb) was identified in an MRSA strain isolated in 1985 in New Zealand (strain 82/2082) (31); type IV (20 to 24 kb) was identified in two community-acquired ORSA strains (38) and in isolates of the Pediatric clone from Poland and Portugal (45); and a new type 2 was identified in three community onset ORSA strains from Adelaide, Australia (45). SCC*mec* II and III contain transposon Tn*554*, which encodes erythromycin and spectinomycin resistance (31). SCC*mec* I and IV carry no other resistance genes (31, 38).

In conclusion, results of *Sma*I PFGE typing, corroborated with those of limited MLST and *spa* typing, allowed us to identify genetic backgrounds and delineate the major U.S. ORSA lineages within a national database generated from PFGE fingerprinting and epidemiologic data from the CDC and U.S. state health laboratory investigations. PFGE has proven to be more discriminating than MLST for monitoring the spread of ORSA isolates in the United States, although periodic typing of selected isolates using MLST will be critical for assessing major changes in the lineages over time.

ACKNOWLEDGMENTS

We thank Mark Enright for providing the MLST data on several of the isolates cited in this paper; we also thank Molly Kellum, Bette Jensen, Glennis Westbrock, and Loretta Carson for pulsed-field electrophoresis typing, Stephen Weber for assistance with SCC*mec* analysis, Matthew Kuehnert and Jeff Hageman for providing isolates, and J. Kamile Rasheed and Leslie McGee for comments on the manuscript.

The use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

REFERENCES

1. **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.
2. **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 from 1996 to 1998. J. Clin. Microbiol. **39:**2197–2205.
3. **Aucken, H. M., M. Ganner, S. Murchan, B. D. Cookson, and A. P. Johnson.** 2002. A new UK strain of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-17) resistant to multiple antibiotics. J. Antimicrob. Chemother. **50:**171–175.
4. **Bannerman, T. L., G. A. Hancook, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus.* J. Clin. Microbiol. **33:**551–555.
5. **Centers for Disease Control and Prevention.** 1997. *Staphylococcus aureus* with reduced susceptibility to vancomycin—United States, 1997. Morb. Mortal. Wkly. Rep. **46**:765–766.
6. **Centers for Disease Control and Prevention.** 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. Morb. Mortal. Wkly. Rep. **48**:707–710.
7. **Centers for Disease Control and Prevention.** 2001. Methicillin-resistant *Staphylococcus aureus* skin or soft tissue infections in a state prison—Mississippi, 2000. Morb. Mortal. Wkly. Rep. **50**:919–922.
8. **Centers for Disease Control and Prevention.** 2002. *Staphylococcus aureus* resistant to vancomycin—United States, 2002. Morb. Mortal. Wkly. Rep. **51**:565–567.
9. **Centers for Disease Control and Prevention.** 2002. Public health dispatch: vancomycin-resistant *Staphylococcus aureus*—Pennsylvania, 2002. Morb. Mortal. Wkly. Rep. **51**:902.
10. **Centers for Disease Control and Prevention.** 2003. Public health dispatch: outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* skin infections—Los Angeles County, California, 2002–2003. Morb. Mortal. Wkly. Rep. **52**:88.
11. **Chang, S., D. M. Sievert, J. C. Hageman, M. L. Boulton, F. C. Tenover, F. P. Downes, S. Shah, J. T. Rudrik, G. R. Pupp, W. J. Brown, D. Cardo, S. K. Fridkin, and the Vancomycin-Resistant** *Staphylococcus aureus* Investigative Team. 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *van*A resistance gene. N. Engl. J. Med. **348**:1342–1347.
12. **Charlebois, E. D., D. R. Bangsberg, N. J. Moss, M. R. Moore, A. R. Moss, H. F. Chambers, and F. Perdreau-Remington.** 2002. Population-based community prevalence of methicillin-resistant *Staphylococcus aureus* in the urban poor of San Francisco. Clin. Infect. Dis. **34:**425–433.
13. **Chung, M., H. de Lencastre, P. Matthews, A. Tomasz, and the Multilaboratory Project Collaborators: 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-Leão, I. Santos Sanches, J. 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.
14. **Crisostoma, M. I., H. Westh, A. Tomasz, M. Chung, D. C. Oliveira, and H. de Lencastre.** 2001. The evolution of methicillin resistance in *Staphylococcus aureus*: similarity of genetic backgrounds in historically early methicillin-susceptible and -resistant isolates and contemporary epidemic clones. Proc. Natl. Acad. Sci. USA **9:**9865–9870.
15. **Deplano, A., A. Schuermans, J. Van Eldere, W. Witte, H. Meugnier, J. Etienne, H. Grundmann, D. Jonas, G. T. Noordhoek, J. Dijkstra, A. van Belkum, W. van Leeuwen, P. T. Tassios, N. J. Legakis, A. van der Zee, A. Bergmans, D. S. Blanc, F. C. Tenover, B. C. Cookson, G. O'Neil, M. J. Struelens, and The European Study Group on Epidemiological Markers of the ESCMID.** 2000. Multicenter evaluation of epidemiological typing of methicillin-resistant *Staphylococcus aureus* strains by repetitive-element PCR analysis. J. Clin. Microbiol. **38**:3527–3533.
16. **Deplano, A., W. Witte, W. J. van Leeuwen, Y. Brun, and M. J. Struelens.** 2000. Clonal dissemination of epidemic methicillin-resistant *Staphylococcus aureus* in Belgium and neighboring countries. Clin. Microbiol. Infect. **6:**239–245.
17. **Dufour, P., Y. Gillet, M. Bes, G. Lina, F. Vandenesch, D. Floret, J. Etienne, and H. Richet.** 2002. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin. Infect. Dis. **35:**819–824.
18. **Enright, M. C., and B. G. Spratt.** 1999. Multilocus sequence typing. Trends Microbiol. **7:**482–487.
19. **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.
20. **Enright, M. C., D. 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.
21. **Feil, E. J., J. E. Cooper, H. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. M. Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. J. Day.** 2003. How clonal is *Staphylococcus aureus*? J. Bacteriol. **185:**3307–3316.
22. **Fridkin, S. K., J. Hageman, L. K. McDougal, J. Mohammed, W. R. Jarvis, T. M. Perl, F. C. Tenover, and the Vancomycin-Intermediate** ***Staphylococcus aureus*** **Epidemiology Study Group.** 2003. Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997–2000. Clin. Infect. Dis. **36**:429–439.
23. **Gillet, Y., B. Issartel, P. Vanhems, J. Fournet, G. Lina, M. Bes, F. Vandenesch, Y. Piémont, N. Brousse, D. Floret, and J. Etienne.** 2002. Association between *Staphylococcus aureus* strains carrying the gene for the Panton-Valentine leukocidin and highly-lethal necrotising pneumonia in young immunocompetent patients. Lancet **359:**753–759.
24. **Gomes, A. R., I. Santos Sanches, M. Aires de Sousa, E. Castaneda, and H. De Lencastre.** 2001. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Colombian hospitals: dominance of a single unique multidrug-resistant clone. Microb. Drug Resist. **7:**23–32.
25. **Groom, A. V., D. H. Wolsey, T. S. Naimi, K. Smith, S. Johnson, D. Boxrud, K. A. Moore, and J. E. Cheek.** 2001. Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian community. JAMA **286:**1201–1205.
26. **Haley, R. W., N. B. Cushion, F. C. Tenover, T. L. Bannerman, D. Dryer, J. Ross, P. J. Sanchez, and J. D. Siegel.** 1995. Eradication of endemic methicillin-resistant *Staphylococcus aureus* infections from a neonatal intensive care unit. J. Infect. Dis. **171:**614–624.
27. **Herold, B. C., L. C. Immergluck, M. C. Maranan, D. S. Lauderdale, R. E.**

Gaskin, S. Boyle-Vavra, C. D. Leitch, and R. S. Daum. 1998. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA **279:**593–598.
28. **Hiramatsu, K., H. Hanaki, T. Ino, K. Yabuta, T. Oguri, and F. C. Tenover.** 1997. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J. Antimicrob. Chemother. **40:**135–146.
29. **Hiramatsu, K., L. Cui, M. Kuroda, and T. Ito.** 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus.* Trends Microbiol. **9:**486–493.
30. **Ito, T., Y. Katayama, and K. Hiramatsu.** 1999. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother. **43:**1449–1458.
31. **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.
32. **Karchmer, A. W.** 2000. Nosocomial bloodstream infections: organisms, risk factors, and implications. Clin. Infect. Dis. **31**(Suppl. 4)**:** S139–S143.
33. **Kim, M., C. H. Pai, J. H. Woo, J. S. Ryu, and K. Hiramatsu.** 2000. Vancomycin-intermediate *Staphylococcus aureus* in Korea. J. Clin. Microbiol. **38:** 3879–3881.
34. **Jones, T. F., M. E. Kellum, S. S. Porter, M. Bell, and W. Schaffner.** 2002. An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus.* Emerg. Infect. Dis. **8:**82–84.
35. **Leclercq, R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. **34:**482–492.
36. **Lina, G., Y. Piémont, F. Godail-Gamot, M. Bes, M. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132.
37. **Lindenmayer, J. M., S. Schoenfeld, R. O'Grady, and J. K. Carney.** 1998. Methicillin-resistant *Staphylococcus aureus* in a high school wrestling team and the surrounding community. Arch. Intern. Med. **158**:895–899.
38. **Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chontrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu.** 2002. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **46:** 1147–1152.
39. **Maguire, G. P., A. D. Arthur, P. J. Boustead, B. Dwyer, and B. J. Currie.** 1996. Emerging epidemic of community-acquired methicillin-resistant *Staphylococcus aureus* infection in the Northern Territory. Med. J. Aust. **164:**721–723.
40. **Maiden, M. C. J., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
41. **Murchan, S., M. E. Kaufmann, A. Deplano, R. de Ryck, M. Struelens, C. E. Zinn, V. Fussing, S. Salmenlinna, J. Vuopio-Varkila, N. E. Solh, C. Cuny, W. Witte, P. T. Tassios, N. Legakis, W. van Leeuwen, A. van Belkum, A. Vindel, I. Laconcha, J. Garaizar, S. Haeggman, B. Olsson-Liljequist, U. Ransjo, G. Coombes, and B. Cookson.** 2003. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J. Clin. Microbiol. **41:**1574–1585.
42. **Naimi, T. S., K. H. LeDell, D. J. Boxrud, A. V. Groom, C. D. Steward, S. K. Johnson, J. M. Besser, C. O'Boyle, R. N. Danila, J. E. Cheek, M. T. Osterholm, K. A. Moore, and K. E. Smith.** 2001. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. Clin. Infect. Dis. **33:**990–996.
43. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed., vol. 20, no. 2. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
44. **O'Brien, F. G., J. W. Pearman, M. Gracey, T. V. Riley, and W. B. Grubb.** 1999. Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J. Clin. Microbiol. **37:**2858–2862.
45. **Okuma, K., K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien, G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito, and K. Hiramatsu.** 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. **40:**4289–4294.
46. **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.
47. **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.
48. **Panlilio, A. L., D. H. Culver, R. P. Gaynes, S. Banerjee, T. S. Henderson, J. S. Tolson, and W. J. Martone.** 1992. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975–1991. Infect. Control Hosp. Epidemiol. **13**:582–586.
49. **Phillips, S., and R. P. Novick.** 1979. Tn*554*—a site-specific repressor-controlled transposon in *Staphylococcus aureus.* Nature **278:**476–478.
50. **Ploy, M. C., C. Grélaud, C. Martin, L. de Lumley, and F. Denis.** 1998. First clinical isolate of vancomycin-intermediate *Staphylococcus aureus* in a French hospital. Lancet **351:**1212.
51. **Roberts, R. B., H. de Lencastre, W. Eisner, E. P. Severina, B. Shopsin, B. N. Kreiswirth, and A. Tomasz, and the MRSA Collaborative Study Group.** 1998. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in 12 New York hospitals. J. Infect. Dis. **178:**164–171.
52. **Roberts, R. B., M. Chung, H. de Lencastre, J. Hargrave, A. Tomasz, D. P. Nicolau, J. F. John, Jr., O. Korzeniowski, and the Tri-State MRSA Collaborative Study Group.** 2000. Distribution of methicillin-resistant *Staphylococcus aureus* clones among health care facilities in Connecticut, New Jersey, and Pennsylvania. Microb. Drug Resist. **6**:245–251.
53. **Rosdahl, V. T., and A. M. Knudsen.** 1991. The decline of methicillin resistance among Danish *Staphylococcus aureus* strains. Infect. Control Hosp. Epidemiol. **12:**83–88.
54. **Richardson, J. F., and S. Reith.** 1993. Characterization of a strain of methicillin-resistant *Staphylococcus aureus* (EMRSA-15) by conventional and molecular methods. J. Hosp. Infect. **25:**45–52.
55. **Sa-Leao, 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.
56. **Salgado, C. D., B. M. Farr, and D. P. Calfee.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. Clin. Infect. Dis. **36:**131–139.
57. **Santos-Sanches, I., 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.
58. **Shopsin, B., M. Gomez, S. O. Montgomery, D. H. Smith, M. Waddington, D. E. Dodge, D. A. Bost, M. Riehman, S. Naidich, and B. N. Kreiswirth.** 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. **37:**3556–3563.
59. **Simor, A. E., M. Ofner-Agostini, E. Bryce, A. McGeer, S. Paton, and M. R. Mulvey.** 2002. Laboratory characterization of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: results of 5 years of national surveillance, 1995–1999. J. Infect. Dis. **186:**652–660.
60. **Smith, T. L., M. L. Pearson, K. R. Wilcox, C. Cruz, M. V. Lancaster, B. Robinson-Dunn, F. C. Tenover, M. J. Zervos, J. D. Band, E. White, and W. R. Jarvis.** 1999. Emergence of vancomycin resistance in *Staphylococcus aureus.* N. Engl. J. Med. **340:**493–501.
61. **Struelens, M. J., R. Bax, A. Deplano, W. G. Quint, and A. Van Belkum.** 1993. Concordant clonal delineation of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis and polymerase chain reaction genome fingerprinting. J. Clin. Microbiol. **31:**1064–1070.
62. **Swaminathan, B., T. J. Barrett, S. B. Hunter, R. V. Tauxe, and the CDC PulseNet Task Force.** 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg. Infect. Dis. **7**:382–389.
63. **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.
64. **Tenover, F. C., R. Arbeit, G. Archer, J. Biddle, S. Byrne, R. Goering, G. Hancock, G. A. Hebert, B. Hill, and R. Hollis.** 1994. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus.* J. Clin. Microbiol. **32:**407–415.
65. **Tenover, F. C., M. V. Lancaster, B. C. Hill, C. D. Steward, S. A. Stocker, G. A. Hancock, C. M. O'Hara, N. C. Clark, and K. Hiramatsu.** 1998. Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. J. Clin. Microbiol. **36:**1020–1027.
66. **Van Belkum, A., W. van Leeuwen, M. E. Kaufmann, B. Cookson, F. Forey, J. Etienne, R. Goering, F. Tenover, C. Steward, F. O'Brien, W. Grubb, P. Tassios, N. Legakis, A. Morvan, N. ElSolh, R. de Ryck, M. Struelens, S. Salmenlinna, J. Vuopio-Varkila, M. Kooistra, A. Talens, W. Witte, and H. Verbrugh.** 1998. Assessment of resolution and intercenter reproducibility of results of genotyping *Staphylococcus aureus* by pulsed-field gel electrophoresis of *Sma*I macrorestriction fragments: a multicenter study. J. Clin. Microbiol. **36:**1653–1659.
67. **Voss, A., D. Milatovic, C. Wallrauch-Schwarz, V. T. Rosdahl, and I. Braveny.** 1994. Methicillin-resistant *Staphylococcus aureus* in Europe. Eur. J. Clin. Microbiol. Infect. Dis. **13:**50–55.
68. **Wei, M. Q., and W. B. Grubb.** 1992. Typing of Australian methicillin-resistant *Staphylococcus aureus* strains by pulsed-field gel electrophoresis. J. Med. Microbiol. **37:**187–191.
69. **Weisblum, B.** 1995. Insights into erythromycin action from studies of its activity as inducer of resistance. Antimicrob. Agents Chemother. **39:**797–805.