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Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolates Collected in 2005 and 2006 from Patients with Invasive Disease: a Population-Based Analysis[▽]

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This study characterizes 1,984 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected in 2005 and 2006 from normally sterile sites in patients with invasive MRSA infection. These isolates represent a convenience sample of all invasive MRSA cases reported as part of the Active Bacterial Core surveillance system in eight states in the United States. The majority of isolates were from blood (83.8%), joints (4.1%), and bone (4.2%). Isolates were characterized by pulsed-field gel electrophoresis (PFGE); SCC*mec* typing; susceptibility to 15 antimicrobial agents; and PCR analysis of staphylococcal enterotoxin A (SEA) to SEH, toxic shock syndrome toxin 1, and Panton-Valentine leukocidin. Thirteen established PFGE types were recognized among these isolates, although USA100 and USA300 predominated, accounting for 53.2% and 31.4% of the isolates, respectively. As expected, isolates from hospital onset cases were predominantly USA100, whereas those from community-associated cases were predominantly USA300. USA100 isolates were diverse (Simpson's discriminatory index [DI] = 0.924); generally positive only for enterotoxin D (74.5%); and resistant to clindamycin (98.6%), erythromycin (99.0%), and levofloxacin (99.6%), in addition to β-lactam agents. USA300 isolates were less diverse (DI = 0.566), positive for Panton-Valentine leukocidin (96.3%), and resistant to erythromycin (94.1%) and, less commonly, levofloxacin (54.6%), in addition to β-lactam agents. This collection provides a reference collection of MRSA isolates associated with invasive disease, collected in 2005 and 2006 in the United States, for future comparison and ongoing studies.

Staphylococcus aureus is an important cause of serious infection in the United States and worldwide. Although traditionally associated with health care-acquired infections, methicillin-resistant *S. aureus* (MRSA) is increasingly causing severe and even fatal disease in community settings as well. Historically, in the United States, MRSA strains associated with hospital onset MRSA (HO-MRSA) disease were resistant to multiple classes of antibiotics and contained the SCC*mec* II element, whereas community-associated MRSA (CA-MRSA) strains were resistant only to β-lactam agents and erythromycin, carried the SCC*mec* IV element, and encoded the Panton-Valentine leukocidin (PVL) toxin (24). More recently, transmission of strains historically associated with CA-MRSA in the United States has been documented to occur in health care facilities (15, 21). In addition, many invasive MRSA infections related to delivery of health care are occurring in the outpatient setting. These health care-associated community onset MRSA (HACO-MRSA) infections, occurring in nonhospitalized patients who have recently documented exposures to health care delivery, made up the largest category of invasive MRSA infections in the United States in 2005 (22).

Identifying unique differences in strain characteristics by geography or type of invasive infection might help to explain the changing epidemiology of MRSA clinical disease and to develop a baseline from which to evaluate temporal trends in molecular characteristics of MRSA associated with disease. To accomplish this, we characterized a large collection of MRSA isolates responsible for invasive disease collected from geographically disparate areas in the United States during 2005 and 2006 to determine the diversity of strain types and correlations between strain characteristics and clinical or epidemiologic characteristics.

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MATERIALS AND METHODS

Surveillance methodology and definitions. Cases of invasive MRSA were reported through clinical microbiology laboratories in acute-care hospitals and all

reference laboratories processing sterile-site specimens for residents of the surveillance areas participating in the Active Bacterial Core surveillance (ABCs) system in the Emerging Infections Program (EIP) of the Centers for Disease Control and Prevention (CDC). This surveillance program's methodology, definitions, and isolate collection method have been published previously (22). In brief, ABCs, an ongoing, population-based, active hospital laboratory surveillance system, began submitting MRSA isolates associated with cases of invasive MRSA infection beginning in January 2005 from 48 (39%) of the clinical microbiology laboratories serving residents of the eight surveillance sites: (i) the state of Connecticut (estimated population, 3.5 million); (ii) the Atlanta, GA, metropolitan area (eight counties; estimated population, 3.5 million); (iii) the San Francisco Bay area, CA (three counties; estimated population, 3.2 million); (iv) the Denver, CO, metropolitan area (five counties; estimated population, 2.3 million); (v) the Portland, OR, metropolitan area (three counties; estimated population, 1.5 million); (vi) Monroe County (Rochester area), NY (estimated population, 733,000); (vii) Davidson County (Nashville area), TN (estimated population, 575,000); and (viii) Ramsey County (St. Paul area), MN (estimated population, 495,000).

A case of invasive MRSA infection was indicated by the isolation of MRSA from a normally sterile body site in a resident of the surveillance area. Personnel used a standard case report form to compile each ABCs site's abstracted data from medical records from hospital and clinic visits. The abstraction is performed by surveillance personnel rather than clinical investigators and relies largely on admission notes, discharge notes, and other notes rather than primary clinical data for determining specific diagnoses. For this analysis, we used health care exposure information to classify cases into mutually exclusive groups: (i) health care-associated infections, which were either community onset infections (cases with a health care risk factor but with culture obtained ≤48 h after hospital admission) or hospital onset infections (cases with culture obtained >48 h after admission, regardless of the presence of other health care risk factors), and (ii) community-associated infections (cases without documented health care risk factors and with culture obtained ≤48 h after hospital admission).

Patients were considered to have a clinical syndrome of bacteremia, pneumonia, cellulitis, osteomyelitis, endocarditis, septic shock, or other infection when there was documentation of such diagnosis in the medical record (e.g., discharge face sheet), regardless of the source of the isolate. A patient with documented bloodstream infection (BSI) and no other recorded clinical syndrome was considered to have uncomplicated BSI (referred to as "BSI-only"). This clinical syndrome likely includes catheter-related infections and other BSI where no primary infection was documented, but available data did not contain sufficient detail to assess this. Most (56.1%) patients with clinical syndromes other than BSI-only had more than one documented syndrome, and the most common accompanying syndrome (84.0%) was documented BSI.

Isolate characterization. The isolates in this collection represent a convenience sample of MRSA cases reported through ABCs. Convenience sampling, also called grab or opportunity sampling, is a method of choosing isolates in an unstructured manner from the population frame. Laboratories whose staff were willing to save, store, and process large numbers of isolates linked to cases were chosen for isolate submission. These considerations lessen the impact of a nonrandom sample.

All isolates were sent to the CDC laboratory for confirmatory identification and testing. Isolates received at the CDC were subcultured onto 5% sheep blood agar and incubated overnight at 35°C. Colonies consistent with *S. aureus* (large, gray to gold, and beta-hemolytic) were confirmed by a positive result for a Staphaurex assay (Remel, Inc., Lenexa, KS); atypical colonies or those negative by Staphaurex were confirmed with a positive result for a tube coagulase test and/or additional biochemical reactions (ID32 Staph; bioMérieux SA, Marcy-l'Etoile, France). Methicillin resistance was confirmed by oxacillin and cefoxitin disk diffusion (6, 7). When multiple-colony morphologies were present, each morphotype received confirmatory identification and methicillin resistance testing, although further characterization was performed only on the predominant MRSA morphotype. All isolates were frozen in sheep blood at −70°C for future use.

To reduce the number of isolates tested, complete testing was performed on all nonblood isolates, all isolates associated with diagnoses other than BSI, and a sample of blood isolates from patients with BSI-only. When applying this testing strategy resulted in fewer than 100 isolates for any submitting site, additional isolates associated with BSI-only were tested.

A representative sample of isolates tested and reported in this paper is available through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (http://www.narsa.net/).

Statistical analysis. We included isolates collected from January 2005 through December 2006. Although the ABCs system is designed to collect population-based surveillance data and previous reports of invasive MRSA cases from this time period could be generalized to the larger population, isolates were collected as a convenience sample and might not be representative of the cases described in previous reports (22).

We compared the clinical syndromes of osteomyelitis, endocarditis, pneumonia (including pneumonia and/or empyema), and soft tissue infections (including cellulitis and abscess) to those in the referent group, comprising BSI-only patients (i.e., with no other staphylococcal diagnosis reported). We chose BSI-only patients as the referent group because these patients most closely represent traditional health care-associated MRSA infections (e.g., central line-associated BSI) and also because most patients with other staphylococcal syndromes also had documented BSI. By using BSI-only patients as the referent group, we hoped to better evaluate the influences of strain type and epidemiologic class on non-BSI staphylococcal syndromes. We further stratified each clinical syndrome by epidemiologic classification (HO-, HACO-, and CA-MRSA). We tested differences in proportions of descriptive characteristics by using the χ^2 test. Analyses were performed using SAS version 9.1.3 (SAS Institute, Inc., Cary, NC).

Associations between strain type and clinical presentation or epidemiologic class were estimated by odds ratio (OR) and 95% confidence intervals (CI) obtained by logistic regression modeling (Proc Logistic in SAS 9.1.3). Both clinical presentation and epidemiologic class were included as independent variables for multivariate logistic regression modeling to obtain an adjusted OR (AOR), which showed the strength of association between the variable of interest and the strain type (e.g., USA300) after adjustment for the other variable in the model. Multivariate logistic regression modeling found no interaction between clinical presentation and epidemiologic class ($P > 0.15$).

Strain typing. Strain typing by pulsed-field gel electrophoresis (PFGE) was performed with the SmaI restriction enzyme as described previously (24), now updated using *Salmonella enterica* serovar Braenderup H9182 as the normalization standard. PFGE testing was performed at the CDC and at EIP laboratories in Colorado, Connecticut, Georgia, Minnesota, and Oregon. Gel images were compared at the CDC by using BioNumerics version 4.01 software (Applied Maths, Austin, TX) and assigned to pulsed-field types at 80% relatedness and to pulsed-field patterns at 95% relatedness by use of Dice coefficients and the unweighted-pair group method using average linkages (24).

spa typing was performed on the single isolate that was nontypeable by PFGE, as recommended. Resultant sequences were queried on a *spa* typing website (http://www.spaserver.ridom.de/) developed by Ridom GmbH and curated by SeqNet.org (http://www.seqnet.org/) (17).

Discrimination index (DI). Simpson's index of diversity was used to compare diversity among PFGE types, as described by the formula of Hunter and Gaston (18).

Detection of staphylococcal toxins. DNA was extracted by suspending five single colonies from overnight growth in 50 μl 0.05 M NaOH and heating them to 95°C for 15 min. After the heating, 18 μl 0.5 N HCl and 400 μl water were added, mixed by inversion, and frozen until use. Five microliters of this DNA extract was used as a PCR template in the subsequent reactions. The presence of genes encoding staphylococcal enterotoxins A, B, C, D, E, and H (SEA to SEH), toxic shock syndrome toxin 1 (TSST-1) (*tst*) and PVL (*lukS-PV*) was assessed by real-time PCR (MX3000P; Stratagene, La Jolla, CA) in two multiplex PCR assays as previously described (26). The presence of genes encoding only TSST-1 and PVL was assessed by real-time PCR (MX3000P; Stratagene) in a single duplex reaction using the same primers, probes, and concentrations.

SCC*mec* typing. Identification of the SCC*mec* complex was performed by a PCR screen for SCC*mec* II and SCC*mec* IV, using DNA extract prepared as described above as a template. A 50-μl PCR mixture was prepared in 1× AmpliTaq Gold buffer (Applied Biosystems, Foster City, CA) with 1.5 mM $MgCl_2$, 0.8 mM deoxynucleoside triphosphates, 2.5 U AmpliTaq Gold (Applied Biosystems), and each of the following primers at a 0.2 μM final concentration: mI3 (5′ CAA AAG GAC TGG ACT GGA GTC CAA A 3′), mI4 (5′ CAA GTG AAT TGA AAC CGC CT 3′) (28), CBM1 (5′ CCC TAT TTC TTT AAT AGG CGT CTA A 3′), and CBM2 (5′ CCT AAA CCT AAT CGA AAC AAG CGT A 3′). The cycling parameters were 1 min at 95°C; 30 cycles of 30 s at 94°C, 60 s at 50°C, and 120 s at 72°C; and 2 min at 72°C. Products were separated by electrophoresis through 2% agarose and visualized by ethidium bromide staining and long-wave UV light. A 180-bp product was interpreted as SCC*mec* type II, and a 200-bp product was interpreted as SCC*mec* type IV. Any isolate that did not yield a product of the expected size with this assay was investigated by more-extensive SCC*mec* typing as described previously (34).

Antimicrobial susceptibility testing. Isolates were tested using the CLSI broth microdilution reference method (3, 5) for susceptibility to chloramphenicol, clindamycin, daptomycin, doxycycline, erythromycin, gentamicin, levofloxacin, linezolid, mupirocin, penicillin, oxacillin, rifampin, tetracycline, trimethoprim-

TABLE 1. Clinical syndromes associated with 1,984 cases of invasive MRSA infection

Clinical syndrome[a]	No. (%) of cases listed as:			
	One of >1 syndrome		Only syndrome	
BSI	1,388	70.0	453	22.8
Pneumonia	319	16.1	57	2.9
Osteomyelitis	204	10.3	51	2.6
Arthritis/joint infection/ bursitis	169	8.5	51	2.6
Cellulitis	283	14.3	42	2.1
Abscess	130	6.6	40	2.0
Sepsis	136	6.9	31	1.6
Urinary tract infection	181	9.1	24	1.2
Empyema/pleural effusion	52	2.6	22	1.1
Endocarditis	134	6.8	11	0.6
Peritonitis	32	1.6	15	0.8
Surgical site infection	61	3.1	9	0.5
Other	56	2.8	7	0.4
Meningitis/VPS infection[b]	10	0.5	5	0.3
Pressure ulcer	50	2.5	4	0.2
Traumatic wound	17	0.9	2	0.1
Pericarditis	6	0.3	0	0.0
No diagnosis identified	47	2.4	47	2.4
Total	3,729		871	

[a] Clinical syndromes were assessed on the basis of documentation of such diagnosis in the medical record (e.g., discharge face sheet), regardless of the source of the isolate. Patients may have more than one clinical diagnosis, with the exception of bloodstream infection only, which is defined as bloodstream infection with no other diagnosis reported.

[b] VPS, ventriculoperitoneal shunt.

sulfamethoxazole, and vancomycin, using MIC plates prepared in-house at the CDC. Cation-adjusted Mueller-Hinton broth was obtained from BBL Microbiology Systems (Sparks, MD). MIC plates were incubated in ambient air for 18 to 24 h as described by the CLSI. Inducible resistance to clindamycin was detected with a D-zone test (2) or by broth microdilution with a combination of 4 μg/ml erythromycin and 0.5 μg/ml clindamycin (3). The following quality control organisms were included on each day of susceptibility testing: *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 (4).

RESULTS

Description of MRSA infections and epidemiologic characteristics. A total of 2,670 invasive MRSA isolates were received and successfully matched to the appropriate cases; 1,984 (74.3%) were chosen for this evaluation by using the specified criteria. Isolates in this collection were obtained from blood (1,662; 83.8%) or other normally sterile body sites, including joint (81; 4.1%), bone (87; 4.4%), pleural fluid (39; 2.0%), peritoneal fluid (21; 1.1%), other body fluid (23; 1.2%), and other (<1% each) sites.

Patients represented by this group of isolates had a variety of invasive MRSA infection types, most often presenting with BSI (69.9%), skin and soft tissue infections (e.g., cellulitis or abscess) (20.9%), pneumonia or empyema (18.6%), or osteomyelitis (10.4%) (Table 1). Only 871 (43.9%) of the isolates were from patients with a single documented invasive MRSA-presenting syndrome; among these patients were 453 with BSI-only, 57 with pneumonia or empyema, 51 with cellulitis or abscess, 51 with arthritis or joint infection, and 42 with osteomyelitis. Although BSI was not documented to occur in non-BSI-only cases, most isolates were recovered from blood.

The median number of isolates evaluated per surveillance site was 248 (range, 47 to 512). The isolates included 447 (22.5%) from hospital onset infections, 1,140 (57.5%) from health care-associated community-onset infections, and 188 (19.1%) from community-associated infections; 18 (0.9%) were from patients with undetermined statuses (Table 2). The distribution of epidemiologic class among isolates described here is similar to what was observed among all invasive MRSA infections reported to this system (22). In all surveillance sites, the proportion of isolates from patients with HACO-MRSA infections was highest, and isolates from hospital onset cases were generally more frequent than those from community-associated cases (data not shown).

Diversity of PFGE patterns and characterization of MRSA strain types. PFGE, performed on 1,923 of 1,984 (96.9%)

TABLE 2. Epidemiologic characteristics of invasive MRSA isolates, according to PFGE type

PFGE type	No. (%)	No. (%) in indicated epidemiologic class			
		HO	HACO	CA	UNK[a]
USA100	1,063 (53.6)	306 (28.8)	669 (62.9)	83 (7.8)	5 (0.5)
USA200	15 (0.8)	6 (40)	9 (60)	0 (0)	0 (0)
USA300	627 (31.6)	76 (12.1)	293 (46.7)	246 (39.2)	12 (1.9)
USA400	6 (0.3)	0 (0)	3 (50)	3 (50)	0 (0)
USA500	74 (3.7)	11 (14.9)	57 (77)	6 (8.1)	0 (0)
USA600	14 (0.7)	6 (42.9)	6 (42.9)	2 (14.3)	0 (0)
USA700	8 (0.4)	2 (25)	3 (37.5)	3 (37.5)	0 (0)
USA800	38 (1.9)	6 (15.8)	25 (65.8)	6 (15.8)	1 (2.6)
USA1000	16 (0.8)	1 (6.2)	10 (62.5)	5 (31.2)	0 (0)
USA1100	8 (0.4)	2 (25)	4 (50)	2 (25)	0 (0)
Iberian	36 (1.8)	8 (22.2)	20 (55.6)	8 (22.2)	0 (0)
Novel type	11 (0.6)	4 (36.4)	7 (63.6)	0 (0)	0 (0)
EMRSA15	4 (0.2)	2 (50)	1 (25)	1 (25)	0 (0)
Group D	2 (0.1)	0 (0)	1 (50)	1 (50)	0 (0)
Nontypeable	1 (0.1)	0 (0)	0 (0)	1 (100)	0 (0)
Not done	61 (3.1)	17 (27.9)	32 (52.5)	12 (19.7)	0 (0)
Total	1,984	447 (22.5)	1,140 (57.5)	379 (19.1)	18 (0.9)

[a] UNK, unknown.

TABLE 3. Molecular characteristics of invasive MRSA isolates, according to PFGE type[a]

PFGE type	No. (%) of isolates tested	DI	No. (%) of isolates						
			With indicated toxin detected by PCR						ND
			TSST-1	PVL	SEA	SEB	SEC	SED	
USA100	063 (53.6)	0.923	2 (0.2)	2 (0.2)	9 (1.5)	2 (0.3)	1 (0.2)	454 (74.4)	453 (42.6)
USA200	15 (0.8)	0.695	13 (86.7)	0 (0)	10 (76.9)	0 (0)	0 (0)	0 (0)	2 (13.3)
USA300	627 (31.6)	0.566	0 (0)	605 (96.5)	0 (0)	0 (0)	0 (0)	3 (1)	313 (49.9)
USA400	6 (0.3)	0.8	0 (0)	4 (66.7)	1 (25)	1 (25)	1 (25)	0 (0)	2 (33.3)
USA500	74 (3.7)	0.881	0 (0)	0 (0)	40 (88.9)	31 (68.9)	0 (0)	1 (2.2)	29 (39.2)
USA600	14 (0.7)	0.846	0 (0)	0 (0)	1 (11.1)	1 (11.1)	0 (0)	0 (0)	5 (35.7)
USA700	8 (0.4)	0.786	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (87.5)
USA800	38 (1.9)	0.899	0 (0)	0 (0)	2 (14.3)	0 (0)	0 (0)	5 (35.7)	24 (63.2)
USA1000	16 (0.8)	0.724	0 (0)	2 (12.5)	0 (0)	6 (75)	0 (0)	0 (0)	8 (50)
USA1100	8 (0.4)	0.464	0 (0)	6 (75)	0 (0)	0 (0)	0 (0)	0 (0)	4 (50)
Iberian	36 (1.8)	0.887	1 (2.8)	1 (2.8)	0 (0)	2 (13.3)	1 (6.7)	1 (6.7)	21 (58.3)
Novel type	11 (0.6)	0.982	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (57.1)	4 (36.4)
EMRSA15	4 (0.2)	0.83	0 (0)	1 (25)	0	0	0	0	4 (100)
Group D	2 (0.1)	NA	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (50)
Nontypeable	1 (0.1)	NA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Not done	61 (3.1)	NA	0 (0)	18 (29.5)	0	0	0	0	61 (100)
Total	1,984		16 (0.8)	639 (32.2)	63 (3.2)	44 (2.2)	3 (0.2)	468 (23.6)	938 (47.3)

[a] NA, not applicable; ND, not done.

isolates collected, classified isolates into 13 recognized PFGE types and 9 unnamed (novel) types (Tables 2 and 3). One isolate from New York could not be digested with SmaI after numerous attempts and was deemed nontypeable by this method. *spa* typing characterized this isolate as t1191, a type unlinked to any named clonal complex (14). USA100 and USA300 were the most common types observed, accounting for 53.3% and 31.5% of isolates, respectively. Among the more common strain types identified among isolates in this study (represented by 20 or more isolates, i.e., ≥1% of the total), each was identified in seven or more states, with the notable exception of USA500. After USA100 and USA300, USA500 was the most common strain type, accounting for 3.7% of all isolates. Two geographically distinct sites accounted for the majority of USA500 strains in our study (29.7% and 58.1%, respectively), although these sites were the fourth and fifth most common contributors of isolates overall. USA500 was more common than USA300 (12.6% and 9.2%, respectively) at one site. Other PFGE types were less common; each accounted for less than 2.0% of isolates and cumulatively accounted for only 7.8% of isolates (Tables 2 and 3).

There was substantial diversity among the 1,063 isolates identified as PFGE type USA100, with 154 unique PFGE patterns observed (DI = 0.924) (Table 3). Together, the three most commonly observed USA100 strain patterns accounted for only 40.7% of all USA100 isolates (data not shown). All USA100 isolates contained the type II SCC*mec* element. In contrast, only 38 unique PFGE patterns were observed among the 627 USA300 isolates (DI = 0.566). A single clone, USA300-0114, was predominant, accounting for 63.5% of USA300 isolates. Although only 73 isolates were classified as USA500, there was considerable diversity among the PFGE patterns (DI = 0.885) (Table 3). All USA300 and USA500 stains contained the type IV SCC*mec* element.

The presence of genes encoding SEA to SEH, TSST-1, and PVL was analyzed by PCR. All isolates were tested for the presence of *lukS*-PV and *tst*, and 1,045 (52.7%) were tested for the presence of enterotoxin genes *sea* to *seh*. As expected, PVL was uniformly present in USA300 (605/627; 96.5%) isolates and was common among USA400 (4/6; 66.7%) and USA1100 (6/8; 75%) isolates. TSST-1 was common only among USA200 isolates (13/15; 86.7%), consistent with characteristics of *S. aureus* isolates from asymptomatic nasal colonization (31). SED, the most frequently detected enterotoxin, was present in 74.5% of USA100 isolates and was also present among isolates of other strain types (Table 3). SEA, SEB, and SEC were less common and did not appear to be specific to a given strain type (Table 3). SEE and SEH were not detected among isolates characterized in this study.

Antimicrobial susceptibility profiles of MRSA isolates. Resistances to erythromycin and levofloxacin were common among all isolates tested, with correspondingly high MIC_{50}s and MIC_{90}s for each agent (Table 4), whereas most isolates were susceptible to daptomycin, doxycycline, tetracycline, gentamicin, linezolid, rifampin, trimethoprim-sulfamethoxazole, and vancomycin. USA100 isolates were resistant to erythromycin, levofloxacin, and clindamycin, with inducible resistance accounting for 25.9% of clindamycin resistance (Table 4). USA300 isolates were uniformly resistant only to erythromycin, although many were resistant to levofloxacin as well (Table 4). Inducible resistance to clindamycin was detected in only 2.9% of USA300 isolates. Interestingly, the MIC_{90} for tetracycline was higher among USA300 than among USA100 isolates (Table 4). Among all invasive MRSA isolates, the MIC_{50} and MIC_{90} for vancomycin were both 1 μg/ml. Six percent of isolates had vancomycin MICs of 2 μg ml, and these were primarily from health care-associated infections (29.2% HO-MRSA and 65.8% HACO-MRSA). One HACO-MRSA isolate exhibited a vancomycin MIC of 4 μg/ml (Table 4).

There are currently no interpretive criteria for mupirocin against *S. aureus*. Therefore, mupirocin testing data are reported here as MICs. Most (94.9%) MRSA isolates in this

TABLE 4. Antimicrobial susceptibility testing results

Antimicrobial	All isolates tested (n = 1,978)						USA100 isolates (n = 1,062)		
	% in category			MIC range	MIC_{50}	MIC_{90}	% in category		
	S	I	R				S	I	R
Chloramphenicol	57.0	42.6	0.4	<2–>32	8	16	30.0	69.4	0.6
Clindamycin[b]	36.8	0.0	63.2	≤0.25–>16	≤0.25	≥16	1.2	0.0	99.9
Erythromycin	5.6	0.5	93.9	≤0.25–>8	>8	>8	0.5	0.4	99.2
Daptomycin	99.6	0.0	0.4[a]	≤0.5–4	≤0.5	1	99.4	0.0	0.6[a]
Doxycycline	98.3	1.7	0.1	≤1–16	≤1	≤1	99.2	0.8	0.0
Tetracycline	94.0	0.1	5.9	≤1–>16	≤1	≤1	98.2	0.0	1.8
Gentamicin	95.8	0.1	4.1	≤2–>32	≤2	≤2	96.3	0.0	3.7
Levofloxacin	18.5	0.2	81.3	≤0.5–>16	>16	>16	0.2	0.0	99.8
Linezolid	99.9	0.0	0.1[a]	≤1–8	2	4	99.9	0.0	0.1[a]
Mupirocin[c]				<4–>128	<4	<4			
Rifampin	97.3	0.7	1.9	≤0.5–>8	≤0.5	≤0.5	97.2	0.8	2.1
Trimethoprim-sulfamethoxazole	94.5	0.0	5.5	≤0.5/9.5–>8/152	≤0.5/9.5	≤0.5/9.5	98.7	0.0	1.3
Vancomycin	99.9	0.1	0.0	≤0.5–4	1	1	99.9	0.0	0.1

[a] For daptomycin and linezolid, these isolates are nonsusceptible.
[b] Resistant isolates include those with inducible clindamycin resistance: 17.3% (n = 342) of all isolates, 25.8% (n = 274) of USA100 isolates, and 2.7% (n = 17) of USA300 isolates.
[c] CLSI breakpoints have not been established for mupirocin.

study demonstrated mupirocin MICs of ≤4 μg/ml; 2.3% of all MRSA isolates had mupirocin MICs of >128 μg/ml. The percentages of USA100 and USA300 isolates with mupirocin MICs of ≤4 μg/ml were 94.0% and 96.5%, respectively (not shown). Invasive MRSA isolates with mupirocin MICs of >128 μg/ml were slightly more common among USA300 (2.9%) than among USA100 (1.9%) isolates (not shown) and were identified in each state from which ≥100 isolates were received (not shown). Resistance to trimethoprim-sulfamethoxazole was detected in 5.5% of isolates, primarily among USA500 and Iberian strain types from patients with health care-associated exposures (HO- or HACO-MRSA).

Association between strain type and clinical presentation. We analyzed strain types associated with distinct clinical syndromes to determine whether there was an association between strain type or strain characteristics and clinical presentation. For each of the unique clinical syndromes evaluated, the presence of PVL correlated directly with the proportion of isolates determined to be USA300. Among toxins SEA to SEH, only SED was prevalent enough to evaluate. SED was present in similar proportions of isolates from all unique clinical syndromes. Although SED was not specific to USA100, non-USA100 strains carrying SED represent less than 3% of the collection; therefore, the presence of SED is largely predictive of USA100 strain type in this analysis. Likewise, 13 of 16 isolates positive for TSST-1 were USA200. Thus, the relative contribution of these virulence factors to any clinical syndrome could not be assessed independent of strain type.

CA-MRSA isolates were primarily USA300 (243; 64.1%), whereas most HO-MRSA (68.5%) and HACO-MRSA (58.0%) isolates were USA100. USA300 was also commonly isolated from patients with health care-associated infections (47% of USA300 isolates were from HACO-MRSA cases, and 12% were from HO-MRSA cases) (Tables 2 and 3). Together, USA100 and USA300 strains accounted for 84.8% of isolates in this study; therefore, our analysis of strain characteristics and association with epidemiologic class was limited to isolates of these two strains that had complete epidemiologic and strain characteristics and that did not have overlapping clinical syndromes (n = 1,068).

We constructed a model estimating the likelihood that a strain was USA300 rather than USA100. Isolates classified as CA-MRSA by epidemiologic criteria were significantly more likely to be USA300 than those classified as HO-MRSA (AOR, 10.4; 95% CI, 6.6 to 16.3) or as HACO-MRSA (AOR, 6.6; 95% CI, 4.6 to 9.6). Even with adjustment for epidemiologic classification in a multivariate model, compared to patients with BSI-only, those presenting with soft tissue infection, endocarditis, pneumonia, or osteomyelitis were more likely to be associated with USA300 than with USA100 (Table 5). However, because PFGE type was strongly correlated with other strain characteristics (e.g., PVL), we could not statistically evaluate relationships between presenting syndrome and toxin genes. Overall, among these invasive MRSA isolates, epidemiologic class was a better predictor of strain type than clinical presentation (Table 5).

DISCUSSION

These data represent a very large collection of MRSA isolates associated with invasive disease from geographically distinct areas of the United States; analysis suggests that among these, there is minimal genetic diversity among USA300 strains. This affirms the conclusions of smaller studies of MRSA isolates, suggesting relatively recent evolution and national expansion of USA300 (20, 27, 32).

USA100 was the most common strain type isolated in this study, most commonly from patients with health care risk factors. This is not surprising; USA100 has a long history as a common health care-associated MRSA strain (24), and the diversity of PFGE patterns observed in this strain is consistent with its longevity as a pathogen (10). Nearly three-quarters of the USA100 isolates tested in this study were PCR positive for SED, which is higher than the percentage reported by Diep

TABLE 4—*Continued*

USA100 isolates (n = 1,062)			USA300 isolates (n = 625)					
MIC range	MIC_{50}	MIC_{90}	% in category			MIC range	MIC_{50}	MIC_{90}
			S	I	R			
<2–>32	16	16	97.0	3.0	0.0	4–16	8	8
≤0.25–>16	≥16	≥16	91.0	0.0	9.0	≤0.25–>16	≤0.25	≤0.25
0.5–>8	>8	>8	5.6	0.5	93.9	≤0.25–>8	>8	>8
≤0.5–4	≤0.5	1	99.7	0.0	0.3[a]	≤0.5–4	≤0.5	1
≤1–8	≤1	≤1	99.5	0.5	0.0	≤1–8	≤1	≤1
≤1–>16	≤1	≤1	89.9	0.0	10.1	≤1–>16	≤1	>16
≤2–>32	≤2	≤2	98.7	0.2	1.1	≤2–>32	≤2	≤2
≤0.5–>16	>16	>16	45.4	0.0	54.6	≤0.5–>16	4	8
≤1–8	4	4	100.0	0.0	0.0	≤1–4	2	4
<4–>128	<4	<4				<4–>128	<4	<4
≤0.5–>8	≤0.5	≤0.5	97.3	1.0	1.8	≤0.5–>8	≤0.5	≤0.5
≤0.5/9.5–>8/152	≤0.5/9.5	≤0.5/9.5	99.4	0.0	0.6	≤0.5/9.5–>8/152	≤0.5/9.5	≤0.5/9.5
≤0.5–4	1	1	100.0	0.0	0.0	≤0.5–2	1	1

and colleagues (14%) in 2006 (8). The significance of this finding for invasive MRSA disease is unknown.

As expected (24), the majority of CA-MRSA isolates were USA300, although USA300 was also a common isolate from patients with health care-associated infections (12% of all USA300 isolates were identified among HO-MRSA cases and 47% were among HACO-MRSA cases). USA500 was more common than USA300 at one site and was less geographically represented than other strain types, although USA500 was the third most commonly observed strain type. PVL, present in 96.5% of USA300 isolates, was the only toxin we tested for that was commonly found among USA300 isolates. Although USA400 and USA1100 were rare in this collection, the prevalences of PVL in these strains (66.7% and 75.0%, respectively) found in this study are similar to those observed in the CDC's larger database of more than 5,000 characterized MRSA isolates received over the past decade (G.E. Fosheim, personal communication).

Resistance to erythromycin, levofloxacin, and clindamycin was common in this collection of isolates. This is likely because of the high proportion of USA100 isolates, although more than half of USA300 isolates in our collection were resistant to levofloxacin. Fluoroquinolone resistance among USA300 isolates was previously reported to be uncommon, but several recent reports, including this one, suggest that fluoroquinolone resistance is increasing among USA300 isolates (16, 26, 33). Six percent of isolates demonstrated vancomycin MICs of 2 μg/ml. This might be because of acceptable variation in the reference broth microdilution method (4), but of note is that most of these isolates were USA100 and health care associated (HO- or HACO-MRSA), as was the single isolate with a vancomycin MIC of 4 μg/ml.

TABLE 5. Effects of epidemiologic class and clinical presentation on strain type estimation and logistic regression for MRSA isolates associated with 1,068 cases of invasive MRSA infection

Comparison	OR for USA300/USA100		
	Adjusted OR	95% CI	Statistical significance (P)
CA vs HO	10.39	6.63–16.28	<0.0001
CA vs HACO	6.60	4.56–9.56	<0.0001
HACO vs HO	1.57	1.10–2.26	0.014206
Soft tissue infection vs BSI only	6.87	4.53–10.43	<0.0001
Endocarditis vs BSI only	2.54	1.51–4.29	0.000455
Osteomyelitis vs BSI only	2.27	1.44–3.58	0.000433
Pneumonia vs BSI only	2.29	1.58–3.34	<0.0001

Although no interpretive criteria currently exist for mupirocin MICs for *S. aureus*, there is increasing evidence that isolates with very high MICs for mupirocin, mediated by *mupA*, are associated with decolonization failure (19). Only 45 (2.3%) isolates in this study demonstrated mupirocin MICs of ≥128 μg/ml; 42 (93.3%) of these were from patients with health care risk factors. These isolates were nearly equally distributed between USA100 and USA300 and were not limited to any geographic region. As hospitals increasingly implement active surveillance and decolonization for MRSA, it may be important to monitor MICs for this agent.

We attempted to correlate strain characteristics to presenting syndrome; however, PFGE type was so closely tied to presence of toxin gene that we were only able to evaluate the relationship between PFGE type and presenting syndrome in a valid manner. Although our data are limited to invasive disease, an absence of documented health care exposures was the strongest predictor of strain type, although the clinical syndromes commonly thought of as presenting among outpatients are also predictive (e.g., endocarditis, soft-tissue infections, pneumonia, and osteomyelitis).

MRSA isolates belonging to strain types USA100, USA200, and USA500 have been associated primarily with health care-associated disease, while strains USA300, USA400, USA1000, and USA1100 were associated most often with CA-MRSA disease (9, 24). Recently, however, the association between strain type and epidemiologic class of disease has become less distinct, as strains historically associated with community disease enter and spread within health care institutions (21, 22). Skin abscesses, cellulitis, and necrotizing pneumonia are the clinical presentations most often associated with CA-MRSA infections (1, 23, 30). Therefore, our finding that soft tissue infection (AOR = 6.9) and invasive pneumonia or empyema (AOR = 2.3) were associated with infection by USA300,

rather than USA100, is not especially surprising. Our data also quantify the magnitude of the association between USA300 and epidemiologic classification, where CA-MRSA patients were 10-fold more likely, and HACO-MRSA patients were 6-fold more likely, to be infected with USA300 than with hospital onset strains. The fact that this association for HACO-MRSA cases is so high suggests that many HACO-MRSA patients, despite documented contacts with health care delivery, are often infected with USA300.

Both endocarditis and osteomyelitis were also found more frequently among patients with USA300 infections than among those with USA100 infections. With the exception of intravenous drug users, patients who develop endocarditis often have health care risk factors, such as indwelling lines, history of surgery, or underlying disease, such as diabetes (12). Recent reports suggest that CA-MRSA infectious endocarditis cases could be increasing (11, 25), and more than 30% of the CA-MRSA patients described in that report had recent or concurrent skin infections. In this study, 15.4% of CA-MRSA endocarditis patients and 14.8% of USA300 endocarditis patients had concurrent or underlying skin or soft tissue infections, compared to 8.1% of USA100 endocarditis patients.

The association between USA300 and osteomyelitis is also unclear. Hematogenous osteomyelitis in children is relatively common, but isolates from pediatric patients were infrequent in our collection, and only 5.4% of osteomyelitis patients in this study were under 15 years of age. There are only a few reports of osteomyelitis in adults caused by CA-MRSA, defined either by epidemiology or by strain characteristics in the literature, and these are very closely linked to a history of boils or other skin infections (13, 29). In this study, less than one-third of USA100 and USA300 isolates associated with osteomyelitis came from patients with documented concurrent or historic skin infection (i.e., abscess or cellulitis) (25.5% or 30.9%, respectively). The presence of decubiti or other ulcers was also common among patients with osteomyelitis, occurring in 34.5% of patients infected with USA100 and 10.3% of patients infected with USA300.

A limitation of this study is the sample of isolates available for evaluation. Isolates were collected as a convenience sample of patients with invasive MRSA. However, the patients from which our sample was obtained have characteristics similar to those of all patients reported to the surveillance system (22). Second, our analysis was limited to only those MRSA isolates recovered from a normally sterile site, usually blood. This limits the interpretation of the findings, in that isolates associated with some clinical syndromes (e.g., pneumonia and cellulitis) were limited to bacteremic cases and might not reflect the full spectrum of those diseases. For instance, there might be greater variability in strain characteristics among strains recovered from skin abscesses or lung tissue than are described in this paper. Finally, this study relies upon retrospective chart review by surveillance personnel and those diagnoses documented in the patient's chart. The clinical syndrome data presented here have not been validated in comparison with clinical observations made by treating physicians.

Despite these limitations, these data confirm that MRSA with PFGE type USA300 associated with invasive disease has limited molecular diversity in the United States and is still strongly associated with disease from patients having no documented exposures to health care facilities. These data also serve as a baseline for tracking changes in susceptibility to antistaphylococcal agents as emerging resistance among USA300 or all MRSA isolates is monitored through this and other systems.

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