Origins of Community Strains of Methicillin-Resistant *Staphylococcus aureus*

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Abstract

To characterize methicillin-resistant *Staphylococcus aureus* (MRSA) strains circulating in the community, we identified predictors of isolating community MRSA and genotyped a sample of MRSA collected from a community-based, high-risk population. Computerized databases of the Community Health Network of San Francisco and the Clinical Microbiology Laboratory were searched electronically for the years 1992–1999 to identify community-onset infections caused by MRSA. Sequential analyses were performed to identify predictors of MRSA strains. The majority (58%) of infections were caused by strains traceable to the hospital or to long-term care facilities. Injection drug use was associated with infections that were not associated with health care settings. Genotypes for 20 of 35 MRSA isolates recovered from injection drug users did not match any of >600 genotypes of clinical isolates. In a nonoutbreak setting, the hospital was the main source of community MRSA; however, the presence of genetically distinct and diverse MRSA strains indicates MRSA strains now also originate from the community.

The global emergence of drug-resistant bacteria is a pressing public health problem. Firmly established in hospitals worldwide, methicillin-resistant *Staphylococcus aureus* (MRSA) has now emerged as a significant community-acquired pathogen [1–14]. Several features appear to distinguish community from nosocomial MRSA strains: (1) absence of hospital-associated risk factors; (2) susceptibility to most antibiotics other than *β*-lactams; (3) distinct genotypes that do not match *S. aureus* strains commonly found in hospitals; (4) presence of type 4 staphylococcal chromosomal cassette *mec (SCCmec)* (the element that contains the methicillin-resistance determinant), not typical of nosocomial MRSA strains; and (5) the presence of genes encoding for toxins such as Pantone-Valentine leukocidin and the many staphylococcal enterotoxins [2,15–21].

Community strains of MRSA may arise in either of 2 ways: hospital strains may be carried into the community, where they then spread person to person [1], or community MRSA may arise de novo when the methicillin-resistance gene complex is acquired by a methicillin-susceptible strain [2,22]. Anecdotal reports, case series, and studies of outbreaks suggest the latter is occurring, but these may not accurately represent strains circulating in the community. To obtain evidence concerning the origins of community-acquired strains of MRSA in San Francisco, California, we took a novel combination approach that used a large database of clinical MRSA isolates to identify a target population at risk for community MRSA. A community-based sample of this target population was assessed for MRSA nasal carriage, and a molecular analysis of MRSA isolates was performed to determine their genotypes.

MATERIALS AND METHODS

Clinical Epidemiology

Setting—The Community Health Network (CHN) of San Francisco is a publicly funded health care delivery system with sites of care including San Francisco General Hospital (SFGH; a university-affiliated 550-bed public teaching hospital and the regional trauma center), 13 neighborhood health centers, a 1000-bed publicly funded long-term care facility (LTCF), a 150-bed long-term care mental health facility, and a 17,000-visit-per-year home health care network.

***S. aureus* bacterial culturing and antibiotic susceptibility testing**—The clinical microbiology laboratory of SFGH performs all clinical antimicrobial susceptibility tests for the CHN. MIC determinations were performed with the Microscan Walkaway instrument (Dade International) in accordance with NCCLS guidelines [23].

Electronic data collection—Antimicrobial susceptibility data were obtained from existing clinical databases for all positive *S. aureus* culture results processed by the CHN centralized clinical laboratory at SFGH. The total number of bacterial cultures processed annually and the inpatient hospital census by year were obtained to determine secular trends in the volume of patients served and changes in bacterial culture ordering practices by health care providers.

Electronic records of previous hospitalizations and clinic visits were obtained for all CHN patients with cultures positive for *S. aureus* for the years 1992–1999 from existing CHN-wide databases. Patient registration records were also examined for previous residence in the county LTCF.

Statistical analysis—To adjust for potential bias arising from multiple *S. aureus* isolates cultured from a single patient, only data for the initial isolate for each calendar year was included in the analysis. When both a methicillin-resistant and susceptible organism were isolated from the same patient during a calendar year, preference was given to the methicillin-resistant isolate.

Fisher's exact test was used to test for significant associations between categorical variables. Univariate and multiple logistic regressions were applied to estimate the OR associated with risk factors for methicillin resistance among *S. aureus* isolates and for multidrug resistance among MRSA isolates. The χ^2 test for trend was used to evaluate evidence for increasing proportion of methicillin resistance among *S. aureus* isolates by calendar year.

Definitions—A hospital-associated *S. aureus* isolate was defined as one cultured from a clinical specimen obtained >72 h after hospital admission. A community-associated *S. aureus* isolate was defined as one cultured during the first 72 h of a patient's hospital admission or from an outpatient. MRSA isolates resistant to ≥3 non–β-lactam antibiotics classes were

classified as multidrug-resistant MRSA (R-MRSA). The duration of hospitalization before collection of each *S. aureus* isolates was calculated, and patients were grouped into 5 mutually exclusive categories: (1) no hospitalizations within the previous 3 years, (2) hospitalization within the previous 6 months, (3) hospitalization within 6–12 months, (4) hospitalization within >1–2 years, and (5) hospitalization within >2–3 years.

Community-Based MRSA Sample

A community-based sample of nasal *S. aureus* colonization was conducted for April–September 1999 in the context of the Urban Health Study, an ongoing community-based research and prevention program among active injection drug users (IDUs) in San Francisco [24].

Microbiological Studies

The anterior nares of each subject consenting to the study were sampled with a cotton swab dampened with normal saline. The swab was inoculated in the field onto a 5% sheep's blood tryptic soy agar plate. After overnight incubation at 37°C, colonies resembling staphylococci were individually inoculated onto mannitol salt and blood agar and incubated overnight. Isolates were identified as *S. aureus* if they produced the appropriate color change on mannitol salt agar and a positive tube coagulase test result.

Susceptibility of nares isolates to ampicillin, ciprofloxacin, tetracycline, gentamicin, erythromycin, trimethoprimsulfameth-oxazole, clindamycin, linezolid, and vancomycin were determined on Mueller-Hinton agar (purchased from BBL) by the disk diffusion method in the SFGH Molecular Epidemiology Reference Laboratory [25]. Results were interpreted in accordance with the NCCLS guideline M7–A5 [23]. Susceptibility to methicillin was tested according to the recommendations of McDougal and Thornsberry [26].

Molecular Studies

mecA, the gene that determines methicillin resistance, was detected in either whole cells or lysostaphin-treated cell lysates by the presence of a 533-bp PCR amplification product that used sense and antisense primers—5′-AAAATCGATGGTAAA-GGTTGGC-3′ and 5′-AGTTCTGCAGTACCGGATTTGC-3′ respectively (GenBank accession no. D86934) [27]. *SCCmec* type was determined by multiplex PCR [28]. Control strains for *SCCmec* types 1, 2, and 3 were as follows: type 1, COL; type 2, N315 and 67-0; and type 3, SFGH clinical isolates 1349 and 2766 (confirmed using the method of Okuma et al. [2]). MRSA isolates were genotyped by PFGE of *Sma*I digests of chromosomal DNA [29,30], *spa* (staphylococcal protein A) typing [31], and multilocus sequencing typing (MLST) [32]. All MRSA strains were evaluated by *SCCmec* type, *spa* typing, and PFGE. We then grouped strains according to matches on the basis of *spa* and PFGE. A random isolate within each group was then chosen for MLST sequence typing. PFGE patterns were compiled with the BioRad Molecular Analyst program and were compared with those in a genotype database composed of 600 MRSA clinical isolates collected 1996–1999 by the Molecular Epidemiology Research Laboratories from the clinical microbiology laboratories at SFGH.

RESULTS

Increasing prevalence of methicillin resistance

Between 1988 and 1999, there were 20,819 *S. aureus*–positive cultures, of which 12,159 were initial isolates. Isolates from samples obtained for culture ≤72 h after hospital admission or from an outpatient setting (i.e., community-associated isolates) were less likely to be methicillin resistant (12.1%) compared with samples obtained for culture >72 h after hospital

admission (i.e., hospital-associated isolates) (27.4%) (OR, 2.1; 95% CI, 1.9–2.4; P <.0001). The proportion of *S. aureus* isolates that were resistant to methicillin increased significantly from 3.1% in 1988 to 26.9% in 1999 (P <.0001, by χ^2 test for trend), increasing among hospital-associated isolates from 16% in 1993 to 42% in 1999 and from 7% in 1993 to 29% in 1999 in the community-associated isolates (figure 1).

MRSA were significantly more likely to be resistant to erythromycin, ciprofloxacin, clindamycin, gentamicin, or trimethoprim-sulfamethoxazole than were methicillin-susceptible *S. aureus* (MSSA) isolates (table 1). Thirty-three percent of MRSA isolates were resistant to ≥3 antibiotics, compared with only 2% of MSSA isolates (P <.001). Among all MRSA isolates, community-associated MRSA isolates were less likely to be resistant to antibiotics than were hospital-associated MRSA isolates (P <.0001).

Risk factors for methicillin resistance

Univariate risk factors for methicillin resistance among 4685 community-associated *S. aureus* isolates were more recent year during which the sample was obtained for culture (OR, 1.2; 95% CI, 1.2–1.3; P <.0001), previous residence in the LTCF (OR, 3.3; 95% CI, 2.7–4.1; P <.0001), previous hospitalization within the previous 3 years (OR, 1.7; 95% CI, 1.5–2.0; P <.0001), age of ≥18 years (OR, 1.6; 95% CI, 1.01–2.5), homelessness (OR, 1.9; 95% CI, 1.5–2.4; P <.0001), and injection drug use (OR, 2.1; 95% CI, 1.5–3.0; P <.0001). Admission to the hospital during the previous year and admission to the LTCF were the only variables predictive of a MRSA isolate among patients with community-associated MRSA by multivariate analysis (table 2). Nonsignificant risk factors evaluated included sex, ethnicity, and history of recent outpatient care visit.

Risk factors for multidrug-resistant *S. aureus*

To determine whether multidrug resistance was associated with a nosocomial MRSA isolate, multivariate analysis was repeated for the 553 community-associated MRSA isolates. Previous LTCF residence and previous hospitalization within 0–6 months were associated with an increased risk (table 2) for R-MRSA, confirming the relationship between multiple resistances and nosocomial source, suggesting that nosocomial isolates accounted for a considerable proportion of the community MRSA group (47% of MRSA with LTCF or hospitalization within 6 months, 58% of MRSA with LTCF or hospitalization within 2 years).

Because multidrug resistance was a marker for a nosocomial strain, to define risk factors for "true" community MRSA (i.e., those purged of endemic hospital clones), univariate analysis was performed for community-associated MRSA, this time excluding community cases with R-MRSA isolates or with admission to the hospital or LTCF within the previous year. Factors associated with methicillin resistance in univariate analysis among these patients with community MRSA according to the more stringent definition were recent year of culture (OR, 1.4; 95% CI, 1.3–1.5; P <.0001), homelessness (OR, 1.7; 95% CI, 1.1–2.6; P =.015), and injection drug use (OR, 2.3; 95% CI, 1.1–4.8; P =.02). The proportion of these "true" community MRSA isolates among all positive *S. aureus* cultures (n =2979) increased significantly, from 3.3% in 1993 to 17.7% in 1999 (P < .001).

Community-based sample of IDUs

A previous study from our group [4] found that an urban poor, largely homeless population had a low MRSA prevalence of 2.8% and that the vast majority of these isolates were associated with hospital or LTCF contact. Among this homeless population, IDUs had significant risk for community-associated MRSA. In the community-based sample of IDUs, 172 (25.3%) of 683 subjects had *S. aureus* isolated from nasal swab cultures, 42 of which were MRSA, for an

overall prevalence of 6.1% and a prevalence of MRSA of 24.4% among those colonized with *S. aureus*.

Genotypes of community-acquired MRSA

Genotypes were determined for 35 of the MRSA isolates (table 3) obtained from the community-based sample of IDUs. These comprised 9 distinct PFGE groups, 8 *spa* types, and 6 sequence types (STs) or clonal complexes (CCs). Five isolates had a unique PFGE type (U) (figure 2) not closely related to one another or to the other isolates.

Fifteen isolates (43%) (PFGE types C and D) were closely related to PFGE genotypes previously identified among >600 clinical isolates collected 1996–1999 at the CHN. All 15 were *SCCmec* type 4. These strains belonged to CC8, an archetypal nosocomial MRSA genotype [2,34]. Twenty isolates (57%) had genotypes not found in the 1996–1999 collection (although many have since been identified among strains collected after 1999), and they were genotypically more diverse, constituting 7 PFGE patterns and 7 *spa* types. Thirteen isolates were *SCCmec* type 4, and 7 were nontypeable. Two isolates, M17 and M45, had similar *spa* types and were members of CC5, another archetypal MRSA nosocomial genotype [2,34]. Stain J28 was a member of CC1, the same as that of the Minnesota and North Dakota community MRSA strains [2,35]. Strain M25 was a member of CC30, a common type among community MRSA isolates in Australia [2]. PFGE groups A and B both were ST59 and strain J35 was ST12/CC12. These are uncommon MRSA sequence types—they have not previously been reported among community strains and are rarely found in nosocomial collections [2,34,36].

Consistent with a community phenotype, isolates with genotypes not found in the database tended to be drug susceptible, with 13 of 20 susceptible to all non–β-lactam antibiotics versus 5 of 15 isolates whose genotypes were in the database, although this difference was not statistically significant. Hospitalization within the previous 12 months was relatively uncommon in this community-based sample of IDUs. Only 9 patients (21%) had been hospitalized (all at SFGH) within the previous year, although the majority (29 [69%] of 42) reported ≥1 visit to the emergency department within the previous year, usually for treatment of skin or soft-tissue infections.

DISCUSSION

MRSA, which has until recently been regarded as almost exclusively a hospital-associated pathogen, has been increasingly identified as a cause of community-onset infections. Some have argued that, because of the dramatically increasing prevalence of MRSA in the hospital, the parallel epidemic in the community is attributable to individuals returning to the community with MRSA from health care facilities [6,37]. However, other evidence—both epidemiological and from molecular typing studies—have suggested that community MRSA strains are not simply feral hospital strains.

The present investigation used a serial, cross-sectional analysis of electronic databases to identify predictors of isolating a community MRSA strain. The clinical epidemiology identified 2 reservoirs for community strains of MRSA. The majority of community-onset MRSA infections identified from CHN electronic databases were almost certainly caused by strains acquired from hospitalization or residence in an LTCF, because these were the only predictors of MRSA identified by multivariate analysis of all MRSA isolates.

When the analysis was performed with community cases purged of those likely to be of health care facility origin, 3 risk factors were identified: homelessness, injection drug use, and year that the sample was obtained for culture. In a previously published community-based study in San Francisco, homelessness was not confirmed to be a significant risk factor for MRSA and

MRSA genotypes that were identified and could be epidemiologically linked to acquisition in the hospital [4]. The community-based survey of IDUs, however, confirmed the predicted high prevalence of MRSA in this population, and the MRSA isolates recovered from this population had the hallmarks of community strains: they were largely susceptible to non–*β*-lactam antibiotics; type 4 *SCCmec,* recently described in other community MRSA isolates [15], predominated; and hospital admission among MRSA carriers within the prior year was lacking.

The molecular typing studies of these community isolates, mirroring the clinical epidemiology, identified 2 potential reservoirs for community MRSA isolates. Clearly, health care facilities were an important contributor, because nearly one half of the MRSA isolates had PFGE genotypes matching those of MRSA clinical isolates in a database of strains collected during 1996–1999. All were members of CC8, which is common among nosocomial MRSA worldwide. These isolates are probably feral hospital-endemic clones.

Community MRSA isolates not matching those within the PFGE database included 2 that were MLST sequence and clonal complex type 5. This CC is historically hospital associated and common among SFGH nosocomial isolates as well. The remaining isolates from the community-based sample were sequence and CC types reported as community-outbreak strains from other locations and not common among nosocomial isolates (CC1 and CC30) or uncommon nosocomial isolates and not previously reported as community isolates (ST59 and CC12).

The striking genetic feature of the community MRSA isolates, regardless of presumed source and despite diversity of genotype, was the predominance of *SCCmec* type 4, which was present in 89% of isolates, including the isolates whose genotypes were represented in the database of CHN strain collection. Type 4 *SCCmec,* although it may be the predominant community type, should not be considered unique to community isolates, as others have observed [34]. Indeed, there is recent evidence of the increasing prevalence of type 4 *SCCmec* within both community and nosocomial strains of MRSA in San Francisco and identification of nosocomial MRSA containing type 4 *SCCmec* [38,39].

Our results indicate that a large proportion of community MRSA strains in San Francisco are feral descendentis of hospital endemic clones that over time have adopted a community phenotype of multiple-drug susceptibility. Others strains appear truly to be community-adapted residents. It is possible that these clones also originally were endemic in hospitals, but, being relatively unfit for an environment of heavy antibiotic exposure, they prefer the more salutary, less antibiotic-selective community setting. Alternatively, these may have recently arisen by horizontal transfer of type 4 *SCCmec* into a methicillin-susceptible background, suggested by the fact that they are members of clonal complexes not common among MRSA, community or nosocomial. The genetic diversity of community MRSA isolates and the presence of nontypeable (perhaps novel) *SCCmec* types also demonstrate mobility and plasticity of *SCCmec* [22]. It is the smallest of the 4 known *SCCmec* elements, 21–24 kb in size (compared with 35 to >60 kb in size of types 1, 2, and 3); it is small enough to be packaged in a bacteriophage and horizontally transmitted, whereas the other 3 types are too large. The complete absence of the other 3 *SCCmec* types, which are readily found in collections of nosocomial strains [2,34,35] in the community isolates, is surprising and suggests a strong counterselection for these elements or strains carrying them in the community or a strong selective advantage for type 4 strains.

Given that the epidemiology and individual strain prevalence of MRSA is known to exhibit significant regional variation, caution must be used in generalizing these specific results to other geographic locations. However, as the trends in the emergence and spread of community-acquired MRSA in San Francisco have been mirrored by community-acquired MRSA trends

in other US and international settings, it is likely that the general mechanisms and findings in such locations will be similar, once investigated.

The findings presented have important therapeutic implications. If community strains continue to spread and increase in prevalence independent of a hospital source, then empirical approaches to therapy for *S. aureus* infection will have to take this into account. In addition, mathematical modeling suggests that the most effective method of controlling drug-resistant bacteria is to reduce their input into hospitals [40]. To the extent that MRSA input in the community is independent of a hospital reservoir, it will be much more difficult—if not impossible—to control.

Acknowledgements

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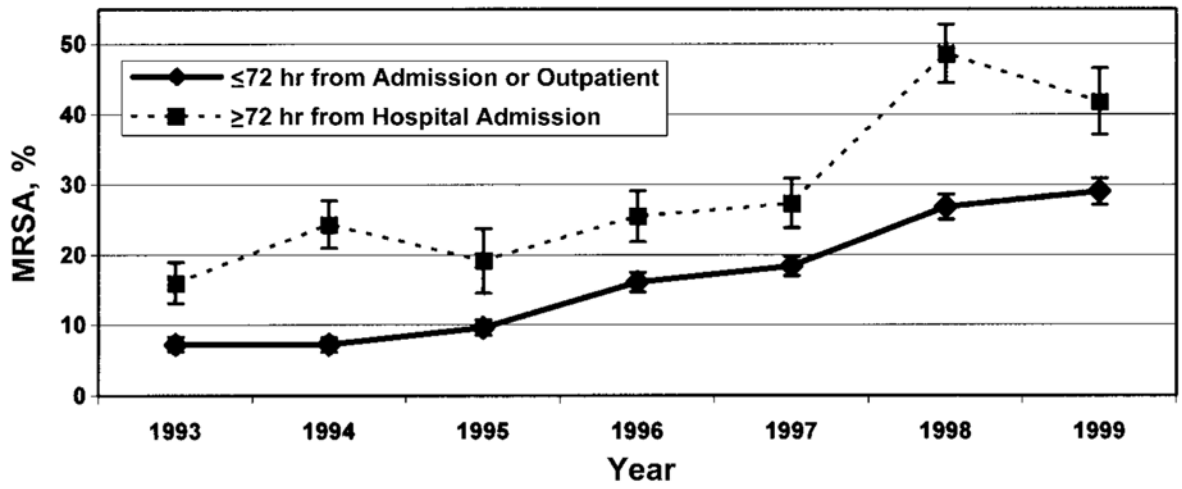
Figure 1.
Percentage of strains of methicillin-resistant *Staphylococcus aureus* (MRSA), by year. *Bars,*
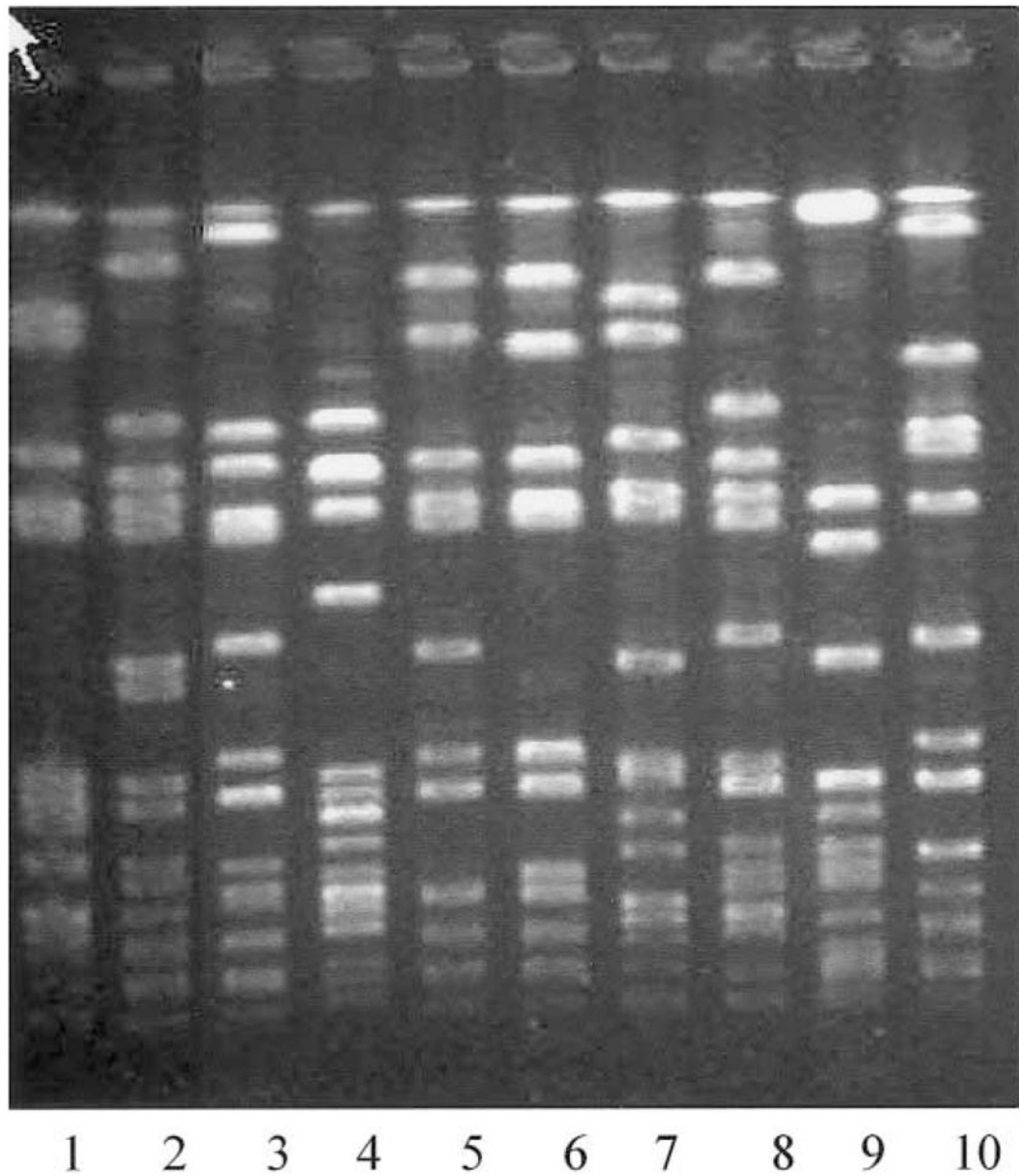SEMs.

Figure 2.
PFGE of prototype strains isolated from a community-based sample of injection drug users. Strains in each lane are as follows: *1,* J50; *2,* J28; *3,* JY39; *4,* M36; *5,* M17; *6,* M45; *7,* J52; *8,* J35; *9,* M25; *10,* reference laboratory strain.

Table 1

Staphylococcus aureus antibiotic resistance profile.

Isolate, source	No. of patient isolates	Agent, % of resistant isolates						
		Erythromycin	Tetracycline	Ciprofloxacin	Clindamycin	Gentamicin	TMP-SMZ
MSSA							
Outpatient or person hospitalized for ≤72 h	4532	29.9	17.2	2.0	2.3	1.5	7.4
Person hospitalized for >72 h	769	23.2	13.1	1.3	2.1	1.2	8.3
MRSA							
Outpatient or person hospitalized for ≤72 h	811	67.3	12.2	44.3	43.5	26.6	15.8
Person hospitalized for >72 h	290	78.6	10.1	64.8	65.1	42.1	18.4

NOTE. MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; TMP-SMZ, trimethoprim-sulfamethoxazole.

Table 2

Multivariate ORs for risk of methicillin resistance and multidrug resistance among *Staphylococcus aureus* and methicillin-resistant *S. aureus* isolates recovered from cultures of samples obtained while the person was as an outpatient or within 72 h of hospital admission.

Outcome, exposure	No. of patient isolates	OR (95% CI)	*P*	% MRSA or % R-MRSA[a]
Risk of methicillin resistance among *S. aureus* (*N* = 4685)				
Previous LTCF residence	55	2.1 (1.1–3.9)	.02	27.3
Previous hospitalization, duration				
0–6 months	1061	3.5 (2.9–4.3)	<.0001	23.3
>6–12 months	234	2.2 (1.5–3.2)	<.0001	15.8
>12–24 months	185	1.5 (0.9–2.4)	.11	11.4
>24–36 months	68	0.7 (0.3–2.1)	.57	5.9
None in previous 3 years	3137	Reference	...	7.8
Risk of multidrug resistance among MRSA (*N* = 553)				
Previous LTCF residence	15	3.5 (1.2–10.5)	.02	60.0
Previous hospitalization, duration				
>0–6 months	247	1.7 (1.1–2.5)	.009	37.7
>6–12 months	37	0.4 (0.2–1.1)	.083	13.5
>12–24 months	21	0.3 (0.1–1.4)	.12	9.5
>24–36 months	4	0.9 (0.1–9.6)	.98	25.0
None in previous 3 years	244	Reference	...	25.8

NOTE. LTCF, long-term care facility; MRSA, methicillin-resistant *S. aureus.*

[a]For the first outcome, the percentages (% MRSA) refer to the percentage of *S. aureus* isolates that were MRSA, and for the second outcome, the percentages (% R-MRSA) refer to the percentage of MRSA isolates that were multidrug resistant.

Table 3

Genotypes and SCCmec types of methicillin-resistant *Staphylococcus aureus* isolates from a community-based sample of injection drug users.

Prototype strain	PFGE type (no. of isolates)[a]	SFGH related[b]	spa Repeat sequences	SCCmec type	ST/CC
J50	A (5)	No	Z1D1M1D1N1K1B1	NT	59/–
J52	B (10)	No	Z1D1M1D1M1N1K1B1	IV	59/–
JY39	C (11)	Yes	Y1H1G1F1M1B1Q1B1L1O1	IV	8/8
M36	D (4)	Yes	Y1H1G1F1M1B1Q1B1L1O1	IV	8/8
M17	U (1)	No	T1J1M1B1M1D1M1G1G1M1K1	IV	5/5
M25	U (1)	No	X1A1K1A1O1M1Q1	NT	30/30
M45	U (1)	No	T1J1M1B1M1D1M1G1M1K1	NT	5/5
J28	U (1)	No	U1J1F1E1	IV	1/1
J35	U (1)	No	U1J1F1Q1P1L1M1	IV	12/12

NOTE. CC, clonal complex of which the multilocus sequencing typing (MLST) sequence type is a member; NT, nontypeable pattern of SCC*mec* with PCR products not classifiable into one of the 4 main types; PFGE, pulsed-field gel electrophoresis; SFGH, San Francisco General Hospital; ST, MLST sequence type.

[a]PFGE type indicates groups of isolates with identical or closely related PFGE patterns. The PFGE types of isolates in this study correspond to the following PFGE clone groups published in Diep et al. [33]: types A and B, clone group P; types C and D, clone group C; unique strains M17 and M45, clone group D; strain M25, clone group Z; strain J28, clone group K. Strain J35, which is a unique isolate in this study, belongs to a recently identified type among CHN isolates, clone group F.

[b]This indicates a PFGE type that is closely related to one identified from the MERL database of MRSA clinical isolates collected during 1996–1999.