Methicillin-Susceptible Staphylococcus aureus as a Predominantly Healthcare-Associated Pathogen: A Possible Reversal of Roles?

Michael Z. David¹,², Susan Boyle-Vavra¹, Diana L. Zychowski¹, Robert S. Daum¹

¹ Department of Pediatrics, The University of Chicago, Chicago, Illinois, United States of America, ² Department of Medicine, The University of Chicago, Chicago, Illinois, United States of America

Abstract

Background: Methicillin-resistant Staphylococcus aureus (MRSA) strains have become common causes of skin and soft tissue infections (SSTIs) among previously healthy people, a role of methicillin-susceptible (MSSA) isolates before the mid-1990s. We hypothesized that, as MRSA infections became more common among S. aureus infections in the community, perhaps MSSA infections had become more important as a cause of healthcare-associated infection.

Methods: We compared patients, including children and adults, with MRSA and MSSA infections at the University of Chicago Medical Center (UCMC) from all clinical units from July 1, 2004-June 30, 2005; we also compared the genotypes of the MRSA and MSSA infecting bacterial strains.

Results: Compared with MRSA patients, MSSA patients were more likely on bivariate analysis to have bacteremia, endocarditis, or sepsis (p = 0.03), to be an adult (p = 0.005), to be in the intensive care unit (21.9% vs. 15.6%) or another inpatient unit (45.6% vs. 40.7%) at the time of culture. MRSA (346/545) and MSSA (76/114) patients did not differ significantly in the proportion classified as HA-S. aureus by the CDC CA-MRSA definition (p = 0.5). The genetic backgrounds of MRSA and MSSA multilocus sequence type (ST) 1, ST5, ST8, ST30, and ST59 comprised in combination 94.5% of MRSA isolates and 50.9% of MSSA isolates. By logistic regression, being cared for in the Emergency Department (OR 4.6, CI 1.5-14.0, p = 0.008) was associated with MRSA infection.

Conclusion: Patients with MSSA at UCMC have characteristics consistent with a health-care-associated infection more often than do patients with MRSA; a possible role reversal has occurred for MSSA and MRSA strains. Clinical MSSA and MRSA strains shared genotype backgrounds.

Introduction

Staphylococcus aureus is among the most common pathogens affecting human beings. It is a common cause of skin and soft tissue infections (SSTIs), bloodstream infections, osteomyelitis, septic arthritis, and device-related infections. Approximately 25-40% of people are asymptomatically colonized with S. aureus [1]. The epidemiology of S. aureus changed at the end of the twentieth century with the emergence of new strains, most often methicillin-resistant (MRSA), that have circulated in the general population. The new strains, known as community-associated (CA)-MRSA, have been dominated by a single genetic background, USA300, a pulsortype corresponding to ST8 by multilocus sequence typing (M. David, unpublished data) [2]. They differ from the older health care-associated (HA)-MRSA strains genotypically, in the populations they infect, and in the types of infections that they cause [3-5].

One distinctive feature of CA-MRSA strains is the almost universal carriage of genes for the Panton-Valentine leukocidin (PVL), a toxin rarely carried by S. aureus strains prior to the 1990s. CA-MRSA strains have become the most common cause of SSTIs in U.S. emergency rooms and in jails, typically among previously healthy people [6,7]. Prior to the late 1990s, when MRSA isolates were restricted to individuals in contact with the health care system, methicillin-susceptible S. aureus (MSSA) strains were almost the exclusive cause of both serious and uncomplicated S. aureus infections among previously healthy people.

In the CA-MRSA era, the relationship between circulating MRSA and MSSA strains in the U.S. has been examined for asymptomatic colonization [8-15], SSTIs [9,16,17], and infections among children [18]. However, the relationship among MSSA and MRSA isolates causing infections of all kinds in children and adults at a single center has not been recently examined.
After noting anecdotally that few patients seemed to be presenting for care from the community with MSSA infections, we hypothesized that, as MRSA infections became more common among *S. aureus* infections in the community, perhaps MSSA infections had become less common. To explore this relationship further, we studied a representative sample of MSSA and MRSA infections at one center to compare the genotypic and phenotypic characteristics of contemporary MSSA and MRSA isolates, risk factors for MSSA and MRSA infection, and clinical syndromes caused by MSSA and MRSA isolates.

**Methods**

**Setting**

The study was approved by the Institutional Review Board (IRB) of the Biological Sciences Division of the University of Chicago. Consent was provided by all subjects or by their parents or guardians for their information to be stored for this study. Informed consent was obtained by telephone in a procedure approved by the IRB. The University of Chicago Medical Center (UCMC) is an academic medical center located on the south side of Chicago serving the surrounding inner-city population as well as tertiary care referral patients.

**MSSA isolate collection**

The first 20 MSSA isolates identified by the Clinical Microbiology Laboratory at UCMC each month were prospectively collected from July 1, 2004 to June 30, 2005. *S. aureus* isolates from 169 unique patients (referred to as patient-isolates when analyzing patient-isolate dyads) were identified; isolates beyond the first obtained from each patient were excluded. Of the 169 identified isolates, 6 could not be located and 5 were found on further testing not to be *S. aureus*. Of the 158 eligible patients with an available MSSA isolate, 60 (38%) were enrolled by telephone and 87 (55%) were enrolled with a waiver of consent because they could not be reached or were deceased in December 2008-June 2009; 11 (7.0%) declined enrollment.

**MRSA isolate collection**

Among 616 consecutive MRSA isolates obtained from UCMC patients in July 1, 2004-June 30, 2005 as described [19], 71 were excluded because the isolate represented asymptomatic carriage, and 545 who had a clinical infection were included in the present study. Clinical and demographic information about the patients and genotypic and phenotypic information about the isolates was tabulated as previously described [19].

**Patient data**

For the 147 enrolled MSSA patients, a physician (MZD) abstracted the electronic and paper medical records at UCMC, determining age, race/ethnicity as recorded in the chart, past medical history, details of the clinical MSSA infection, and putative risk factors for exposure to MRSA. Each patient contacted for enrollment was also asked to complete a questionnaire concerning the above demographic, medical, and risk factor topics on the telephone; 65% (39/60) of those contacted by telephone completed this questionnaire. We applied the CDC case definition, used to distinguish patients with CA- from HA-*S. aureus* infections, to assess the CA- or HA- status of MSSA isolates. The CDC case definition classifies a patient as having a CA infection if the isolate was obtained from an outpatient of from an inpatient <48 hours after admission and lacks the following risk factors for exposure to the health care system: hospitalization, hemodialysis, or surgery in the previous year, presence of an indwelling catheter at the time the culture is obtained [20]. Our definition differed from the CDC criteria in that we considered surgery only in the previous 6 months, rather than 12 months, to be a risk factor for HA-MRSA infection.

**Studies on MSSA isolates**

Antimicrobial susceptibility testing to oxacillin, clindamycin, erythromycin, rifampin, trimethoprim-sulfamethoxazole, vancomycin, and gentamicin was performed with the Vitek 2 system (bioMérieux Vitek, Inc., Durham, NC). A D-zone test was performed for inducible clindamycin resistance for isolates found to be susceptible to clindamycin and resistant to erythromycin according to CLSI guidelines [21]. D-zone test positive isolates were considered to be resistant to clindamycin. Isolates reported to have intermediate susceptibility to an antibiotic were considered to be resistant. The MLST was determined for each MSSA isolate as described [22]. Clonal complexes were assigned using the eBURST algorithm as described. The presence of lukF-PV and lukS-PV encoding the Panton-Valentine leukocidin (PVL) toxin was performed by PCR as described [23].

**Statistical analysis**

Data were tabulated for each demographic, medical history, and other patient characteristic factors. The CDC definition was used to classify MSSA and MRSA isolates as CA- or HA-*S. aureus* [20]. The risk factor data were compared for MSSA and MRSA using χ-square or Fisher Exact for dichotomous variables, or Student’s *t*-test for continuous variables. Logistic regression models were developed to test the independent association of all patient and clinical variables with *p* < 0.05 on univariate analysis (Stata, v. 10, Statacorp, College Station, TX).

**Results**

Among the 147 MSSA isolates from enrolled patients, 33 were excluded when they were determined to be colonizing and not from a site of infection; 114 from clinical infections were analyzed further. In the same period at UCMC, there were 545 MRSA isolates from different patients with infections; 71 isolates representing asymptomatic colonization were excluded [19].

Patients with an MSSA isolate were more likely to have private insurance than MRSA patients (*p* = 0.001). The racial/ethnic make-up of the MSSA and MRSA patient groups differed (*p* < 0.001); the MRSA group included a higher percent of African Americans than the MSSA group (75.5% vs. 46.9%). Patients with an MSSA isolate were more like to be an adult (75%) than were MRSA patients (60.6%) (*p* = 0.005). The most common MSSA infectious syndromes were SSTI (47.4%), bactereemia, endocarditis, or sepsis (19.3%), and osteomyelitis or septic arthritis (9.7%). Compared with MRSA patients, MSSA patients were more likely to have bactereemia, endocarditis, or sepsis (*p* = 0.03). Patients with a MRSA isolate were more likely to have an SSTI than MSSA patients (*p* < 0.001) (Table 1).

MSSA isolates were more often obtained from inpatients more than 48 hours after hospital admission than were MRSA (26.3% vs. 19.5%), although the difference was not significant (*p* = 0.1). There was no significant difference in the percent of MRSA and MSSA patients who had surgery in the previous 6 months, a hospital or long-term care facility stay or hemodialysis in the previous year, or an indwelling catheter at the time of culture. There was no significant difference in the percent of MSSA and MRSA patients, by self-report (*p* = 0.3) or laboratory report at UCMC (after 1993) (*p* = 0.4), who had MRSA isolated in the past (Table 2).
MRSA (346/545) and MSSA (76/114) patients did not differ significantly in the proportion that would be classified as HA-
S. aureus by the CDC CA-MRSA definition (p = 0.5).

MSSA and MRSA patients did not differ significantly in the likelihood that they had a comorbid condition, including an
immunocompromised state, diabetes mellitus, cystic fibrosis,
cancer, or HIV infection. Patients with MSSA were more likely
to be transplant recipients than MRSA patients (p = 0.002). MRSA
patients were more likely than MSSA patients to have been in jail
(p = 0.005); MRSA patients lived in larger households than MSSA
patients (p = 0.047) (Table 3).

At the time of culture, MSSA patients were more likely than
MRSA patients to be in the intensive care unit (21.9% vs. 13.6%)
or another inpatient unit (45.6% vs. 40.7%) whereas MRSA
patients were more likely to be in the emergency department
(23.1% vs. 8.8%) (Figure 1).

The MSSA isolates were more polyclonal than were the MRSA
isolates. Among the MSSA isolates, there were 24 STs
(representing 12 clonal clusters [CC] and 2 STs that did not belong
to a defined CC). Among the MRSA isolates, there were 11
STs (in 6 CC). There was substantial overlap in the ST/CC
repertoire of the MRSA and MSSA isolates. ST1, ST3, ST8,
ST30, and ST59, all common genetic backgrounds of clinical
MRSA isolates in the U.S. and other parts of the world,
comprised, in aggregate, 94.5% of the MRSA isolates; among
the MSSA isolates, these STs comprised 50.9% of the aggregate
MSSA isolates (Figure 2, Table 4). Of MRSA isolates, 33% (180)
carried SCCmec type II and 67.7% (358) carried SCCmec type IV,
and 1.3% (7) did not SCCmec elements typable by the routine PCR
assays used.

PVL gene carriage (PVL+) was common among the MRSA
isolates; significantly fewer MSSA isolates were PVL+ (58.2% vs.
7.0%, p < 0.001). Among the 9 PVL+ MSSA isolates, 6 were ST3,
1 was ST1 and 1 was ST121. The proportion of PVL+ isolates
did not differ significantly among the ST3 MSSA (6/8, 75%) and ST3
MRSA (290/321, 90.3%) backgrounds (p = 0.2). The syndromes
caused by the 6 ST8, PVL+ MSSA isolates were uncomplicated
SSTIs in 3 and an abscess associated with a transcutaneous gastric
tube, a surgical wound infection, and a central venous
catheter-associated bacteremia in 1 patient each. The ST121, PVL+ MSSA
isolate was obtained from a patient with septic arthritis and
pyomyositis; the ST1 PVL+ MSSA isolate came from a patient
with an uncomplicated SSTI. Among the 44 invasive MSSA
infections, just 2 (4.6%) were caused by PVL+ strains.

In Model 1, including a history of incarceration as a covariate,
African American race (OR 0.37, CI 0.15-0.91, p = 0.03) and
a history of having ever been incarcerated (7.9 CI 1.7–36.9,
9.008) were independently associated with a MRSA infection.
Care in the Emergency Department (OR 3.4 compared with
patients from inpatient non-ICU units, CI 0.84–14.0, p = 0.09)
and an increasing number of people in the household (OR 1.3,
CI 0.98–1.7, p = 0.07) trended toward significance among MRSA
patients. Only 184 patients were included in Model 1 because data
were available about history of incarceration from only 195 (48
MSSA and 147 MRSA) patients, and 11 of these lacked data on
other variables in the model (Table 3a). In Model 2, omitting the
history of incarceration (n = 455), only care in the Emergency
Department (OR 4.6 compared with patients from inpatient non-
ICU units, CI 1.5–14.0, p = 0.008) was significantly associated
with MRSA infection. Being a transplant patient trended toward
a significant association with MSSA infection (OR 0.40, CI 0.13–
1.1, p = 0.08) (Table 5b).

Discussion

Our data yield three major conclusions. First, MSSA has, by
several criteria become a predominantly health-care-associated
pathogen among patients at UCMMC. Moreover, MRSA patients
are no longer predominantly patients with recent exposure to the
health care setting. Thus, a possible role reversal has occurred for
MSSA and MRSA strains; before the mid-1990s, MRSA was almost
exclusively a health care-associated pathogen, and S. aureus infections

---

### Table 1. Demographic and clinical characteristics of patients with MSSA and MRSA infections.

|                      | MRSA, No. of patients, n=545 (%) | MSSA, No. of patients, n=114 (%) | p-value |
|----------------------|----------------------------------|----------------------------------|---------|
| **Clinical syndrome**|                                  |                                  |         |
| Bacteraemia,         | 63 (11.6)                        | 22 (19.3)                        | 0.03    |
| endocarditis, or     |                                  |                                  |         |
| sepsis               |                                  |                                  |         |
| Osteomyelitis or     | 33 (6.1)                         | 11 (9.7)                         | 0.2     |
| septic arthritis     |                                  |                                  |         |
| Pneumonia            | 46 (8.4)                         | 8 (7.0)                          | 0.6     |
| Skin and soft tissue infection | 354 (65.0) | 54 (47.4) | <0.001 |
| Urinary tract infection | 22 (4.0) | 2 (1.8) | 0.4 |
| Other*               | 27 (5.0)                         | 17 (14.9)                        | <0.001  |
| **Type of Infection**|                                  |                                  |         |
| Invasive             | 175 (32.1)                       | 44 (38.6)                        | 0.2     |
| Non-Invasive         | 370 (67.9)                       | 70 (61.4)                        | –       |
| **Age Group**        |                                  |                                  |         |
| Pediatric (<18.0 years) | 215 (39.5) | 29 (25) | 0.005 |
| Adult                | 330 (60.6)                       | 85 (75)                          | –       |
| **Gender**           |                                  |                                  |         |
| Male                 | 268 (49.2)                       | 56 (49.1)                        | 1.0     |
| Female               | 277 (50.8)                       | 58 (50.9)                        | –       |
| **Race**             |                                  |                                  |         |
| African American     | 406 (74.5)                       | 53 (46.9)                        | <0.001  |
| Caucasian            | 84 (15.4)                        | 35 (31.0)                        | –       |
| Latino               | 11 (2.0)                         | 8 (7.1)                          | –       |
| Native American      | 3 (0.6)                          | 1 (0.9)                          | –       |
| Unknown              | 41 (7.3)                         | 17 (14.2)                        | –       |
| **Type of insurance**|                                  |                                  |         |
| Public assistance    | 378 (69.4)                       | 68 (59.7)                        | 0.04    |
| Private              | 132 (24.2)                       | 45 (39.5)                        | 0.0001  |
| Uninsured            | 19 (3.5)                         | 1 (0.9)                          | 0.2     |
| Unknown              | 16 (2.9)                         | 0 (0)                            | –       |

*Includes abdominal abscess, toxic shock syndrome, cholecystitis, conjunctivitis, peritonitis, empyema, neurosurgical device infection, uncertain site of culture, and upper respiratory infection.

Abbreviations: MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-susceptible Staphylococcus aureus.

doi:10.1371/journal.pone.00018217.0001
Table 2. Presence of CDC risk factors for HA-MRSA* among patients with MSSA and MRSA infections.

| Risk Factor                        | MRSA, No. of patients, n=545 (%) | MSSA, No. of patients, n=114 (%) | p-value |
|------------------------------------|----------------------------------|---------------------------------|---------|
| Inpatient culture obtained >48 hours after admission | 106 (19.5)                      | 30 (26.3)                       | 0.1     |
| Hospital stay, past year           | 225/458 (49.1)                   | 57 (50.0)                       | 0.9     |
| Surgery, past 6 months             | 209/486 (43.0)                   | 43 (37.7)                       | 0.3     |
| Hemodialysis, past year            | 35 (6.4)                         | 9 (8.0)                         | 0.6     |
| Indwelling catheter                | 69 (12.7)                        | 16 (14.0)                       | 0.7     |
| Previous MRSA isolation            |                                  |                                 |         |
| Laboratory report                  | 58 (10.6)                        | 15 (13.2)                       | 0.4     |
| Self-report only                   | 9/281 (3.2)                      | 1 (0.9)                         | 0.3     |
| Lived in long-term care facility, past year | 16/290 (5.5)                  | 2 (1.8)                         | 0.1     |

*Absence of these risk factors comprise the CDC case definition for community-associated MRSA infections. Denominators for HA-MRSA risk factors exclude those interviewed patients who answered that they did not know information requested of them and those patients about whom risk factor information could not be determined from chart review. For all 659 patients it was determined whether MRSA had been isolated from them at UCH since 1994, but for 295 patients, it could not be determined if MRSA had been isolated from them at another health care facility. The information regarding a stay in a long-term care facility was determined only for those patients lacking another health-care risk factor.

Abbreviations: HA-, health care associated; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-susceptible Staphylococcus aureus.

doi:10.1371/journal.pone.0018217.t002

Table 3. Additional Risk Factors and Putative Risk Factors for Exposure to MRSA.

| Risk Factor                        | MRSA, No. of patients, n=545 (%) | MSSA, No. of patients, n=114 (%) | p-value |
|------------------------------------|----------------------------------|---------------------------------|---------|
| Antibiotic use in past 6 months    | 229 (42.1)                       | 57 (50)                         | 0.1     |
| Immunocompromised*                 | 80 (14.7)                        | 22 (19)                         | 0.2     |
| Diabetes                           | 103 (18.9)                       | 24 (21)                         | 0.6     |
| Cancer                             | 87 (16.0)                        | 22 (19)                         | 0.4     |
| Transplant                         | 12 (2.2)                         | 9 (8.0)                         | 0.002   |
| Implant, hardware or other foreign body | 88 (16.1)                  | 20 (18)                         | 0.7     |
| HIV-infected                       | 13 (2.4)                         | 4 (3.5)                         | 0.5     |
| Attend daycare                     | 31/215 (14.4)                    | 3/29 (10)                       | 0.8     |
| Work in prison or jail             | 15 (2.8)                         | 2 (1.8)                         | 0.8     |
| Been in prison or jail             | 41 (7.5)                         | 1 (0.9)                         | 0.005   |
| Travel in previous 6 months        | 56 (10.3)                        | 9 (7.9)                         | 0.4     |
| Intravenous drug use               | 29 (5.3)                         | 2 (1.8)                         | 0.1     |
| Household healthcare contact†      | 65/303 (21.5)                    | 10/49 (20)                      | 0.9     |
| Household hospitalized contact†    | 67/295 (22.7)                    | 7/47 (15)                       | 0.2     |
| Number people in household, mean ± s. d.* | 3.66±2.16                   | 3.15±1.51                       | 0.047   |
| Number bedrooms in household mean ± s. d.† | 2.88±1.28                  | 2.87±0.89                       | 1.0     |
| Number persons/bedroom in household, mean ± s. d.* | 0.89±0.03                 | 1.02±0.07                       | 0.1     |

*Patients either had an inborn immunodeficiency or were infected with HIV at the time of culture or that they were taking immunosuppressive drugs at the time of culture. Patients with diabetes mellitus, liver disease, cancer, or a rheumatologic disease were not considered to be immunocompromised in the absence of immunosuppressive drug therapy.

†Denominators indicated are the number of patients interviewed who answered the relevant questions.

For MRSA n = 424; for MSSA, n = 79.

For MRSA n = 277; for MSSA, n = 45.

For MRSA, n = 276; for MSSA, n = 45.

Abbreviations: MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-susceptible Staphylococcus aureus.

doi:10.1371/journal.pone.0018217.t003
in the community were nearly always MSSA. We found that MSSA patients were more likely to have bacteremia, endocarditis, or sepsis, to be transplant patients, to be adults, and to be in an intensive care unit than MRSA patients. MSSA patients were also less likely than MRSA patients to have an SSTI or to be treated in the emergency department and released. MSSA and MRSA patients had a similar likelihood of having an invasive infection. MRSA infections were not more likely than MSSA infections to occur among patients with a number of chronic diseases.

The second conclusion is that there was considerable overlap in the MLST genotypes of MRSA and MSSA strains. Although the MSSA strains we studied were more polyclonal than the MRSA strains, as others have noted [24], we found that both the MRSA and MSSA isolates from patients seeking care for infections mostly fell into 5 STs: ST1, ST5, ST8, ST30, and ST59, all common MRSA genetic backgrounds in various regions of the world. Thus, despite the emergence of novel CA-MRSA strains during the previous decade in Chicago, the genotypic backgrounds among MSSA strains causing infections at our center maintained substantial genotypic overlap with MRSA strains.

Third, we have demonstrated that MSSA strains causing clinical infections rarely carry genes for the PVL toxin (7.0%), in contrast to MRSA strains (58.2%) even in the era of epidemic CA-MRSA. The low prevalence of PVL gene carriage in MSSA strains is consistent with reports from European centers prior to the emergence of CA-MRSA [24]. Further research is needed to determine why PVL toxin genes are almost universally carried by CA-MRSA strains but remain rare among MSSA and HA-MRSA.

Figure 1. Percent of patients with MRSA and MSSA infections who had cultures obtained in various sites of care; emergency department patients include only those cultured and not admitted to the hospital. doi:10.1371/journal.pone.0018217.g001

Figure 2. Percent of MSSA and MRSA isolates from UCMC, July 1, 2004-June 30, 2005, belonging to ST1, ST5, ST8, ST30, ST59, and other genetic backgrounds. doi:10.1371/journal.pone.0018217.g002
### Table 4. MLST of MRSA and MSSA isolates causing infections at UCMC.

| Clonal complex type/MLST | MRSA | | | | MSSA | | | |
|-------------------------|------|---|---|---|------|---|---|---|
|                         | Number (n=545) | Percent | Number (n=114) | Percent |
| **Clonal complex 1**    |      |   |      |   |
| 1                       | 21   | 3.9 | 5    | 4.4 |
| 188                     | 0    | 0  | 1    | 0.9 |
| 573                     | 0    | 0  | 1    | 0.9 |
| **Clonal complex 5**    |      |   |      |   |
| 5                       | 170  | 31.3 | 9  | 7.9 |
| 5slv†                   | 1    | 0.2 | 1    | 0.9 |
| 105                     | 3    | 0.6 | 0    | 0   |
| 231                     | 14   | 2.6 | 0    | 0   |
| **Clonal complex 8**    |      |   |      |   |
| 8                       | 321  | 58.9 | 14  | 12.3 |
| 8slv†                   | 2    | 0.3 | 0    | 0   |
| 72                      | 1    | 0.2 | 5    | 4.4 |
| 1181                    | 0    | 0  | 1    | 0.9 |
| **Clonal complex 9**    |      |   |      |   |
| 9                       | 0    | 0  | 1    | 0.9 |
| 109                     | 0    | 0  | 4    | 3.5 |
| **Clonal complex 12**   |      |   |      |   |
| 12                      | 0    | 0  | 1    | 0.9 |
| 12slv†                  | 0    | 0  | 1    | 0.9 |
| **Clonal complex 15**   |      |   |      |   |
| 15                      | 0    | 0  | 8    | 7.0 |
| 582                     | 0    | 0  | 1    | 0.9 |
| **Clonal complex 20**   |      |   |      |   |
| 20                      | 0    | 0  | 3    | 2.6 |
| **Clonal complex 22**   |      |   |      |   |
| 22                      | 6    | 1.1 | 0    | 0   |
| **Clonal complex 30**   |      |   |      |   |
| 30                      | 0    | 0  | 25   | 21.9 |
| 30slv†                  | 0    | 0  | 1    | 0.9 |
| 36                      | 3    | 0.6 | 0    | 0   |
| 39                      | 0    | 0  | 1    | 0.9 |
| **Clonal complex 59**   |      |   |      |   |
| 59                      | 2    | 0.4 | 5    | 4.4 |
| **Clonal complex 97**   |      |   |      |   |
| 97                      | 0    | 0  | 2    | 1.8 |
| **Clonal complex 121**  |      |   |      |   |
| 121                     | 0    | 0  | 1    | 0.9 |
| **Clonal complex 182**  |      |   |      |   |
| 182slv†                 | 0    | 0  | 1    | 0.9 |
| **Singletons**          |      |   |      |   |
| 580                     | 0    | 0  | 1    | 0.9 |
| 1159                    | 0    | 0  | 1    | 0.9 |

*CC1 was subsumed by the CC15 by the MLST administrators.
†slv, single locus variant.
Abbreviations: MLST, multilocus sequence typing; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-susceptible Staphylococcus aureus.
doi:10.1371/journal.pone.0018217.t004
Table 5. (a) Logistic regression Model 1. including variables demonstrating significant association with MRSA infection on bivariate analysis (n = 184); (b) Logistic regression Model 2. same as Model 1. excluding variable for ever been incarcerated (n = 455).

| Characteristic | OR (95% CI) | p-value |
|---------------|-------------|---------|
| **Patient characteristics** |          |         |
| Non-African American race* | 0.37 (0.15–0.91) | 0.03 |
| Pediatric Age Group | 0.86 (0.29–2.6) | 0.8 |
| Public Insurance or Uninsured† | 1.9 (0.77–4.6) | 0.2 |
| Number of people in household‡ | 1.3 (0.98–1.7) | 0.07 |
| Ever been incarcerated | 7.9 (1.7–36.9) | 0.008 |
| Transplant patient | 0.48 (0.11–2.1) | 0.3 |
| **Other factors** |          |         |
| Location of care |          |         |
| Inpatient (non-ICU) | ref. | – |
| ICU | 1.6 (0.48–5.4) | 0.5 |
| Emergency Department | 3.4 (0.84–14.0) | 0.09 |
| Outpatient clinic | 0.46 (0.15–1.4) | 0.2 |
| SSTI | 1.2 (0.44–3.0) | 0.8 |
| Slh. Model 2 |          |         |
| Patient characteristics |          |         |
| Non-African American race | 0.61 (0.34–1.1) | 0.1 |
| Pediatric Age Group | 0.95 (0.47–1.9) | 0.9 |
| Public Insurance or Uninsured* | 1.4 (0.79–2.5) | 0.3 |
| Number of people in household‡ | 1.08 (0.92–1.3) | 0.3 |
| Transplant patient | 0.40 (0.13–1.1) | 0.08 |
| Other factors |          |         |
| Location of care |          |         |
| Inpatient (non-ICU) | ref. | – |
| ICU | 1.3 (0.58–2.7) | 0.6 |
| Emergency Department | 4.6 (1.5–14.0) | 0.008 |
| Outpatient clinic | 1.2 (0.47–1.9) | 0.7 |
| SSTI | 1.3 (0.74–2.4) | 0.3 |

*Public Insurance or uninsured (n = 131) compared with privately insured (n = 33).
†Indicates odds ratio for every additional person in the household.
‡African American (n = 353) compared with all others of known race (n = 102).

Abbreviations: SSTI, skin or soft tissue infection.

doi:10.1371/journal.pone.0018217.t005

strains; these genes presumably impart either a survival advantage or they represent virulence determinants in these strains that became widespread in the late 1990s.

Most MSSA strains that were PVL+ were ST8, the same ST of strains with the USA300 pulsortype, and most ST8 strains were obtained from uncomplicated SSTIs, the most common type of infection caused by CA-MRSA in many studies in the U.S. These data indicate an association between ST8 S. aureus strains, regardless of the methicillin resistance phenotype, and presence of an SSTI.

Others have also found that MSSA strains commonly cause invasive infections.16, 19–25 The importance of ST8 (consistent with USA300) among MSSA invasive disease isolates, however, is unclear. We found that ST8 MSSA isolates were infrequently responsible for invasive disease although others have established an important role for ST8 isolates among invasive infections in children [17,25]. We also found few invasive infections caused by PVL+ MSSA strains. The results of these studies may differ because we also studied adults or because of temporal or geographic variation in the MRSA epidemic.

Our study suggests that patients living in poverty and underserved populations are at higher risk for a MRSA infection, but not an MSSA infection. Private insurance was more common among MSSA than MRSA patients, and MRSA patients were more commonly treated in the emergency department. This is consistent with our finding that MSSA infections reflect epidemiologic characteristics of nosocomial or health-care associated infections. Our patients with MSSA isolates were not as likely as patients with CA-MRSA infections to be previously healthy and presenting for care from the community.

Other studies have compared MRSA and MSSA patients, but they have been limited by a lack of genotyping data, or limited to isolates obtained from skin infections and/or CA-S. aureus infections (defined by various criteria) [4,16–18,26,27]. McCarthy et al. examined MRSA and MSSA infections among military veterans in Atlanta in 2007–2008 and found that MRSA patients were more likely to have had a previous MRSA infection and to have had a stay in a long-term care facility in the previous year, but less likely to have had a biopsy in the previous year [27]. We did not find the same distinctions, perhaps because of differences in the populations studied.

There are limitations to our study. We examined S. aureus infections at one medical center in one city. The MSSA patients studied were not a complete cohort, but the 114 patients and isolates that we examined were a carefully designed sample that is likely representative of all MSSA infections at our institution.

In conclusion, MSSA has reversed roles with MRSA. In the era of epidemic CA-MRSA infections, MSSA isolates have assumed the role of a nosocomial pathogen among the debilitated, while CA-MRSA strains continue to predominate among patients in our inner-city community.

Author Contributions
Conceived and designed the experiments: MZD SBV RSD. Performed the experiments: MZD DLZ. Analyzed the data: MZD. Contributed reagents/materials/analysis tools: RSD SBV MZD. Wrote the paper: MZD RSD. Procured funding: RSD.

References
1. Lowy FD (1998) Staphylococcus aureus infections. N Engl J Med 339: 520–532.
2. Tenover FC, McDougal LK, Goering RV, Killinger G, Prejan SJ, et al. (2006) Characterization of a strain of community-associated methicillin-resistant Staphylococcus aureus widely disseminated in the United States. J Clin Microbiol 44: 108–118.
3. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Bouord DJ, et al. (2003) Comparison of community- and health care-associated methicillin-resistant Staphylococcus aureus infection. JAMA 290: 2976–2984.
4. Kaplan SL, Hulten KG, Gonzalez BE, Hammerman WA, Lamberth L, et al. (2005) Three-year surveillance of community-acquired methicillin-resistant Staphylococcus aureus in children. Clin Infect Dis 40: 1785–1791.
5. Herold BC, Inmergold LC, Maranan MC, Lauderdale DS, Gaskin RE, et al. (1998) Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk. JAMA; 279: 593–598.
6. David MZ, Mennella C, Mansour M, Boyle-Vavra S, Daum RS (2008) Predominance of methicillin-resistant Staphylococcus aureus among pathogens...
causing skin and soft tissue infections in a large urban jail: Risk factors and recurrence rates. J Clin Microbiol 46: 3222–3227.

7. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, et al. (2006) Methicillin-resistant S. aureus infections among patients in the emergency department. N Engl J Med 355: 666–674.

8. Tenover FC, McAllister S, Fosheim G, McDougal LK, Carey RB, et al. (2008) Characterization of Staphylococcus aureus isolates from nasal cultures of individuals in the United States, 2001-2004. J Clin Microbiol 46: 2837–2841.

9. Lowy FD, Aiello AE, Bhat M, Johnson-Lawrence VD, Lee MH, et al. (2007) Staphylococcus aureus colonization and infection in New York state prisons. J Infect Dis 196: 911–918.

10. Donker GA, Deurenberg RH, Driessen C, Sebastian S, Nys S, et al. (2009) The population structure of Staphylococcus aureus among general practice patients from the Netherlands. Clin Microbiol Infect 15: 137–143.

11. Grundman H, Hori S, Enright MC, Webster C, Tami A, et al. (2002) Determining the genetic structure of the natural population of Staphylococcus aureus: A comparison of multilocus sequence typing with pulsed-field gel electrophoresis, randomly amplified polymorphic DNA analysis, and phage typing. J Clin Microbiol 40: 4544–4546.

12. Cespedes C, Said-Salim B, Miller M, Lo SH, Kreiswirth BN, et al. (2005) The clonality of Staphylococcus aureus nasal colonization. J Infect Dis 191: 444–452.

13. Melles DC, Gorkink RFJ, Boeldens HA, Snijders SV, Peeters JK, et al. (2004) Natural population dynamics and expansion of pathogenic clones of Staphylococcus aureus. J Clin Invest 114: 1782–1790.

14. Ruiny R, Arnaud-Lefèvre L, Barbier F, Ruppe E, Cocojaru R, et al. (2009) Comparisons between geographically diverse samples of carried Staphylococcus aureus. J Bacteriol 191: 5577–5583.

15. Montevecce S, Lidicke C, Slickers P, Ehricht R (2009) Molecular epidemiology of Staphylococcus aureus in asymptomatic carriers. Eur J Clin Microbiol Infect Dis 28: 1159–1165.

16. Miller LG, Perdreau-Remington F, Bayer AS, Diep B, Tan N, et al. (2007) Clinical and epidemiologic characteristics cannot distinguish community associated methicillin-resistant Staphylococcus aureus infection from methicillin-susceptible S. aureus infection: A prospective investigation. Clin Infect Dis 44: 471–482.

17. Oroschek RC, Hunsdal DA, Fritz SA, Loughman JA, Mitchell K, et al. (2009) Contribution of genetically restricted, methicillin-susceptible strains to the ongoing epidemic of community-acquired Staphylococcus aureus infections. Clin Infect Dis 49: 536–542.

18. Mongkolrattanothai K, Aldag JC, Mankin P, Gray BM (2009) Epidemiology of community-onset Staphylococcus aureus infections in pediatric patients: An experience at a children’s hospital in central Illinois. BMC Infect Dis 9: 112.

19. David MZ, Gilkman D, Crawford SE, Peng J, King KJ, et al. (2008) What is community associated methicillin-resistant Staphylococcus aureus? J Infect Dis 197: 1235–1245.

20. Klevens MR, Morrison MA, Nadle J (2007) Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA 298: 1763–1771.

21. National Committee for Clinical Laboratory Standards (NCCLS) (2004) Performance standards for antimicrobial disk susceptibility testing: 14th Informational Supplement, M100-S14. Villanova, PA: NCCLS.

22. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 38: 1008–1015.

23. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, et al. (1999) Involvement of Panton-Valentine-leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 29: 1129–1132.

24. Chambers HF, Deloe FR (2009) Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol 7: 629–641.

25. McCaskill ML, Mason EO, Kaplan SL, Hammerman W, Lamberth LB, et al. (2007) Increase of the USA300 clone among community-acquired methicillin-susceptible Staphylococcus aureus causing invasive infections. Pediatr Infect Dis J 26: 1122–1127.

26. Sattler CA, Mason EO, Kaplan SL (2002) Prospective comparison of risk factors and demographic and clinical characteristics of community-acquired, methicillin-resistant versus methicillin-susceptible Staphylococcus aureus infection in children. Pediatr Infect Dis J 21: 910–917.

27. McCarthy NL, Sullivan PS, Gaynes R, Rinderud D (2010) Risk factors associated with methicillin resistance among Staphylococcus aureus infections in veterans. Infect Control Hosp Epidemiol 31: 36–41.