Methicillin-Resistant
Staphylococcus aureus USA300
Clone in Long-Term Care Facility

Pierre Tattevin,1 Binh An Diep, Michael Jula, and Françoise Perdreau-Remington

We performed a longitudinal analysis of 661 methicillin-resistant Staphylococcus aureus (MRSA) isolates obtained from patients in a long-term care facility. USA300 clone increased from 11.3% of all MRSA isolates in 2002 to 64.0% in 2006 (p<0.0001) and was mostly recovered from skin or skin structures (64.3% vs. 27.0% for non-USA300 MRSA; p<0.0001).

S
since 2001, a dramatic increase in methicillin-resistant Staphylococcus aureus (MRSA) infections has been observed in the United States, mostly related to emergence of the USA300 clone in the community (1) and subsequently in hospitals (2,3). Residents of long-term care facilities (LTCFs) are at risk for colonization with antimicrobial drug–resistant bacteria, including MRSA. After they have been colonized, these residents are at increased risk for infections (4). Although it is assumed that transfer of patients between acute-care hospitals and LTCFs provides an ongoing cycle for the introduction of MRSA between these facilities (5), few studies have described the molecular epidemiology of MRSA in LTCFs. We report the prevalence and distribution of MRSA genotypes among clinical isolates obtained over a 10-year period (1997–2006) at the main LTCF in San Francisco, California, USA.

The Study

The San Francisco Laguna Honda Hospital is a 1,000-bed LTCF. We conducted a retrospective review of electronic records for all cultures positive for S. aureus that originated from hospital residents during 1997–2006. Colonization screening was not performed during the study period. Data collection was approved by the Committee on Human Research, Office of Research Administration, at the University of California, San Francisco.

Isolates were tested for oxacillin resistance by the salt agar method, and the presence of the mecA gene was confirmed by PCR. Susceptibility to other antimicrobial drugs was determined by using microbroth dilution with the MicroScan WalkAway 96 instrument (Dade Behring, Deerfield, IL, USA). Inducible clindamycin-resistance testing (D-zone test) was performed by using the agar disk-diffusion method for isolates obtained during 2005–2006. Results were interpreted in accordance with guidelines (M7–A5) of the Clinical and Laboratory Standards Institute (Wayne, PA, USA; www.clsi.org).

Nonduplicated MRSA isolates were genotyped by pulsed-field gel electrophoresis (PFGE) after digestion of chromosomal DNA with Smal (6), spa typing (7), and multilocus sequence typing (MLST) (8). USA300 was defined by the presence of Panton-Valentine leukocidin (PVL) genes (lukF-PV and lukS-PV) and the arginine catabolic mobile element (ACME), detected by PCR. Staphylococcal cassette chromosome mec (SCCmec) type was identified by using a PCR-based protocol (9). All strains underwent spa typing and were tested for PVL and ACME genes. MLST was conducted for 12 strains that could not be characterized otherwise.

Chi-square tests were used for bivariate analysis, and χ² tests for trend were used to evaluate secular trends. All statistical analysis was conducted by using Stata version 9.1 (Stata Corp., College Station, TX, USA).

S. aureus was isolated from 1,284 patients. Of these isolates, 744 (57.9%) were MRSA. The proportion of MRSA among S. aureus isolates increased from 38.1% (56/147) in 1997 to 72.3% (99/137) in 2006 (p<0.0001; Figure 1). Median age (interquartile range [IQR]) was 67 years (54–78 years) for patients with MRSA and 64 years (49–78 years) for those with methicillin-susceptible S. aureus (MSSA) (p = 0.22). Male:female ratio was 1:2.2 for MRSA and 1:1.7 for MSSA (p = 0.12). For MRSA specimen sources, we observed a decrease in the proportion of urinary or respiratory specimens from 69.7% (131/188) during 1997–2000 to 49.5% (236/477) during 2001–2006, and a concomitant increase in the proportion of skin or skin structure specimens from 22.3% (42/188) to 42.8% (204/477) (p<0.0001; Figure 2). No change in specimen sources was observed for MSSA.

Three PFGE clonal groups (USA100 [ST5, SCCmec type II], USA300 [ST8, SCCmec type IV], and USA500 [ST8, SCCmec type IV]) accounted for 85.2% (563/661) of genotyped MRSA isolates. USA300 was first isolated in 2001 and accounted for 11.3% of all MRSA (7/62) in 2002, 30.1% (25/83) in 2003, 47.9% (45/94) in 2004, 49.5% (49/99) in 2005, and 64.0% (55/86) in 2006 (p<0.0001; DOI: 10.3201/eid1506.080195

Author affiliations: Pontchaillou University Hospital, Rennes, France (P. Tattevin); and San Francisco General Hospital, San Francisco, California, USA (B.A. Diep, M. Jula, F. Perdreau-Remington)

Current affiliation: San Francisco General Hospital, San Francisco, California, USA

References

1. Current affiliation: San Francisco General Hospital, San Francisco, California, USA
In contrast to reports characterizing USA300 strains as typically not multidrug resistant (7), we found that up to 30.9% of USA300 isolates were multidrug resistant. This finding suggests that USA300 isolates were acquired under antimicrobial drug pressure in the LTCF or during a stay in another hospital rather than while in contact with the community. Results from this study and others illustrate the fitness trait of USA300 clonal lineage (12). To prevent further spread of multidrug-resistant USA300 in LTCFs would require enhanced infection control policies, which may include isolation or cohorting of infected patients, antimicrobial drug stewardship, and systematic use of alcohol-based handwashing products. However, given that these policies have proven difficult to implement in tertiary care hospitals, it may be even more challenging in LTCFs, which have limited staff and infection control resources (5,13–15).

This study had some limitations. Increased incidence of MRSA-positive cultures could be related to changes in sampling policies in this institution (e.g., more frequent sampling, surveillance cultures). However, the increased incidence rate for MRSA is not likely related to these changes because the annual number of cultures positive for any pathogen gradually decreased over the study period, a 2-fold increase in MRSA incidence and an increase to 73% in the rate of methicillin resistance among S. aureus. Moreover, because USA300 has a tropism for skin and skin structure infections (3), its emergence caused a shift in MRSA specimen sources.

Although primarily encountered in urinary and respiratory specimens until 2001 in this study, as in previous studies performed in LTCFs, MRSA isolates have mostly originated from skin or skin structure since 2002. This finding has important implications for MRSA transmission and should be taken into account when designing infection control policies in LTCFs. For example, nasal decolonization as a means to prevent MRSA infection implies that MRSA reservoirs reside in endogenous sources. However, skin–skin and skin–fomite contact may represent common alternative routes of transmission for USA300 (11).

In contrast to reports characterizing USA300 strains as typically not multidrug resistant (7), we found that up to 30.9% of USA300 isolates were multidrug resistant. This finding suggests that USA300 isolates were acquired under antimicrobial drug pressure in the LTCF or during a stay in another hospital rather than while in contact with the community. Results from this study and others illustrate the fitness trait of USA300 clonal lineage (12). To prevent further spread of multidrug-resistant USA300 in LTCFs would require enhanced infection control policies, which may include isolation or cohorting of infected patients, antimicrobial drug stewardship, and systematic use of alcohol-based handwashing products. However, given that these policies have proven difficult to implement in tertiary care hospitals, it may be even more challenging in LTCFs, which have limited staff and infection control resources (5,13–15).

This study had some limitations. Increased incidence of MRSA-positive cultures could be related to changes in sampling policies in this institution (e.g., more frequent sampling, surveillance cultures). However, the increased incidence rate for MRSA is not likely related to these changes because the annual number of cultures positive for any pathogen gradually decreased over the study period, a 2-fold increase in MRSA incidence and an increase to 73% in the rate of methicillin resistance among S. aureus. Moreover, because USA300 has a tropism for skin and skin structure infections (3), its emergence caused a shift in MRSA specimen sources.

Although primarily encountered in urinary and respiratory specimens until 2001 in this study, as in previous studies performed in LTCFs, MRSA isolates have mostly originated from skin or skin structure since 2002. This finding has important implications for MRSA transmission and should be taken into account when designing infection control policies in LTCFs. For example, nasal decolonization as a means to prevent MRSA infection implies that MRSA reservoirs reside in endogenous sources. However, skin–skin and skin–fomite contact may represent common alternative routes of transmission for USA300 (11).

In contrast to reports characterizing USA300 strains as typically not multidrug resistant (7), we found that up to 30.9% of USA300 isolates were multidrug resistant. This finding suggests that USA300 isolates were acquired under antimicrobial drug pressure in the LTCF or during a stay in another hospital rather than while in contact with the community. Results from this study and others illustrate the fitness trait of USA300 clonal lineage (12). To prevent further spread of multidrug-resistant USA300 in LTCFs would require enhanced infection control policies, which may include isolation or cohorting of infected patients, antimicrobial drug stewardship, and systematic use of alcohol-based handwashing products. However, given that these policies have proven difficult to implement in tertiary care hospitals, it may be even more challenging in LTCFs, which have limited staff and infection control resources (5,13–15).

This study had some limitations. Increased incidence of MRSA-positive cultures could be related to changes in sampling policies in this institution (e.g., more frequent sampling, surveillance cultures). However, the increased incidence rate for MRSA is not likely related to these changes because the annual number of cultures positive for any pathogen gradually decreased over the study period,
References

1. Carleton HA, Diep BA, Charlebois ED, Sensabaugh GF, Perdreau-Remington F. Community-adapted methicillin-resistant *Staphylococcus aureus* (MRSA): population dynamics of an expanding community reservoir of MRSA. J Infect Dis. 2004;190:1730–8. DOI: 10.1086/425019

2. Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF, King MD, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus USA300* genotype as a major cause of health care–associated blood stream infections. Clin Infect Dis. 2006;42:647–56. DOI: 10.1086/499815

3. King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus USA300* clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med. 2006;144:309–17.

4. Bradley SF. Methicillin-resistant *Staphylococcus aureus*: long-term care concerns. Am J Med. 1999;106(SA):2S–10S; discussion 48S–52S. DOI: 10.1016/S0002-9343(99)00349-0

5. Kerttula AM, Lyytikainen O, Vuopio-Varkila J, Ibrahem S, Agthe N, Broas M, et al. Molecular epidemiology of an outbreak caused by methicillin-resistant *Staphylococcus aureus* in a health care ward and associated nursing home. J Clin Microbiol. 2005;43:6161–3. DOI: 10.1128/JCM.43.12.6161-6163.2005

6. Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, et al. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin Microbiol. 2006;44:108–18. DOI: 10.1128/JCM.44.1.108-118.2006

7. Shopin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol. 1999;37:3556–63.

8. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol. 2000;38:1008–15.

9. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2002;46:2155–61. DOI: 10.1128/AAC.46.7.2155-2161.2002

10. Domnio PY, Preney L, Gautier-Lerestif AL, Avril JL, Lafforgue N. Changes in staphylococcal cassette chromosome type and antibiotic resistance profile in methicillin-resistant *Staphylococcus aureus* isolates from a French hospital over an 11 year period. J Antimicrob Chemother. 2004;53:808–13. DOI: 10.1093/jac/dkh185

11. Miller LG, Diep BA. Colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis. 2008;46:752–60. DOI: 10.1086/526773

12. Diep BA, Chambers HF, Graher CJ, Szwowski JD, Miller LG, Han LL, et al. Emergence of multidrug-resistant, community-associated, methicillin-resistant *Staphylococcus aureus* clone USA300 in men who have sex with men. Ann Intern Med. 2008;148:249–57.

13. Lucet JC, Grenet K, Armand-LeFevre L, Harnal M, Bouvet E, Regnier B, et al. High prevalence of carriage of methicillin-resistant *Staphylococcus aureus* at hospital admission in elderly patients: implications for infection control strategies. Infect Control Hosp Epidemiol. 2005;26:121–6. DOI: 10.1086/502514

14. Kramen T, Hu J, Pottinger J, Herwaldt LA. Survey of long-term-care facilities in Iowa for policies and practices regarding residents with methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant enterococci. Infect Control Hosp Epidemiol. 2005;26:811–5. DOI: 10.1086/502498

15. Cretnik TZ, Vovko P, Petelj M, Juterek B, Harlander T, Kolman J, et al. Prevalence and nosocomial spread of methicillin-resistant *Staphylococcus aureus* in a long-term-care facility in Slovenia. Infect Control Hosp Epidemiol. 2005;26:184–90. DOI: 10.1086/502524

Address for correspondence: Françoise Perdreau-Remington, Department of Medicine, Division of Infectious Diseases, San Francisco General Hospital, 1001 Potrero Ave, San Francisco, CA 94143-0811, USA; email: fpr@epi-center.ucsf.edu

Table. Analysis of MRSA clones obtained in a long-term care facility, San Francisco, California, USA, 1997–2006*

| Clonal group | Antimicrobial drug resistance, % | Specimen source, % |
|--------------|---------------------------------|--------------------|
|              | Ery    | Cli   | Cip   | Sxt    | Gen   | Tet    | SST   | Respiratory tract | Urine | Blood† |
| USA100 (n = 299) | 96.2   | 84.9  | 95.9  | 3.3    | 6.1   | 2.6    | 22.7  | 36.4             | 35.1  | 5.8   |
| USA300 (n = 182) | 87.8   | 15.0  | 79.4  | 0.6    | 6.1   | 13.2   | 64.3  | 18.1             | 10.4  | 7.1   |
| USA500 (n = 82)  | 84.7   | 27.8  | 71.7  | 28.2   | 20.8  | 6.0    | 30.5  | 28               | 31.7  | 9.8   |
| LFT (n = 98)     | 87.3   | 65.6  | 62.8  | 10.9   | 13.4  | 7.8    | 37.2  | 26.6             | 26.6  | 9.6   |
| All MRSA (n = 744) | 91.8   | 64.6  | 87.0  | 7.1    | 21.6  | 5.1    | 32.7  | 25.1             | 23.1  | 6.3   |

*MRSA, methicillin-resistant *Staphylococcus aureus*; Ery, erythromycin; Cli, clindamycin; Cip, ciprofloxacin; Sxt, trimethoprim-sulfamethoxazole; Gen, gentamicin; Tet, tetracycline; SST, skin and skin structure; LFT, low frequency types.

†Blood and other typically sterile sites.

from 1,433 in 1997 to 862 in 2006, as did the incidence of MSSA-positive cultures, from 91 in 1997 to 38 in 2006.

Although our study was limited by its reliance on retrospective data collection, it illustrates the need for further investigations in LTCFs regarding risk factors and appropriate interventions to minimize further transmission of MRSA. LTCFs, long thought to be reservoirs of nosocomial MRSA clones, now emerge as an important reservoir for USA300 and could play a role in the emergence of multidrug-resistant USA300.

This study was supported by an unrestricted grant to F.P.-R. from Pfizer. P.T. was supported by a grant from the Société de Pathologie Infectieuse de Langue Française and by the Pontchaillou University Hospital, Rennes, France. B.A.D. was supported by a Microbial Pathogenesis and Host Defense Postdoctoral Fellowship, University of California, San Francisco (5T32AI060537-02).

Dr Tattevin is an infectious diseases physician at Pontchaillou University Hospital in Rennes, France. He is currently working as a postdoctoral fellow at the San Francisco General Hospital. His research interests include MRSA, sepsis, and endocarditis.