

Outbreak of Skin Infections in College Football Team Members Due to an Unusual Strain of Community-Acquired Methicillin-Susceptible *Staphylococcus aureus*[∇]

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We report a skin and soft-tissue infection outbreak among football team members due to a USA300 methicillin-susceptible *Staphylococcus aureus* (MRSA) strain with genes coding for Pantone-Valentine leukocidin and the arginine catabolic mobile element. We postulate that the strain is a community-associated USA300 MRSA strain that lost methicillin resistance but retained important virulence factors.

In the United States, most community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have been caused by the USA300 clone (12). Outbreaks caused by USA300 have been reported in at least 38 states, and it accounts for >50% of all *S. aureus* infections (12). USA300 belongs to multilocus sequence type clonal complex 8, sequence type 8, and *spa* type 008. It is characterized by the presence of SCCmec IV, which confers methicillin resistance, the arginine catabolic mobile element (ACME), and genes coding for Pantone-Valentine leukocidin (PVL) (2).

During the fall of 2007, we detected an outbreak of skin infections among football players at a New England college. The outbreak was characterized by rapid spread among players and abscess formation, features characteristic of previously described CA-MRSA outbreaks among members of athletic teams. The causative organism was methicillin-susceptible *S. aureus* (MSSA), however, and it showed an unusual resistance profile (resistance to erythromycin and ciprofloxacin). The epidemiologic and clinical pattern of the outbreak and the resistance profile prompted us to investigate the cases and look for molecular similarities between the outbreak strain and USA300.

A case was defined as a college football player with a ciprofloxacin-resistant MSSA skin infection with onset between preseason training and the end of the season. Cases were detected by active surveillance for cellulitis and/or skin abscess among players and athletic trainers. Incision and drainage were performed on all abscesses, and specimens were sent for routine cultures. On day 16 of the outbreak, all 110 football players and eight athletic trainers were screened for *S. aureus* colonization. The anterior nares and pharynx were sampled using rayon-tipped swabs (Fisherfinest*; Fisher Scientific, Ontario, Canada), and specimens from each person were combined for microbiological evaluation.

Isolates from cases were typed by pulsed-field gel electrophoresis (PFGE) using restriction endonuclease SmaI (11).

Bionumerics software (version 5.0; Applied Maths, Kortrijk, Belgium) was used to compare isolates to each other and to the USA300 MRSA control strain. *spa* typing (6) and PCR to determine the presence or absence of PVL genes (9) and the ACME-specific *arcA* gene (2) were also performed.

Eight of the 110 football players met the outbreak case definition (attack rate, 7%; Table 1). All of the isolates had the same antibiotic susceptibility profile, with susceptibility to oxacillin, cefazolin, ceftriaxone, piperacillin-tazobactam, clindamycin, gentamicin, rifampin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin and resistance to penicillin, erythromycin, ciprofloxacin, and bacitracin. The isolates were indistinguishable by PFGE, with a macrorestriction fragment pattern typical of USA300 (Fig. 1). The outbreak isolate was *spa* type 008 and contained PVL-encoding and ACME-specific *arcA* genes, consistent with a USA300 designation. Compared with the USA300 control, the outbreak isolates were missing an approximately 30-kb portion of the SmaI fragment of *mecA*.

Of the 118 players and athletic trainers, 62 (53%) were colonized with MSSA but none was colonized with the outbreak strain. Five (4%) were colonized with MRSA. One of the eight infected players was colonized with a nonoutbreak MSSA strain.

Several features of our outbreak are similar to those previously described in reported USA300 MRSA outbreaks, such as the rapidity of spread and the severity of tissue inflammation and abscess formation. The finding that none of the players or trainers was colonized with the outbreak strain, despite a high MSSA colonization rate, is also consistent with USA300 MRSA outbreaks in football teams (1, 8). It is important to note, however, that it is possible that some carriers were not detected because broth enrichment was not used with our selective culture media.

The outbreak strain is a USA300 strain by PFGE and contains genes coding for PVL and ACME. The outbreak strain differs from USA300 MRSA only by an approximately 30-kb deletion consistent with loss of SCCmec IV. Our isolate is similar to USA300 MSSA strains previously described in non-outbreak-associated settings (3, 10, 13).

We believe that our outbreak isolate represents a strain of MSSA that evolved from USA300 by losing SCCmec IV. USA300

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TABLE 1. Demographic and clinical description of the eight cases of MSSA infection associated with a New England college football team outbreak in 2007

Case no.	Age (yr)	Outbreak day	Infection date	Infection site	I and D ^a plus antibiotics	Skin infection in past year
1	19	1	8/27/2007	Leg	Yes	Yes ^b
2	20	2	8/28/2007	Knee	Yes	No
3	22	3	8/29/2007	Forearm	Yes	No
4	18	4	8/30/2007	Forearm	Yes	No
5	19	12	9/7/2007	Thigh	Yes	No
6	19	12	9/7/2007	Chest	Yes	No
7	18	22	9/17/2007	Knee	Yes	No
8	21	55	10/20/2007	Elbow	Yes	No

^a I and D, incision and drainage.

^b Arm lesion 3 weeks before outbreak.

MRSA is thought to have evolved from MSSA through horizontal transfer of mobile elements (2). SCCmec IV and ACME were likely acquired from *S. epidermidis*, given that they were highly prevalent in *S. epidermidis* years before they were detected in *S. aureus* (4). Since SCCmec IV and ACME integrate at the same attachment site, *orfX*, it has been hypothesized that they may have been acquired in association (2, 7). Diep et al. (2) proposed that ACME from *S. epidermidis* is excised by *ccrAB* recombinase from an adjacent SCC element and then integrated into the USA300 MRSA genome by *ccrAB* from an existing SCCmec IV element (2). The dependence of ACME on *ccrAB* from an existing SCCmec element may explain the common attachment site.

Our outbreak isolate is similar to the ACME-positive, SCCmec

IV-negative USA300 strain described by Goering et al. (5). Their analysis of the junction between ACME and the attachment site, *orfX*, did not reveal any SCCmec IV elements. Since the insertion of ACME is most likely dependent on SCCmec recombinase (*ccr*) genes, the absence of SCCmec IV in this isolate suggests that it has been deleted. Donnio et al. (3) showed that an MSSA strain with an unusual resistance pattern (resistant to erythromycin and fluoroquinolones) could be derived from a dominant epidemic MRSA strain with loss of a 40-kb DNA fragment corresponding to the SCCmec region (3). Further analysis of their USA300 MSSA isolates revealed small fragments of SCCmec, further supporting this idea (3).

This outbreak of severe skin and soft-tissue infections among healthy athletes provides evidence that USA300 MRSA is capable of losing its methicillin resistance while maintaining its virulence. If loss of resistance represents the latest evolutionary development for *S. aureus*, it has important implications for infection surveillance and prevention and control programs, which are increasingly based on detection of methicillin resistance as a trigger for interventions.

REFERENCES

1. Begier, E. M., K. Frenette, N. L. Barrett, P. Mshar, S. Petit, D. J. Boxrud, K. Watkins-Colwell, S. Wheeler, E. A. Cebelinski, A. Glennen, D. Nguyen, and J. L. Hadler. 2004. A high-morbidity outbreak of methicillin-resistant *Staphylococcus aureus* among players on a college football team, facilitated by cosmetic body shaving and turf burns. *Clin. Infect. Dis.* **39**:1446–1453.
2. Diep, B. A., S. R. Gill, R. F. Chang, T. H. Phan, J. F. Chen, M. G. Davidson, F. Lin, J. Lin, H. A. Carleton, E. F. Mongodin, G. F. Sensabaugh, and F. Pedreau-Remington. 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet* **367**:731–739.
3. Donnio, P. Y., D. C. Oliveira, N. A. Faria, N. Wilhelm, A. Le Coustumier, and H. de Lencastre. 2005. Partial excision of the chromosomal cassette containing the methicillin resistance determinant results in methicillin-susceptible *Staphylococcus aureus*. *J. Clin. Microbiol.* **43**:4191–4193.
4. Feng, Y., C. J. Chen, L. H. Su, S. Hu, J. Yu, and C. H. Chiu. 2008. Evolution and pathogenesis of *Staphylococcus aureus*: lessons learned from genotyping and comparative genomics. *FEMS Microbiol. Rev.* **32**:23–37.
5. Goering, R. V., L. K. McDougal, G. E. Fosheim, K. K. Bonnstetter, D. J. Wolter, and F. C. Tenover. 2007. Epidemiologic distribution of the arginine catabolic mobile element among selected methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* **45**:1981–1994.
6. Harmsen, D., H. Claus, W. Witte, J. Rothganger, H. Claus, D. Turnwald, and U. Vogel. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J. Clin. Microbiol.* **41**:5442–5448.
7. Highlander, S. K., K. G. Hultén, X. Qin, H. Jiang, S. Yerrapragada, E. O. Mason, Jr., Y. Shang, T. M. Williams, R. M. Fortunov, Y. Liu, O. Igboeli, J. Petrosino, M. Tirumalai, A. Uzman, G. E. Fox, A. M. Cardenas, D. M. Muzny, L. Hemphill, Y. Ding, S. Dugan, P. R. Blyth, C. J. Buhay, H. H. Dinh, A. C. Hawes, M. Holder, C. L. Kovar, S. L. Lee, W. Liu, L. V. Nazareth, Q. Wang, J. Zhou, S. L. Kaplan, and G. M. Weinstock. 2007. Subtle genetic

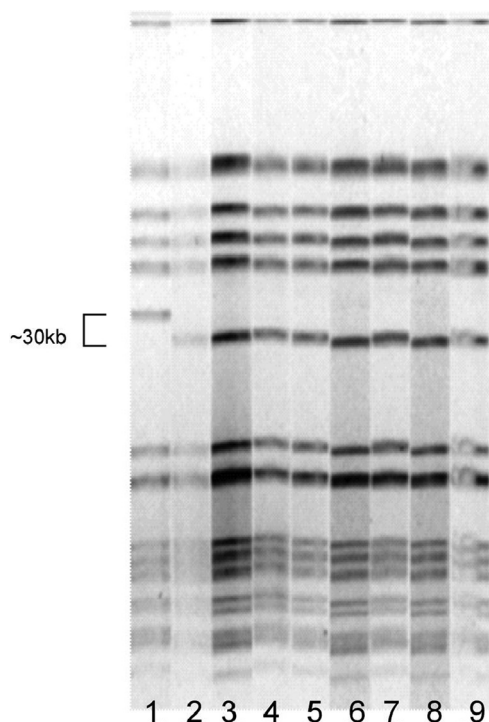


FIG. 1. PFGE results of the eight cases of MSSA infection associated with a New England college football team outbreak in 2007, suggesting a single source of infection (lanes 2 to 9). Lane 1 is a reference strain of USA300 MRSA which differs from the outbreak strain by a single band.

- changes enhance virulence of methicillin resistant and sensitive *Staphylococcus aureus*. *BMC Microbiol.* **7**:99.
8. **Kazakova, S. V., J. C. Hageman, M. Matava, A. Srinivasan, L. Phelan, B. Garfinkel, T. Boo, S. McAllister, J. Anderson, B. Jensen, D. Dodson, D. Lonsway, L. K. McDougal, M. Arduino, V. J. Fraser, G. Killgore, F. C. Tenover, S. Cody, and D. B. Jernigan.** 2005. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. *N. Engl. J. Med.* **352**:468–475.
 9. **Lina, G., Y. Piémont, F. Godail-Gamot, M. Bes, M. O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin. Infect. Dis.* **29**:1128–1132.
 10. **McCaskill, M. L., E. O. Mason, Jr., S. L. Kaplan, W. Hammerman, L. B. Lamberth, and K. G. Hultén.** 2007. Increase of the USA300 clone among community-acquired methicillin-susceptible *Staphylococcus aureus* causing invasive infections. *Pediatr. Infect. Dis. J.* **26**:1122–1127.
 11. **McDougal, L. K., C. D. Steward, G. E. Killgore, J. M. Chaitram, S. K. McAllister, and F. C. Tenover.** 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J. Clin. Microbiol.* **41**:5113–5120.
 12. **Miller, L. G., and B. A. Diep.** 2008. Colonization, fomites, and virulence: rethinking the pathogenesis of community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Clin. Infect. Dis.* **46**:752–760.
 13. **Orscheln, R. C., D. A. Hunstad, S. A. Fritz, J. A. Loughman, K. Mitchell, E. K. Storch, M. Gaudreault, P. L. Sellenriek, J. R. Armstrong, E. R. Mardis, and G. A. Storch.** 2009. Contribution of genetically restricted, methicillin-susceptible strains to the ongoing epidemic of community-acquired *Staphylococcus aureus* infections. *Clin. Infect. Dis.* **49**:536–542.