Presence and Molecular Epidemiology of Virulence Factors in Methicillin-Resistant Staphylococcus aureus Strains Colonizing and Infecting Soldiers†

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Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as an important cause of skin and soft-tissue infections (SSTI). The understanding of the molecular epidemiology and virulence of MRSA continues to expand. From January 2005 to December 2005, we screened soldiers for MRSA nasal colonization, administered a demographic questionnaire, and monitored them prospectively for SSTI. All MRSA isolates underwent molecular analysis, which included pulsed-field gel electrophoresis (PFGE) and PCR for Panton-Valentine leukocidin (PVL), the arginine catabolic mobile element (ACME), and the staphylococcal cassette chromosome mec (SCCmec). Of the 3,447 soldiers screened, 134 (3.9%) had MRSA colonization. Of the 3,066 (89%) who completed the study, 39 developed culture-confirmed MRSA abscesses. Clone USA300 represented 53% of colonizing isolates but was responsible for 97% of the abscesses (P < 0.001). Unlike colonizing isolates, isolates positive for USA300, PVL, ACME, and type IV SCCmec were significantly associated with MRSA abscess isolates. As determined by multivariate analysis, risk factors for MRSA colonization were a history of SSTI and a history of hospitalization. Although various MRSA strains may colonize soldiers, USA300 is the most virulent when evaluated prospectively, and PVL, ACME, and type IV SCCmec are associated with these abscesses.

Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a cause of infections in persons within the general community (community-associated methicillin-resistant Staphylococcus aureus [CA-MRSA]) (14, 31). Diseases caused by CA-MRSA range from cutaneous infection to life-threatening systemic illness (13, 14, 28, 31); however, the majority of disease manifests as suppurative skin and soft-tissue infections (SSTI) (13, 14, 28, 31, 32). CA-MRSA is of particular importance to the military, as soldiers are counted among the epidemiological groups who appear to be particularly at risk (10, 11). Pulsed-field type USA300 appears to have eclipsed other pulsed-field types, as nearly all recently reported MRSA outbreaks in the community have involved USA300 (13, 20, 28, 31, 33). Indeed, large analyses of clinical isolates obtained in metropolitan areas and university-affiliated emergency departments have demonstrated that USA300 is the predominant cause of MRSA SSTI in these settings (14, 21, 24, 31). Additionally, in some regions, USA300 is emerging as a hospital-associated pathogen (24, 37, 39). The factors responsible for USA300’s emergence as a significant pathogen remain unclear. USA300 does possess numerous genetic factors that are believed to augment its virulence, including Panton-Valentine leukocidin (PVL) and the arginine catabolic mobile element (ACME) (4, 5, 13, 20, 31, 42). Much of the clinical research on MRSA infections in the general community is limited to the retrospective analysis of clinical isolates. No large prospective investigations have been conducted to validate the observations that USA300 appears to be the most virulent MRSA clone.

During 2005, a cluster-randomized, double-blind, placebo-controlled trial was undertaken at Fort Sam Houston, TX, in which participants with MRSA nares colonization received intranasal mupirocin or placebo in an attempt to decrease colonization and subsequent SSTI (10). This investigation demonstrated no decrease in infection rate in either the mupirocin-treated individuals or within the larger study group (10). We evaluated molecular and epidemiological data obtained in that trial to identify risk factors for MRSA colonization and to prospectively describe which MRSA pulsed-field types were responsible for colonization and which pulsed-field types went on to cause subsequent infection, and to determine the presence of PVL and ACME in colonizing and abscess-causing isolates.

MATERIALS AND METHODS

Study participants. Study participants were U.S. Army personnel enrolled in the Health Care Specialist Course from 10 January 2005 to 16 December 2005.

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This is a 16-week course held at Fort Sam Houston, TX, which trains soldiers to become combat medics. The first 14 weeks of the course are conducted entirely outside of a health care setting. Soldiers were enrolled into the trial on the first day of their training after giving informed consent. The Brooke Army Medical Center Institutional Review Board approved the protocol (ClinicalTrials.gov; number NCT00289588).

**Specimen collection and identification.** Following enrollment, soldiers had their anterior nares cultured on the first day of training and again 8 to 10 weeks later. Cultures were obtained using BBL CultureSwabs with Stuart’s transport media (BD, Sparks, MD). Specimens were transported to the clinical microbiology laboratory and plated onto selective culture media, BBL CHROMagar Staph aureus and BBL CHROMagar MRSA media (BD Diagnostics, Sparks, MD). Isolates were identified by the characteristic colony morphology and color specific to these medium types (12). Both plates were incubated and examined for growth at 24 h per the manufacturer’s specifications. MRSA plates were read a second time at 48 h, also according to the manufacturer’s specifications.

During a 16-week period following enrollment, participants were observed prospectively to assess for clinical skin and soft-tissue abscesses. For any illness that occurred during this time, participants were seen in the same health care system, thus allowing the complete capture of all clinical infections. SSTI were prospectively to assess for clinical skin and soft-tissue abscesses. For any illness that occurred during this time, participants were seen in the same health care system, thus allowing the complete capture of all clinical infections. SSTI were identified by a daily review of clinic records and administrative codes from the hospital system, thus allowing the complete capture of all clinical infections. SSTI were defined on the anterior nares screening culture, and we defined an MRSA skin and soft-tissue abscess as any incised, drained, and cultured abscess that yielded MRSA. Thirty-five (45%) of the 78 MRSA-colonized participants were found to have nasopharyngeal colonization with MRSA, and 1,316 (38%) were colonized with methicillin-susceptible Staphylococcus aureus (MSSA). Seventy-one (53%) of the 134 MRSA-colonized participants were colonized with the USA300 pulsed-field type (Table 1.). USA800 was the second most common pulsed-field type, found in 45 participants (34%), and USA600 accounted for colonization in 12 participants (9%). Pulsed-field types colonizing only one participant each included USA100, USA400, USA700, and USA1100.

Two isolates possessed undefined PFGE clonal types. Of the 134 subjects colonized with MRSA, 65 (49%) carried a PVL-positive strain, 64 (48%) carried an ACME-positive strain, and 119 (89%) carried a type IV SCCmec-positive strain (Table 1.). USA300 was more highly associated with PVL than the other colonizing MRSA pulsed-field types, as PVL was found in 62 of 71 (87%) USA300 colonizing isolates and only 3 of 63 (5%) other colonizing pulsed-field types (P < 0.001). Similarly, ACME was found in 57 of 71 (80%) USA300 colonizing isolates and only 7 of 63 (11%) other colonizing pulsed-field types (P < 0.001).

Of the 3,447 participants who enrolled, 3,066 (89%) were available for follow-up. At the second nasal culture, 78 (2.5%) of the 3,066 participants were found to have nasal colonization with MRSA. Thirty-five (45%) of the 78 MRSA-colonized participants were colonized with the USA300 pulsed-field type. USA800 was the second most common pulsed-field type, found in 30 participants (39%), and USA600 accounted for colonization in 7 participants (9%). Other pulsed-field types included USA100 (one participant) and USA400 (four participants), and we could not determine the pulsed-field type of one isolate.

**Characteristics of MRSA abscess isolates.** Of the 3,066 participants who completed the investigation, 39 developed culture-proven MRSA soft-tissue abscesses. USA300 caused a significant number of infections, as 38 of the 39 (97%) isolates...
cultured from the MRSA abscesses were USA300 ($P < 0.001$) (Table 1). One abscess was caused by a USA800 strain. PVL was present in 38 of 39 (97%) abscess isolates overall, and of the USA300 abscess isolates, 38 of 38 (100%) were PVL positive. The 39 participants who developed MRSA abscesses, 11 participants had the identical MRSA strain recovered from the anterior nares at either the initial or terminal nasal screening culture. Of these 11 isolates, 10 were USA300 and 1 was USA800. The remaining 28 participants did not have MRSA nares colonization detected at either sampling.

**Comparison between colonizing and abscess isolates.** There was a significant difference between the colonizing pulsed-field types and the MRSA abscess pulsed-field types, as USA300 was more likely to be associated with abscess development than with colonization ($P < 0.001$) (Table 1). In terms of virulence and resistance properties, there were significant differences between colonizing isolates and the MRSA abscess isolates in terms of PVL positivity (65 of 134 [49%] and 38 of 39 [97%]; $P < 0.001$), ACME positivity (64 of 134 [48%] and 34 of 39 [87%]; $P < 0.001$), and type IV SCCmec positivity (119 of 134 [89%] and 39 of 39 [100%]; $P = 0.03$) (Table 1). Additionally, 66 of 134 colonizing isolates, and 20 of 39 abscess isolates, were from the placebo arm of the trial. When only those placebo-arm isolates were analyzed, there were significant differences between colonizing isolates and the MRSA abscess isolates for USA300 (33 of 66 [50%] and 19 of 20 [95%]; $P < 0.001$), for PVL positivity (28 of 66 [42%] and 19 of 20 [95%]; $P < 0.001$), and for ACME positivity (27 of 66 [41%] and 17 of 20 [85%]; $P < 0.001$). Finally, in the treated patients who were colonized with MRSA at the initial screening, there were two MRSA abscesses in the placebo group and three MRSA abscesses in the mupirocin group. All five abscesses were USA300, and all abscess isolates had the same PFGE pattern as the participant’s colonizing isolate.

**Risk factors for MRSA colonization.** After bivariate correlational analysis, MRSA-colonized participants were more likely to have received antibiotics in the past 6 months, were more likely to have been hospitalized in the prior year, and were more likely to have a history of previous SSTI in the past 6 months (Table 2). After multivariate analysis using logistic regression, the significant risk factors for MRSA colonization were a history of SSTI and a history of hospitalization (Table 3). Of the 134 participants with MRSA colonization, 18 (13%) reported a history of SSTI within the prior 6 months. Of these 18 participants, 11 initially were colonized with USA300, and 7 initially were colonized with a non-USA300 pulsed-field type (5 with USA800 and 2 with USA600). The association for having a history of SSTI between USA300-colonized participants and non-USA300-colonized participants was not statistically significant. Of the 134 MRSA-colonized participants, 25 (19%) reported a history of admission to a hospital in the prior year. The causes for the admission of these MRSA-colonized participants were the following: respiratory tract infection, 12; orthopedic injury, 7; SSTI, 3; cholecystectomy, 1; pelvic inflammatory disease, 1; and concussion, 1. The initial colonization results of these 25 participants were the following: USA300, 12; USA800, 12; and USA600, 1. The association of having a history of hospital admission between USA300-colonized participants and non-USA300-colonized participants was not statistically significant.

**Antimicrobial susceptibility results.** MRSA susceptibilities by pulsed-field type and whether the isolates were colonizing or abscess strains are described in Table 4. Clindamycin sus-
ceptibility was more likely to be found in abscess isolates than in colonizing isolates (P < 0.02).

**DISCUSSION**

In this prospective investigation, we have demonstrated that although many MRSA strains may be circulating within a population, USA300 is the most likely pulsed-field type to cause skin and soft-tissue abscesses. Additionally, this study strengthens the notion that PVL and ACME are important virulence factors for MRSA for soft-tissue abscess development.

Prior investigations have identified USA300 as a particularly virulent pulsed-field type, causing the majority of reported MRSA infections in community settings (13, 20, 21, 24, 28, 31). USA300 also is emerging as a significant hospital-associated MRSA infections in community settings (13, 20, 21, 24, 28, 31). In a murine model conducted by Voyich et al., PVL did not demonstrate PVL's importance. In a cumulative total of 150 isolates from experimental animal models has yet to be clearly determined. In a murine model conducted by Voyich et al., PVL did not appear to be a significant factor in the formation of skin abscesses (43). However, Labandeira-Rey et al. found in a murine pneumonia model that PVL appears to be an important virulence determinant in the development of necrotizing pneumonia (22).

In our investigation, ACME was detected in 48% of colonizing isolates and 87% of skin and soft-tissue abscess isolates (P < 0.001). ACME is a bicomponent exotoxin that causes dermal necrosis and possesses particular cytolytic activity against neutrophils and monocytes (38), and whether caused by MRSA or MSSA, it is associated with suppurative cutaneous disease and necrotizing infections (15, 19, 45). In one report of surgically drained community-associated soft-tissue abscesses, PVL was found in 89% of 48 MSSA isolates tested (19). Likewise, in an investigation of purulent SSTI in university-affiliated emergency departments, 23 of 55 (42%) MSSA isolates tested possessed PVL (31). Although PVL commonly is found in S. aureus isolates responsible for suppurative infections, currently it is found in fewer than 15% of colonizing MRSA isolates in the general community (17). The overall significance of PVL in experimental animal models has yet to be clearly determined. In a murine model conducted by Voyich et al., PVL did not appear to be a significant factor in the formation of skin abscesses (43). However, Labandeira-Rey et al. found in a murine pneumonia model that PVL appears to be an important virulence determinant in the development of necrotizing pneumonia (22).

In our investigation, ACME was detected in 48% of colonizing isolates and 87% of skin and soft-tissue abscess isolates (P < 0.001). ACME, which is found adjacent to SCCmec, recently has been noted to be a potentially important virulence factor (5, 6). ACME appears to have been horizontally acquired from *Staphylococcus epidermidis* and is thought to augment virulence by enhancing growth and survival on human skin (5). In a rabbit bacteremia model using isogenic USA300 strains with type IV SCCmec and ACME deletions, Diep et al. demonstrated ACME's importance (6). In this elegant study, the deletion of ACME resulted in diminished pathogenicity, whereas the absence of type IV SCCmec did not (6). ACME may be found in MSSA and MRSA isolates, including clones other than USA300 (9, 16), but it appears to be present in nearly all USA300 isolates (6, 16). There is a strong association between ACME and USA300, but the overall significance of ACME, like that of PVL, has yet to be fully determined.

It is not entirely clear what patient factors are associated with inducible clindamycin resistance (USA300, 4 of 71/71). Morano et al., 213 of 218 MRSA isolates tested possessed PVL (31). In our study, PVL was found in only 49% of colonizing isolates but in 97% of abscess isolates (P < 0.001). PVL is a bicomponent exotoxin that causes dermal necrosis and possesses particular cytolytic activity against neutrophils and monocytes (38), and whether caused by MRSA or MSSA, it is associated with suppurative cutaneous disease and necrotizing infections (15, 19, 45). In one report of surgically drained community-associated soft-tissue abscesses, PVL was found in 89% of 48 MSSA isolates tested (19). Likewise, in an investigation of purulent SSTI in university-affiliated emergency departments, 23 of 55 (42%) MSSA isolates tested possessed PVL (31). Although PVL commonly is found in S. aureus isolates responsible for suppurative infections, currently it is found in fewer than 15% of colonizing MRSA isolates in the general community (17). The overall significance of PVL in experimental animal models has yet to be clearly determined. In a murine model conducted by Voyich et al., PVL did not appear to be a significant factor in the formation of skin abscesses (43). However, Labandeira-Rey et al. found in a murine pneumonia model that PVL appears to be an important virulence determinant in the development of necrotizing pneumonia (22).

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with the acquisition of MRSA colonization within the community. Indeed, risk factors appear to vary even with gender (17). In our population, we found that MRSA-colonized participants were more likely to report a history of hospitalization and a history of SSTI. In a previous investigation, we also found that a history of SSTI had been a risk factor for colonization (11). We also noted that colonized participants were more likely to report a history of antibiotic use in the past 6 months; however, this was not statistically significant in multivariate analysis. Similarly to our findings, Hidron et al. reported that risk factors for MRSA colonization at the time of admission to an urban Atlanta hospital included antimicrobial use, a history of hospitalization, and a diagnosis of SSTI (18). Gorwitz et al. also recently reported that among men, recent health care exposure was associated with MRSA colonization (17). In contrast to our study, a recent meta-analysis demonstrated that previous antimicrobial exposure was a risk factor for MRSA colonization (40). Notably, several factors that have been postulated to increase the risk for MRSA colonization, namely the sharing of towels and soap and a history of contact sports, were not associated with MRSA colonization in our investigation (20, 33).

A question raised by our study is that if USA300 is more virulent than other pulsed-field types, why is it not able to handily outcompete these other strains in the anterior nares? In this population, roughly one-half of participants were colonized with MRSA pulsed-field types other than USA300, yet these non-USA300 pulsed-field types were responsible for only one of the observed skin and soft-tissue abscesses. There are several possible explanations for this discrepancy. First, nasal colonization with USA300 may be transient, or the time between colonization and infection may be brief. Our sampling technique may have been insufficient to detect all colonization, and the limited sampling in our investigation could fail to identify this dynamic intermittent carrier state (44). Nevertheless, we were able to find that 11 of the 39 participants who developed USA300 MRSA abscesses were colonized with the same strain at either the initial or terminal anterior nares culture. Second, it also is possible that USA300 is simply not a persistent nasal colonizer or that it preferentially colonizes other anatomic sites. For example, Kazakova et al. found no nasal colonization during an outbreak investigation of USA300 abscesses among professional football players (20). Additionally, extranasal sites of colonization and environmental exposures may serve as important reservoirs for USA300 (27).

Our noting a wide variety of MRSA strains in our study population is consistent with previous observations. Coombs et al. reported 22 different CA-MRSA clones circulating in Western Australia, which builds on similar findings in that nation (3, 4). Similarly to our findings, Hidron et al. reported 22 different CA-MRSA clones circulating in Western Australia, which builds on similar findings in that nation (3, 4). Gorwitz et al. reported 22 different CA-MRSA clones circulating in Western Australia, which builds on similar findings in that nation (3, 4).

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