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Evaluation for a Novel Methicillin Resistance (*mecC*) Homologue in Methicillin-Resistant *Staphylococcus aureus* Isolates Obtained from Injured Military Personnel

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A total of 102 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected from 50 injured service members (June 2009 to December 2011) at U.S. military treatment facilities were analyzed for the conventional *mecA* gene and *mecC* homologue by using standard PCR-based methods. The prevalence of the *mecC* homologue was zero.

Management and prevention of infections due to multidrug-resistant organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), are critical components of combat care. Among deployed military personnel, skin and soft tissue infections due to MRSA are widespread, resulting in significant morbidity and affecting the operational readiness of the troops (1–3). The control and prevention of MRSA infections require reliable identification of the organism and subsequent isolation of infected/colonized individuals (4). Rapid screening for MRSA using PCR-based assays has led to faster detection of MRSA and, consequently, earlier isolation of patients colonized with MRSA (5). Hence, some major U.S. military treatment facilities (MTFs), including Walter Reed National Military Medical Center (WRNMMC) and San Antonio Military Medical Center (SAMMC), have switched from standard culture techniques to the use of the Xpert MRSA PCR assay (Cepheid) for identifying MRSA colonization. Injured service members are either screened at hospital admission (WRNMMC) or upon admission to the intensive care unit (SAMMC) for nasal MRSA carriage using the Xpert MRSA assay.

The Xpert MRSA assay is a real-time PCR assay that detects the staphylococcal cassette chromosome *mec* element (SCC*mec*)-*orfX* junction. The SCC*mec* is a transposon-encoded genetic region that carries the *mecA* gene. The *mecA* gene confers methicillin resistance among *S. aureus* isolates (6) by encoding an altered penicillin binding protein (PBP), specifically PBP2a, that confers reduced affinity to all β-lactams. In recent years, however, a novel MRSA isolate carrying a novel *mecA* gene (*mecC* or $mecA_{LGA\ 251}$) has been identified (7). While MRSA isolates with this novel gene are phenotypically PBP2a positive, they are not detected by the Xpert (Cepheid) assay used for the identification of MRSA (8). Therefore, the exclusive use of the Xpert MRSA assay (Cepheid) method to screen injured service members for MRSA carriage could result in the misclassification of an *S. aureus* isolate as susceptible to methicillin, when it actually is not, thereby raising questions about the adequacy of the current infection control policies at two of the major U.S. MTFs. Thus, to evaluate the appropriateness of our current infection control measures, we examined all MRSA isolates collected from U.S. service members injured during deployment and transitioned through Germany for the presence of the novel *mecA* homologue.

Since June 2009, U.S. military personnel injured during deployment to Iraq and Afghanistan and admitted to a participating U.S. MTF (WRNMMC and SAMMC), after receiving initial care at Landstuhl Regional Medical Center (Germany), have had their data (e.g., demographics, trauma history, inpatient care, and clinical outcome) captured through a trauma registry with a supplemental infectious disease module. As part of the registry, a repository of bacterial isolates has been established. Using standard PCR-based methods and supplies (5 PRIME MasterMix; 5 PRIME Inc., Maryland), both colonizing and infecting MRSA isolates stored in the bacterial repository were analyzed for the conventional *mecA* gene and the novel homologue (*mecC*). Primers, validated in a prior analysis (9), for the traditional *mecA* gene and the homologue *mecC* were run in separate thermocycle reactions (Bio-Rad iCycler; Bio-Rad, California) for each isolate in duplicate. Thermocycle conditions included 95°C for 2 min, followed by 30 cycles of 30 s at 94°C, a cycle at the annealing temperature (60°C and 50°C for *mecA* and *mecC*, respectively) for 30 s, another 30-s cycle at 72°C, and a final extension for 4 min at 72°C. The resulting PCR products were run on 2% agarose gels at 150 V for 1 h and stained with ethidium bromide. Representative strains, USA300 and 10-001051 sequence type (ST) 130, were used as positive controls for *mecA* and *mecC*, respectively. Reactions yielded PCR fragments of 776 kb for *mecA* and 304 kb for the novel homologue.

A total of 364 *S. aureus* isolates were collected in the repository from June 2009 through December 2011, of which 102 (28%) isolates were identified as MRSA by using standard culture. The strains were isolated from 50 injured service members who were predominantly young (median age of 25.1 years [interquartile range, 22.7 to 30.4]) enlisted (76%) males (98%) serving in the Marine Corp (28%) or the Army (62%) (Table 1). These demographic characteristics are reflective of injured service members being hospitalized at the U.S. MTFs; however, the cohort was

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TABLE 1 Baseline characteristics of U.S. military personnel subjects with information on the source of the MRSA isolates

Baseline characteristic[a]	Value ($n = 50$ patients)
No. (%) of males	49 (98)
Median age in yrs (IQR)	25.1 (22.7–30.4)
Branch of service [no. of subjects (%)]	
Marine	14 (28)
Army	31 (62)
Navy	1 (2)
Missing branch	3 (6)
Rank [no. of subjects (%)]	
Enlisted	38 (76)
Officer	4 (8)
Warrant	3 (6)
Median injury severity score (IQR)	21.0 (18.0–26.0)
Theater of operation [no. of subjects (%)]	
Operation Iraqi Freedom (Iraq)	5 (10)
Operation Enduring Freedom (Afghanistan)	44 (88)
Missing	1 (2)
Sources of the isolates (%)	
Wounds	42
Nares	17
Respiratory (includes tracheal aspirate, sputum, and BAL fluid)	16
Groin	9
Not specified/other	8
Bone	4
Urine	1
Catheter related	1
Bloodstream	1
Intra-abdominal	1

[a] IQR, interquartile range; BAL, bronchoalveolar lavage.

comprised of more severely injured individuals (median injury severity score was 21 [interquartile range, 18 to 26]). Of the 102 MRSA isolates, 26% were classified as colonizing and were isolates obtained either from the groin (9%) or nares (17%), while 74% were classified as infectious and collected primarily from wounds (42%) or a respiratory source (16%). The conventional *mecA* gene was identified in 100% of the MRSA isolates analyzed, while PCR analysis resulted in zero prevalence (exact 95% confidence interval, 0 to 0.036) of the novel *mecC* homologue.

This represents the first study to analyze MRSA isolates collected from a population of injured military personnel for the novel MRSA homologue (*mecC*). Currently, MRSA isolates carrying *mecC* have been reported primarily from European countries, including Germany (7–9). To our knowledge, there have been no reports of the homologue from the U.S. While MRSA isolates carrying *mecC* were first isolated from ruminants, they have now been identified as a cause of human infections (7–9). Despite the medical evacuation of service members to Germany before being transferred to a U.S. MTF, no isolates with the novel *mecA* homologue were detected in this study.

One of the primary objectives of this cross-sectional analysis was to evaluate the adequacy of the current infection control policy implemented at major U.S. MTFs. Our results support the continued use of the Xpert (Cepheid) assay for the identification of individuals colonized with MRSA. The study is limited by the relatively small sample size. Furthermore, only about one-third of our isolates were classified as colonizing. While isolates carrying the novel gene (*mecC*) were rare prior to 2003, their frequency appears to be increasing (10). Therefore, ongoing surveillance for MRSA isolates with the *mecC* homologue among U.S. military personnel should continue, especially given their frequent downrange exposure to livestock.

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