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SHORT REPORT
Methicillin-resistant *Staphylococcus aureus* infection in combat support hospitals in three regions of Iraq

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SUMMARY

Staphylococcus aureus is a leading cause of infections in deployed service members. Based on a molecular epidemiological study of 182 MRSA isolates from patients in three U.S. Army combat support hospitals in separate regions in Iraq, USA300 clone was the most predominant (80%) pulsotype. This finding suggested that strain carriage from the home country by military personnel is epidemiologically more important than local acquisition.

Key words: CA-MRSA, infection, Iraq.

Multidrug resistance in bacterial pathogens is a major public health challenge worldwide. These organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) infections in military treatment facilities have complicated the care of combat casualties of coalition forces serving in support of Operation Iraqi Freedom (OIF) [1]. About half of antimicrobial-resistant isolates from patients in the U.S. combat support hospitals (CSHs) in Iraq were MRSA in recent years [2], and this led us to investigate the molecular epidemiological significance of these MRSA isolates.

MRSA have been classified as being either hospital-associated (HA) or community-acquired (CA) [3]. In contrast to HA-MRSA, CA-MRSA infections typically occur in young individuals without prior exposure to healthcare institutions within 48 h of hospital admission, or from persons who have not been hospitalized within at least 1 year before the date of MRSA isolation [4]. Recently, CA-MRSA has emerged as a significant pathogen in the USA and Europe [5]. Whether this is also true at CSHs in Iraq has not been reported. Methicillin resistance in *S. aureus* is mediated by the *mec*A gene located in the staphylococcal chromosomal cassette (SCC*mec*) of which there are six types identified [6]. HA-MRSA are typically resistant to multiple antibiotics and tend to carry SCC*mec* I, II and III while CA-MRSA strains carry SCC*mec* IV or V complex, and a *S. aureus*-specific exotoxin, Panton–Valentine leukocidin (PVL) gene [3, 7]. Ten major MRSA pulsotypes identified on the North America continent have been designated as USA100, 200, 300, etc. to USA1100 using pulsed-field gel electrophoresis (PFGE) analysis [8]. Of these,

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USA300 and 400 have been primarily associated with CA-MRSA infections [3, 4].

In order to understand the origin of the specific group of MRSA bacteria isolated from U.S. service members from CSHs in Iraq, molecular characterization including PFGE typing, SCC*mec* typing and PVL identification was conducted.

From November 2007 to March 2009, 182 MRSA isolates were submitted from three U.S. Army CSHs located at Ibn Sina (IS), Camp Cropper (CO) and Al Asad (AA) in Iraq and sent to the Walter Reed Army Institute of Research (WRAIR) for molecular characterization. Phenotypic testing including biotyping and antimicrobial susceptibility testing (AST), was performed using the Dade Behring Microscan (Dade Behring Microscan Inc., USA) as outlined by the Clinical and Laboratory Standards Institute (CLSI Standards M7 and M100) before shipment to WRAIR. Basic epidemiological information such as isolation date and infection sources was recorded at patients' primary visit. All MRSA isolates received were genotypically characterized by PFGE as described previously and pulsotype patterns were grouped according to the classification system described previously [8]. Isolates with $\geqslant 80\%$ similarity were considered to be of the same pulsotype using the Dice coefficient with BioNumerics software 6.1 (Applied Maths, Belgium). PFGE electronic images of ten CDC representative MRSA isolates of USA100 to USA 1100 were used for comparison with PFGE patterns of MRSA samples isolated from the CSHs in Iraq. SCC*mec* typing was conducted by a multiplex PCR with six pairs of primers targeting the *mec* gene complex and *ccr* gene complex as described previously [6]. PVL gene identification was also conducted by PCR as described previously [7].

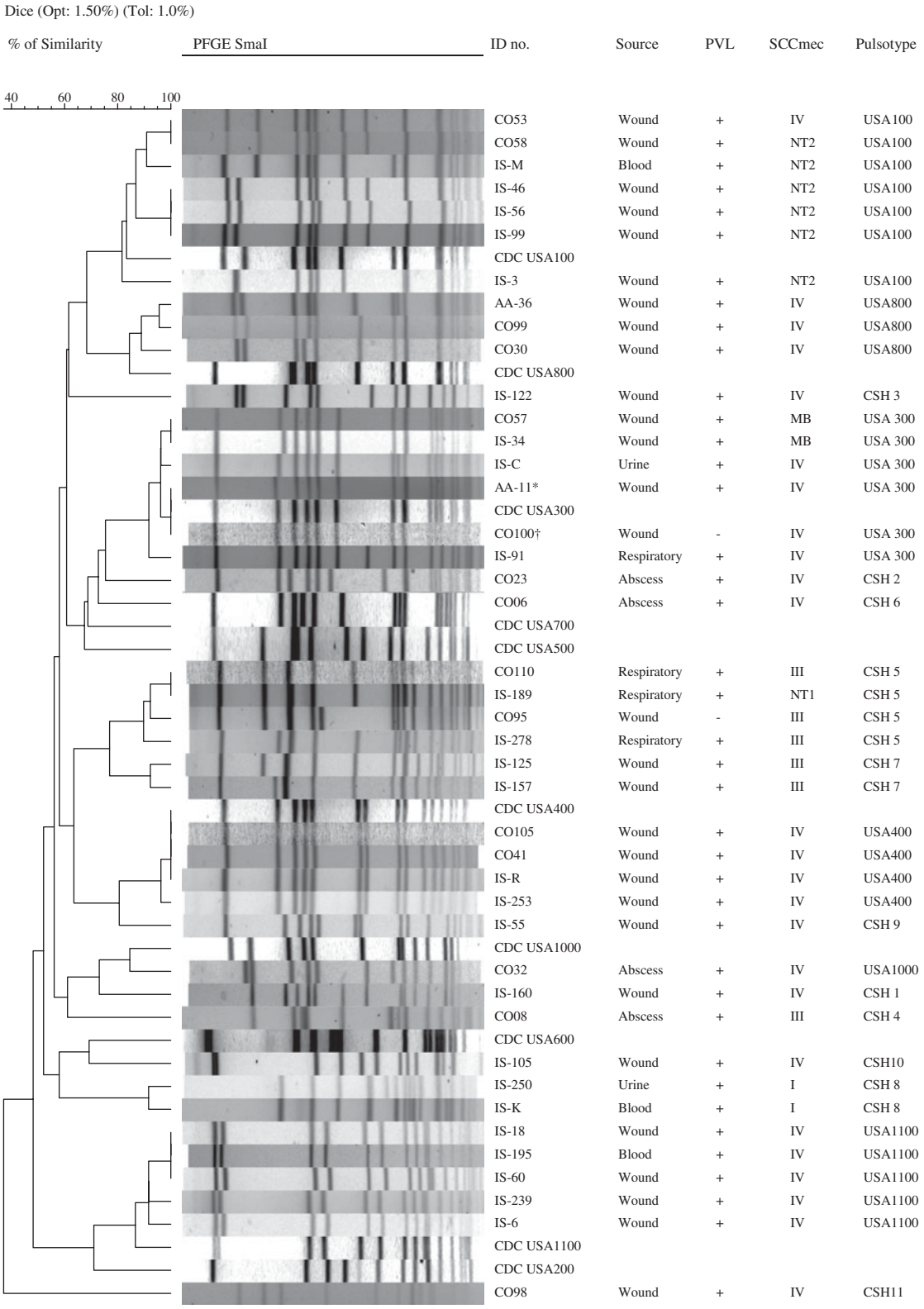
The epidemiological information and genotyping results from this study are summarized in Figure 1. Three hospitals in Ibn Sina, Camp Cropper and Al Asad, in Iraq contributed 81, 78 and 23 MRSA isolates, respectively. About 95% (172/182) of the isolates were from wounds and abscesses; the remainder comprised blood (3), respiratory (4), urine (2) and other sources (1). Seventeen different pulsotypes were identified; 80% (146/182) were typed as USA300, and other types identified were USA100 (7 strains), USA400 (4), USA800 (3), USA1000 (1) and USA1100 (5). The remaining 16 strains (10%) displayed 11 unique pulsotypes not represented in the CDC database [8] and these were tentatively designated as CSH1–CSH11 (Fig. 1).

SCC*mec* typing revealed that all of the strains of pulsotypes USA400, USA800, USA1000, USA1100, CSH1, 2, 3, 6, 9, 10, and 11 isolates as well as 98% of USA300 isolates were of SCC*mec* IV. The non-SCC*mec* IV isolates were distributed in pulsotype US100 and CSH4, 5, 7 and 8. All CSH4, CSH7 and three of four CSH5 isolates were identified as SCC*mec* III and two CSH8 isolates as SCC*mec* I. The remaining CSH5 strain and six of seven USA100 strains could not be grouped with the primers used [6] and they were tentatively designated as SCC*mec* NT (new type) 1 and NT2, respectively (Fig. 1). Ninety-six percent (177/182) of the isolates carried the *PVL* gene, four of the five PVL-negative isolates were of pulsotype USA300 and the fifth was of tentative pulsotype CSH5.

Three of four respiratory isolates were pulsotype CSH5, two of them of SCC*mec* III and one of SCC*mec* NT1. However, one respiratory isolate typed as USA300 and SCC*mec* IV. One isolate from blood was of pulsotype USA1100, SCC*mec* IV while another blood isolate typed as CSH8 and SCC*mec* I as did one of the urine isolates (Fig. 1).

AST results showed that 0–10% of isolates were susceptible to various β-lactam antibiotics and 10% were susceptible to erythromycin. Susceptibility to non-β-lactams was as follows: gentamicin 87%, linezolid 96%, nitrofurantoin 100%, rifampin 95%, trimethoprim–sulfamethoxazole 91%, tetracycline 91% and vancomycin 100%; 77% were susceptible to clindamycin.

A unique feature of this study is that it examined MRSA isolates from patients being treated at one of three hospitals in Iraq rather than isolates from patients following evacuation to either Germany or the USA. The majority (80%) of the isolates typed as USA300, SCC*mec* IV and carried the PVL gene, which are typical features of CA-MRSA [5]. Most were resistant to β-lactam antibiotics, but susceptible to non-β-lactams which is consistent for CA-MRSA described in other studies [4]. USA patients seen at CSHs are typically not held as in-patients, but rather are treated and released, or evacuated to a higher echelon of care. This fact coupled with the phenotypic and genotypic characteristics of the isolates suggest that CA-MRSA was the major cause of wound and other MRSA infections in this study. The identification of the same pulsotype from separate CSHs located in different regions in Iraq provided epidemiological significance, implying the same genetic origin of MRSA infection. Several distinct genetic



ID no.	Source	PVL	SCCmec	Pulsotype
CO53	Wound	+	IV	USA100
CO58	Wound	+	NT2	USA100
IS-M	Blood	+	NT2	USA100
IS-46	Wound	+	NT2	USA100
IS-56	Wound	+	NT2	USA100
IS-99	Wound	+	NT2	USA100
CDC USA100				
IS-3	Wound	+	NT2	USA100
AA-36	Wound	+	IV	USA800
CO99	Wound	+	IV	USA800
CO30	Wound	+	IV	USA800
CDC USA800				
IS-122	Wound	+	IV	CSH 3
CO57	Wound	+	MB	USA 300
IS-34	Wound	+	MB	USA 300
IS-C	Urine	+	IV	USA 300
AA-11*	Wound	+	IV	USA 300
CDC USA300				
CO100†	Wound	-	IV	USA 300
IS-91	Respiratory	+	IV	USA 300
CO23	Abscess	+	IV	CSH 2
CO06	Abscess	+	IV	CSH 6
CDC USA700				
CDC USA500				
CO110	Respiratory	+	III	CSH 5
IS-189	Respiratory	+	NT1	CSH 5
CO95	Wound	-	III	CSH 5
IS-278	Respiratory	+	III	CSH 5
IS-125	Wound	+	III	CSH 7
IS-157	Wound	+	III	CSH 7
CDC USA400				
CO105	Wound	+	IV	USA400
CO41	Wound	+	IV	USA400
IS-R	Wound	+	IV	USA400
IS-253	Wound	+	IV	USA400
IS-55	Wound	+	IV	CSH 9
CDC USA1000				
CO32	Abscess	+	IV	USA1000
IS-160	Wound	+	IV	CSH 1
CO08	Abscess	+	III	CSH 4
CDC USA600				
IS-105	Wound	+	IV	CSH10
IS-250	Urine	+	I	CSH 8
IS-K	Blood	+	I	CSH 8
IS-18	Wound	+	IV	USA1100
IS-195	Blood	+	IV	USA1100
IS-60	Wound	+	IV	USA1100
IS-239	Wound	+	IV	USA1100
IS-6	Wound	+	IV	USA1100
CDC USA1100				
CDC USA200				
CO98	Wound	+	IV	CSH11

Fig. 1. Dendrogram of representative pulsotype of 182 MRSA isoates, in which each MRSA pulsotype was compared with sample isolation source, SCC*mec* type and PVL identification. * Represents 137 MRSA isolates from CSHs in Ibn Sina (IS), Cropper (CO) and Al Alad (AA) characterized as USA300, SCC*mec* IV and PVL+. † Represents four MRSA isolates characterized as USA300, SCC*mec*IV and PVL−. SCC*mec* NT1 and NT2 represent two untypable SCC*mec* patterns. MB, Only displays a *mec*A band by the PCR test. Pulsotype of USA100–USA1100 was compared with all samples.

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lineages, defined by PFGE and multi-locus sequence typing (MLST), have been associated with CA-MRSA in different continents [5]. However, dissemination of the USA300 clone of MRSA in Europe and other continents has been reported through travel and healthcare personnel exchange [9, 10]. A recent study found about 4% of tested soldiers at a training facility in the USA carried MRSA; 53% were USA300 and 34% were USA800 clones. SCC*mec* IV dominated in both pulsotypes but the PVL gene was most common in USA300 [11]. Our data and previous studies suggest that some soldiers may be colonized with CA-MRSA before deployment and subsequently cross-infect fellow soldiers during deployment due to confined living conditions and limited opportunities for personal hygiene. CA-MRSA may then cause wound infections and abscesses when the subjects are injured or host immunity reduced.

We found that MRSA strains from most non-wound infections (respiratory and blood) were non-USA300 pulsotype or of unique pulsotypes [8] and non-SCC*mec* IV or untypable SCC*mec* MRSA strains were mainly associated with these isolates. These isolates may be HA-MRSA or CA-MRSA acquired locally.

In conclusion, we have shown that genetic lineages associated with CA-MRSA infection may have played an important role in wound infection in the CSHs in Iraq. The predominance of the USA300 clone, SCC*mec* IV and PVL gene positivity support this view. CA-MRSA carried by subjects from the USA may be the major cause for MRSA wound infections in the battle theatre and this study therefore provides important epidemiological information relevant to the development of preventive strategies for MRSA control for U.S. service members in Iraq and the public communities.

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DECLARATION OF INTEREST

None.

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