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An Exploratory Study of Methicillin-Resistant *Staphylococcus aureus* and SCC*mec* Elements Obtained from a Community Setting Along the Texas Border with Mexico

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Abstract

An exploratory study of methicillin-resistant *Staphylococcus aureus* (MRSA) and SCC*mec* elements in bacteria along the Mexican border of south Texas was performed. Between September and December of 2008, 375 swabs of anterior nares were self-collected by students attending the University of Texas-Pan American (UTPA) and cultured for MRSA. Fifty seven bacterial isolates were kept for further analysis that included suspected MRSA and other SCC*mec*-containing bacteria. Isolates were examined for the presence of *nuc*, *mec*A, *lukS*-PV, and *spa* genes using PCR. SCC*mec* and *spa* typing were also performed. Seven *S. aureus* isolates were found of which six were classified as MRSA. SCC*mec* typing showed five of the six MRSA strains to be type IV, while one MRSA strain, and most of the non-*S. aureus* strains, were untypeable, producing results that were indicative of mixed SCC*mec* types. Five of the six MRSA strains contained known *spa* types (two of which corresponded to USA300 and one to USA600), while one strain had a novel *spa* type. Only one isolate, a USA300 MRSA, was positive for *lukS*-PV. Easy access by the Texas border community to antibiotics in Mexico without a prescription, and the strong partition in SCC*mec* types between MRSA and non-*S. aureus* bacteria suggest that this border region of Texas may be uniquely suited for the study of emerging SCC*mec* types, their horizontal transfer, and perhaps other aspects of antibiotic resistance in bacteria.

Introduction

Staphylococcus aureus is a bacterium that is commonly isolated from the anterior nares of humans where it is considered a natural inhabitant. Pathogenically, *S. aureus* is characterized as not normally causing disease in man except when an ‘opportunity’, such as a cut or lowered immune system, presents itself, in which case the infection can normally be treated successfully with antibiotics. However, some strains of *S. aureus* have been shown to be more highly resistant to antibiotics, which makes infection difficult to treat and the strains more serious pathogens. Increased resistance to antibiotics has been shown to be due in part to the presence of a genomically located mobile DNA element referred to as the Staphylococcus Cassette Chromosome, which contains the *mec*A gene (SCC*mec*) [12]. The *mec*A gene product is a modified penicillin-binding-protein (PBP2a), which confers resistance to the large group of

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beta-lactam antibiotics, such as methicillin [21]. SCC*mec* also carries a gene coding for a recombinase that facilitates the movement of SCC*mec* to other bacteria that do not have the element. In some bacteria SCC*mec* has been found to also contain plasmids or other mobile DNA elements carrying diverse antibiotic resistance genes, thus expanding the range of antibiotic resistance beyond beta-lactams. When a strain of *S. aureus* acquires a copy of SCC*mec*, the bacterium is referred to as a methicillin-resistant *S. aureus*, or MRSA for short. Historically, MRSA have been associated primarily with health care facilities, such as hospitals, where antibiotics are commonly used and where SCC*mec* presumably has given *S. aureus* a fitness advantage. These strains of MRSA are referred to as healthcare-associated MRSA (HA-MRSA). More recently however, strains of MRSA have been isolated from apparently healthy individuals residing in the community, who had no apparent link to the healthcare system, and are referred to as community-associated MRSA (CA-MRSA) [6]. CA-MRSA are generally characterized as demonstrating less antibiotic resistance but more virulence due to the presence of virulence factors such as the Panton-Valentine leukocidin (PVL), a cytotoxin associated with disease [10]. For both healthcare and scientific reasons, there is much interest in understanding the level of diversity within the SCC*mec* element, and the geographic range of both the different strains of MRSA and the SCC*mec* elements they possess. Useful to such studies are a number of bacterial-typing methods that have been developed for both MRSA and SCC*mec*. In particular, sequence analysis of the different SCC*mec* elements has shown that most MRSA can be grouped into at least one of five established SCC*mec* types, where types I through III are typical of HA-MRSA and types IV and V of community-associated strains [1]. More recently, SCC*mec* elements of types VI [15], VII [9], and VIII [22] have been proposed, along with reports of untypeable elements, raising the question of even more SCC*mec* types, and the need of a more standardized and comprehensive system for SCC*mec* typing [3]. Similarly, it has been shown that the number and sequence of DNA repeats within the Short Sequence Repeat (SSR) region of the *spa* gene in *S. aureus* can be used to show phylogenetic relationships between different MRSA isolates [19].

Although MRSA and the SCC*mec* elements have been studied throughout the United States and the world, relatively little work has been undertaken along the U.S. border with Mexico. The Texas/Mexico border region represents an environment largely unique from the rest of the United States that is characterized by relatively high poverty, a largely Hispanic population, and relatively easy access by U.S. and Mexican citizens to antibiotics without a prescription [2,17]. It is of public health and scientific importance, therefore, to determine if MRSA/SCC*mec* in the border region is evolving differently than elsewhere in the United States, and whether the uniquely transitional environment of the border region may promote the emergence of new, more serious strains of MRSA that have the potential to spread throughout the rest of the United States. To date, limited studies have been undertaken to study MRSA/SCC*mec* along the U.S./Mexico border, and those that have, have focused on strains obtained from a hospital setting [5,14,17]. Thus very little is currently known about MRSA, and their SCC*mec* elements, in communities located along the Texas border with Mexico. Herein, we report on an exploratory study of MRSA and SCC*mec* in a Texas border community in order to provide needed baseline data on MRSA and SCC*mec* elements in this important transitional region.

Materials and Methods

Sample Collection and Culture

Between June and December of 2008, 375 swabs of the anterior nares were self-collected by student volunteers attending the University of Texas-Pan American (UTPA), located along the Texas border with Mexico. The student population at UTPA is comprised primarily of

individuals of Hispanic descent living along the Texas middle Rio Grande Valley and across the border in Mexico. The study was approved by the UTPA Institutional Review Board and informed consent by students volunteering samples was obtained. Swabs were returned to their paper wrappers after collection then immediately taken to the laboratory and placed in 5 ml of Iso-Sensitest broth (Oxoid Ltd., Basingstoke, Hampshire, England) supplemented with 2.3% NaCl, 4 μg/ml cefoxitin, 8 μg/ml colistin, 8 μg/ml of aztreonam [8]. Inoculated broths were incubated for 48 h at 33–35°C, then cultured on Mannitol Salt Agar (MSA, Accumedia Manufacturing Inc., Lansing, Michigan) for 24 h. Colonies showing a yellow to gold color characteristic of *S. aureus*, as well as representatives of other diverse colony phenotypes, were purified and frozen at −80°C in nutrient broth containing 20% glycerol. Students also returned with the sample a questionnaire in which they identified whether they had stayed or worked in a hospital within the last 2 years.

Template Preparation

MSA plates were inoculated with strains frozen at −80°C and cultured overnight at 33–35°C. An aliquot of cells was suspended in 100 μl of water, heated at 100°C for 10 min, then frozen at −20°C until use. 1 μl was used as template in PCR reactions.

Spa Typing

Spa typing of the *spa* gene's short sequence repeat (SSR) region was performed according to Shopsin et al. [19], using primers spa1 (5′-AAGACGATCCTTCGGTGAG-3′) and 1517r. Sequencing reactions were performed using GenomeLab™ DTCS *Quick Start* Kit for Dye Terminator Cycle *Sequencing* (Beckman Coulter, Inc. Fullerton, CA) and were resolved on a Beckman Coulter CEQ 8800 sequencing machine. *spa* sequences were determined from both strands of the SSR region. *spa* repeat sequence type and nomenclature were determined utilizing the Ridom utilities and nomenclature available at http://spaserver.ridom.de/.

SCC*mec* Typing

SCC*mec* typing for types I through V was performed according to Boye et al. [1]. Control strains for each of the five SCC*mec* types (I–V), a gift from the Centers for Disease Control, were used both as positive controls and to act as DNA markers for interpreting the results of the UTPA isolates.

PCR Detection of *nuc*, *mecA*, and *lukS*-PV Genes

The PCR method of Reischl et al. [16], was used for the detection of Panton-Valentine leukocidin-positive strains by targeting the *lukS*-PV gene. The *nuc* and *mecA* genes (indicative respectively of *S. aureus* and resistance to beta-lactam antibiotics) were detected using real-time PCR utilizing the previously reported primers nuc1 and nuc2 [7] and LTmecAF and LTmecAR [20].

Bacterial Identification

Bacterial identification was performed to the genus level for isolates presented in Table 1 using MicroScan's POS COMBO 20 dried gram-positive panels, and results interpreted using the Micro Scan autoSCAN-4 system (Dade MicroScan Inc., West Sacramento, CA).

Antibiotic Sensitivity Testing

Antibiotic sensitivity was performed on Mueller Hinton agar according to CLSI recommendations for *Staphylococcus* species (Performance standards for antimicrobial susceptibility testing: Seventeenth Informational supplement. CLSI document M100-S17 (ISBN 1-56238-625-5). Clinical and Laboratory Standards Institute, 940 West Valley Road,

Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007). Antibiotics tested were cefoxitin, gentamicin, vancomycin, trimethoprin-sulfamethoxazole, erythromycin, ciprofloxacin, chloramphenicol, and rifampicin.

Results

Fifty seven bacteria were isolated from 375 nasal swabs. Characterization of a sub-set of these isolates is presented in Table 1, all of which were shown to be *Staphylococcus* species. Seven of the 57 bacteria tested positive for both the *nuc* and *spa* genes, and were considered to be *S. aureus*. There was complete agreement between the *nuc*/*spa* tests and no cases existed where an isolate tested positive for *nuc* and not *spa*, or visa versa. Of the seven *S. aureus* isolates, six of them were found to posses the *mecA* gene and were considered to be MRSA.

Control strains for SCC*mec* types I–V all typed correctly and confirmed the proper functioning of the test, which identified a bacterial landscape surprisingly rich in different SCC*mec* types. Five of the six MRSA strains were shown to be of type IV, characteristic of community-associated strains. One of the MRSA strains, #28, was untypeable, giving results characteristic of mixed SCC*mec* types (Table 1 and Fig. 1). SCC*mec* type IV, and novel-typing patterns indicative of mixed SCC*mec* types, were common among the non-*S. aureus* strains examined (Table 2). Eight strains did not produce amplification products during SCC*mec* typing, of which two were *mecA*-positive by PCR.

Spa sequence typing identified two strains as USA300, commonly identified with CA-MRSA, along with a USA600 strain. One isolate, #90, was found to contain a novel *spa* type not present in the *spa* database at http://spaserver.ridom.de/, (Table 1).

Only one isolate (#38), a USA 300 MRSA strain, was positive for the *lukS*-PV gene indicating the potential to produce the Panton-Valentine leucocidin cytotoxin.

Antibiotic resistance profiles were determined for both the *S. aureus* and a subset of non-*S. aureus* isolates (Table 1).

Discussion

MRSA, through acquisition of the mobile SCC*mec* element, acquires genes that confer resistance to a wide range of antibiotics. In areas with higher antibiotic use, such as healthcare facilities, MRSA have been shown to acquire different types of SCC*mec* elements that confer an increased level of antibiotic resistance, as compared to elements associated with a community setting. Communities along the Texas border with Mexico have access to antibiotics in Mexico without a prescription, thus presumably creating an atypical community environment with higher than normal levels of antibiotic use, and perhaps misuse. This exploratory study sought to provide an initial characterization of MRSA and SCC*mec* elements in this environment since such data are currently lacking. Samples were collected from a community environment among presumably healthy individuals where the predominant SCC*mec* type is type IV, [13]. It was therefore not surprising that five of the six MRSA isolated in this study were SCC*mec* type IV. In fact, even among non-*S. aureus* staphylococcal strains, SCC*mec* type IV was the most common of the five SCC*mec* types examined (Table 2). One of the predominant type IV strains of MRSA in the U.S. is the USA300 strain, [13], and two of the six strains did possess the *spa* type associated with this strain. However only one of these two strains contained the *lukS*-PV gene, suggesting that the two USA300 strains might not be closely related.

Only the two USA300 strains gave the same *spa* type, suggesting a relatively high level of diversity among the MRSA isolates and demonstrating the absence of a predominant strain of

MRSA among the isolates collected in this study. Similarly, only one strain, #90, gave a *spa* type not present in the database of *spa* types used in this study, suggesting that the study area was not enriched with novel strains of MRSA. However the study was limited in scope and it is possible that an examination of a larger number of MRSA strains could reveal a predominant strain(s) for the region.

Characteristic of bacteria with SCC*mec* type IV elements associated with CA-MRSA strains, most of the MRSA strains (four out of six) were resistant primarily to beta-lactam antibiotics (Table 1). However two of these strains were found to contain a *mec*A gene but were not resistant to cefoxitin. A possible explanation for this apparent contradiction is that the strains may express heterogeneus resistance to beta-lactams, which is known to occur with some MRSA strains [18]. Among the non-beta-lactam antibiotics tested, resistance to erythromycin was relatively more prevalent, the significance of which is not clear, but might reflect its use in the region. It is also interesting that, like HA-MRSA, the non-*S. aureus* staphylococcal strains examined generally showed resistance to more types and numbers of antibiotics than the CA-MRSA strains, including resistance to three or four different classes of antibiotics (Table 1). However, although intriguing, it is not yet known if the increased resistance observed among the non-*S. aureus* strains is located on the SCC*mec* element. If the increased resistance to antibiotics observed in the non-*S. aureus* staphylococcal strains is found to be located on the SCC*mec* element, these bacteria may act as a source of antibiotic resistance for *S. aureus* via the SCCmec element, and would have to be viewed as an integral part of the problem MRSA represents to the healthcare system. It is noteworthy that none of the isolates were resistant to vancomycin, a concern with multi-drug resistance among MRSA.

The high level of genetic diversity observed among the SCC*mec* elements is particularly interesting (Table 2). Although the *S. aureus* strains were predominately of SCC*mec* type IV, the SCC*mec* elements in non-*S. aureus* staphylococci were largely untypeable, giving results consistent with a combination of the five established SCC*mec* types (Fig. 1). There is at least one other report where untypeable SCC*mec* elements, from *Staphylococcus epidermidis* in Finland, have been reported to appear as combinations of established SCC*mec* types [11]. These results suggest that the nasal-derived MRSA are co-existing with other *staphylococcal* species that contain different, and predominantly atypical, SCC*mec* elements. This apparent partitioning of SCC*mec* types between *S. aureus* and other staphylococcal species co-habiting the anterior nares raises obvious questions regarding the role of these non-*S. aureus* species in the emergence of SCC*mec* elements in *S. aureus*.

Undoubtedly, to eventually understand the dynamics of SCC*mec* and antibiotic resistance in staphylococcal populations, it will be necessary to understand the dynamics of emerging resistance, including horizontal transfer and fixation of diverse SCC*mec* elements among the staphylococci [4]. In this regard, the pronounced partitioning of SCC*mec* types between *S. aureus* and other *Staphylococcus* species may represent a useful system of study to answer such questions. In addition, the transitional border environment of south Texas, with its easy access to antibiotics without a prescription, presents itself as a potentially unique and advantageous region through which to perform such studies.

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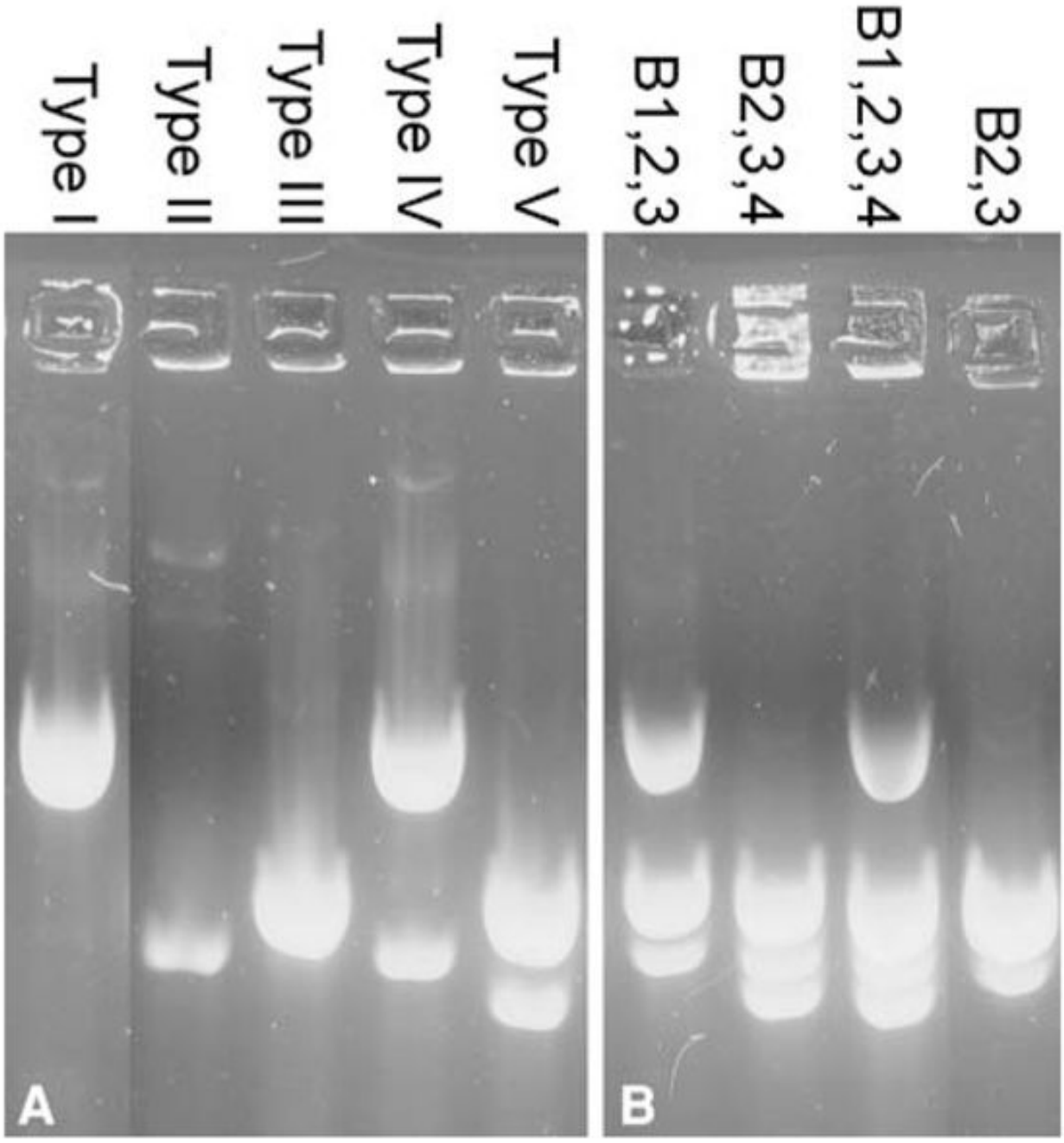

Fig. 1.

A composite image of different lanes from a 2% agarose gel showing **a** the five expected SCC*mec* types (Types I–V) obtained from the control strains and **b** examples of four other untypeable SCC*mec* types demonstrating a mixed pattern. The nomenclature for the untypeable SCC*mec* patterns is “B” for band, followed by which of the four possible bands are present, where band #1 is at the *top* of the gel and band#4 is at the *bottom*

Table 1

Summary table of *S. aureus* and a sub-set of non-*S. aureus* gram-positive staphylococci

Isolate #	N/M[a]	SCC*mec*	Spa Type[b]	Antibiotic resistance[c]
28[d–e]	n/m	B1,2,3[g]	t741	None
38[f–d–e]	n/m	Type IV	t008 (USA300)	FOX
51[d]	n/m	Type IV	t008 (USA300)	FOX, E
54[d]	n/m	Type IV	t004 (USA600)	FOX
78[d]	n/m	Type IV	t688	FOX
90[d]	n/m	Type IV	15–12–16–2–16–2–25–25–24[h]	E
19	n	NR	t209	E
21	m	B2,3	NR	FOX, E, STX
22	m	B2,3,4	NR	FOX, E, CIP
25	m	B1,2,3,4	NR	FOX, GM
27	m	B1,2,3	NR	FOX, E
31	m	B1,2,3	NR	E
42	m	NR	NR	FOX, E
43	m	Type I	NR	E
75	NR	Type III	NR	None
84	m	Type IV	NR	FOX, E, STX, CIP
85	m	Type IV	NR	FOX, E,

[a] *n nuc* positive, *m mec*A positive, *NR* neither the *nuc* or *mec*A genes were detected

[b] *NR* No gene(s) were detected by PCR

[c] *E* erythromycin, *C* chloramphenicol, *GM* gentamicin, *FOX* cefoxitin, *STX* Trimethoprim-sulfamethoxazole, *CIP* Ciprofloxacin

[d] Presumptive MRSA

[e] MRSA isolates from individuals that had worked in a healthcare facility within the past 2 years, (none of the MRSA isolates came from individuals that had been admitted to a hospital within the past 2 years)

[f] Only isolate among the 57 examined that was positive for *luk*S-PV

[g] See Fig. 1 for explanation of untypeable nomenclature

[h] Repeat succession given since spa type was not found in the database (novel *spa* type)

Table 2

SCC*mec* types of non-*S. aureus* staphylococci

	Typeable					**Untypeable**					
SCC*mec* types	I	II	III	IV	V	B1,2,3	B1,2,3,4	B2,3	B2,3,4	No bands	Inconclusive
# of isolates	1	0	6	11	0	4	2	3	14	8	1