Investigating a rare methicillin-resistant \textit{Staphylococcus aureus} strain: first description of genome sequencing and molecular characterization of CC15-MRSA

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Purpose: Methicillin resistant \textit{Staphylococcus aureus} CC15 strains (CC15-MRSA) have only been sporadically described in literature. This study was carried out to describe the genetic make-up for this rare MRSA strain.

Methods: Four CC15-MRSA isolates collected in Riyadh, Saudi Arabia, between 2013 and 2014 were studied. Two isolates were from clinical infection and 2 from retail meat products. Whole genome sequencing was carried out using Illumina HiSeq2500 genome analyzer.

Results: All the CC15-MRSA isolates had the multilocus sequence typing profile ST1535, 13–13–1–1–81–11–13, which is a single locus variant of ST15. Of the 6 contigs related to the SCC element, one comprised a recombinase gene \textit{ccrAA}, a \textit{ccrC-PM1} paralog, and a helicase, another one included \textit{mvaS}, \textit{dru}, \textit{mecA} and 1 had \textit{yobV} and \textit{Q4LAG7}. The SCC element had 5 transposase genes, namely 3 identical paralogs of \textit{tnpIS431} and 2 identical paralogs of \textit{tnpIS256}. Two identical copies of a \textit{tnpIS256}-based insertion element flank the \textit{aacA-aphD} gene. Two copies of this insertion element were present with 1 located in the SCC element and another inserted into the \textit{sasc} gene. A short 3 kb region, which lacks any bacteriophage structural genes and site-specific DNA integrase, was inserted into the \textit{hbl} gene. The \textit{hsdM} and the 5'-part of the \textit{hsdS} gene are replaced by a copy of the \textit{hsdM/hsdS} paralogs from \textit{vSa} giving rise to a new chimeric paralog of \textit{hsdS} in \textit{vSaA}.

Conclusion: CC15-MRSA shows a novel SCCmeC-V/SCCfus composite element. Its variant of \textit{hsdM/hsdS} probably facilitated uptake of foreign mobile genetic elements that promoted emergence of CC15-MRSA. Close surveillance is needed to monitor spread and emergence of further CC15 MRSA strains.

Keywords: whole genome sequencing, MRSA, MLST, clonal complex, SCCmec, Saudi Arabia

Introduction

In recent years, the landscape of the molecular epidemiology of methicillin resistant \textit{Staphylococcus aureus} (MRSA) has been characterized by the emergence and dissemination of new strains. Clonal complex 15 (CC15) is ubiquitous and widely described in the literature, but these isolates are mostly methicillin susceptible \textit{S. aureus} (MSSA).\textsuperscript{1} CC15-MSSA was recently identified as a predominant nasal colonizer in a report from Saudi Arabia.\textsuperscript{2} Previously, methicillin resistant CC15 strains (CC15-MRSA) have only been sporadically described in literature.\textsuperscript{3,5} In a large scale genotyping study of MRSA isolates, no CC15-MRSA was identified.\textsuperscript{1} Two isolates of CC15-MRSA associated with nasal colonization have been reported in Iran and Saudi Arabia.\textsuperscript{3,5} While whole genome sequencing data are available for CC15-MSSA, there are, to the best of our knowledge, no publications on the genomic data for the rare CC15-MRSA. Recently, we reported the first
identification of CC15-MRSA from clinical infections and retail meat products in the Middle East.\textsuperscript{6,7} In light of the emergence of CC15-MRSA in our setting and to provide much-needed insight into the genetic make-up of this rare MRSA clone, we have carried out whole genome sequencing of these isolates.

**Materials and methods**

The human isolates were identified as part of a larger MRSA study for which ethical approval was obtained from the Institutional Review Board, King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia. Patient consent was waived as the study involved use of archived isolates from specimens submitted for routine diagnostic tests and without use of patient identifiers. Four CC15-MRSA isolates collected in Riyadh, Saudi Arabia between 2013 and 2014 were studied. Two isolates (RUH-2 and RUH-71) were from patients with sepsis and wound infection, respectively, while the other 2 (RUH-98 and RUH-99) were from retail camel meat. 

*S. aureus* identification and confirmation of methicillin resistance was performed as previously described.\textsuperscript{6,7} Genomic DNA was extracted using Qiagen DNA isolation kit (Qiagen, Hilden, Germany) in accordance with manufacturer’s instructions. Whole genome sequencing was carried out using the Illumina HiSeq2500 genome analyzer.

Sequencing reads were assembled de novo with SPAdes and the final assembly was done with SPAdes version 3.10.1 (http://bioinf.spbau.ru/spades).\textsuperscript{8} Contigs shorter than 500 nt were dropped. Reads were mapped to the SPAdes contigs but also to the reference sequence (ST15-MSSA strain ST20130938, GenBank: CP012972.1) with the Burrow–Wheeler aligner “bwa” using the local aligning algorithm “mem” (“bwa” version 0.7.12-r1039, https://github.com/lh3/bwa).\textsuperscript{10} We also used “bwa-mem” to map the whole SPAdes contigs on the reference sequence (ST15-MSSA strain ST20130938, GenBank: CP012972.1). Read mappings and coverage were visually inspected with “tablet” (“tablet” version 1.14.10.21, https://ics.hutton.ac.uk/tablet/).\textsuperscript{11} We manually scaffolded and annotated the contigs from isolate RUH-2, which cover the genomic islands of *Sa* and *b*, a 3 kb element inserted into the *hlb* gene and the SCC element. We used the GenomeDiagram module from Biopython to draw sketches from the manually annotated sequences.\textsuperscript{12}

The reads and the SPAdes contigs were submitted to NCBI sequence database. The manually scaffolded and annotated regions were submitted to Genbank as short sequences.

**Results**

For each isolate, a de-novo assembly of the genomic sequence was carried out. The assemblies comprised 73 and 71 contigs for the human isolates RUH-2 and RUH-71, respectively. Isolates RUH-98 and RUH-99 from camel meat had 72 and 66 contigs, respectively. The overall G/C content for the chromosomal contigs was 33%. All the CC15-MRSA isolates had the MLST profile 13–13–1–1–81–11–13. All of the 4 isolates sequenced carried a 30-kb plasmid harboring additional antibiotic resistance genes, namely *cadD*, *cadX*, *blal*, *blaR*, *blaZ*, *lnuA*, *aadD*. In addition, isolate (RUH-71, from human wound infection) harbored another putative plasmidic contig encoding *tetK*. A comparison of the genomic features of the 4 CC15-MRSA isolates reported in this study (RUH-2, RUH71, RUH-98, RUH-99), with CC15-MSSA sequences in the NCBI GenBank (VCU006, MPROS1797, 08–02119, ST20130938, ST20130940, ST20130941) is given in Table S1.

The 6 contigs related to the SCC element (Figure 1) were identical in all isolates. One contig comprised a recombinase gene “cerAA”, *ccrC-PM1*, *fusC* and a helicase; another contig included *mvaS*, *dru*, *mecA* and 1 contig had *yoB* and *Q4LAG7* (putative protein associated with SCCmec V/N). The SCC element presumably comprises 5 transposase genes, namely 3 identical paralogs of tnpIS431 (size 675 nt) and 2 identical paralogs of tnpIS256 (size 1173 nt) (Figure 1). Two identical copies of a tnpIS256-based insertion element flank the bifunctional kanamycin resistance determinant *aacA-aphD*. Two copies of this insertion element were present in the genome with 1 copy located in the SCC element and another copy inserted into the *sasC* gene encoding a surface protein.

The CC15-MRSA isolates had a short 3 kb region inserted into the *hlb* gene (Figure 2). The 3 kb insertion element lacks any bacteriophage structural genes and site-specific DNA integrase. The insertion element comprised 5 genes, including *scn* (staphyloccocal complement inhibitor) and *chp* (chernotaxis inhibitor), but *sak* (staphylokinase) was absent (Figure 2).

The CC15-MRSA isolates showed a variant of *hsdM/hsdS* at the major pathogenicity island *vSaC* compared with the reference CC15-MSSA genome (Figure 3). The *hsdM* and the 5’-part of the *hsdS* gene were replaced by a copy of the *hsdM/hsdS* paralogs from *vSaF*. This gives rise to a new chimeric paralog of *hsdS* in *vSaA* (Figure 3). The chimeric *hsdS* has an intact reading frame. We can see this recombination in all of the 4 CC15-MRSA isolates. Furthermore, a *Sau3AI* restriction system is present in all of the CC15 isolates analyzed, while the type IV restriction system *SauUSI* is absent (Table S1).

**Discussion**

All the CC15-MRSA isolates had the MLST profile 13–13–1–1–81–11–13, which is a single locus variant of ST15. This
MLST profile has been assigned to ST1535 (https://pubmlst.org/bigsdb?db=pubmlst_saureus_isolates&page=profiles) and comprises pta-81 instead of pta-12 in canonical ST15. This pta-81 differs from pta-12 by only 1 single-nucleotide polymorphism, which was present in all our isolates. Three of the 4 isolates assigned to ST1535 in the PUBMLST database are MSSA (https://pubmlst.org/bigsdb?db=pubmlst_saureus_isolates&page=profiles). The fourth is MRSA isolate.
MPROS1797, which has a similar SCC element as the CC15 MRSA in this study (https://www.ncbi.nlm.nih.gov/biosample/SAMEA2664415; Table S1). Due to the presence of repeats, the SCC element could not be scaffolded into a single contiguous sequence. The overall constellation of the SCC element as shown in Figure 1 was interpreted as a novel SCCmec-V/SCCfus composite element. A very similar element has also been found by microarray hybridization in CC97-MRSA from Saudi Arabia.13 Furthermore, reports from Saudi Arabia have described MRSA isolates from other lineages that also harbored SCCfus in addition to SCCmec IV or V elements.6,13 Insertion elements flanked by 2 antiparallel copies of a transposase are common in bacteria, and often found in association with antibiotic resistance genes. The sasC gene, which is interrupted by insertion of another copy of the tnpIS256-based insertion element, has been linked with biofilm production in S. aureus.14

In S. aureus, an insert in the hlb gene is typically a prophage comprising several structural genes encoding the capsule, head and tail of the phage alongside an integrase at the terminus. It also frequently carries virulence associated genes like sea, sep (N315), see, chp, sak and scn in

**Figure 2** The hlb-3kb-insert in CC15-MRSA.
**Notes:** The hemolysin beta gene (hlb) is interrupted by a 3 kb insertion element in CC15-MRSA genomes.
**Abbreviation:** MRSA, methicillin-resistant Staphylococcus aureus.

**Figure 3** hsdM/hsdS recombination in CC15-MRSA.
**Notes:** The Figure shows the contents of genomic islands vSaα and vSaβ in isolate RUH-2 (ST1535/CC15, MF185202, MF185203) and in ST20130398 (ST15/CC15, Genbank accession CP012972.1). The reference genome CP012972.1 comprises two distinct paralog of hsdM/hsdS in genomic islands alpha and beta. The mapping of the sequencing reads from isolate RUH-2 onto the reference sequence CP012972.1 reveals, that hsdM-alpha and the 5’-end of hsdS-alpha are missing in RUH-2, while the coverage of hsdM-beta and the 5’-end of hsdS-beta is doubled with respect to other chromosomal genes, indicating that this stretch of DNA is duplicated in RUH-2. We extracted the duplicated region of vSaβ from the SPAdes contigs and were able to link it to contigs mapping to vSaα.
**Abbreviation:** MRSA, methicillin-resistant Staphylococcus aureus.
of the $\text{ssl}_10$ and $\text{ssl}_11$ superantigen-like genes. A second pair typically located in the genomic island the specificity determinate). One pair of $\text{hsdM}/\text{hsdS}$ genes resides in genomic island $\text{CC15-MRSA}$ isolates showed a different variant of the $\text{hsdM}/\text{hsdS}$ system and the type II R-M locus facilitated uptake of foreign mobile genetic elements, that is, of SCCmec/SCC$fus$ by the ancestral $\text{CC15-MSSA}$ promoting emergence of $\text{CC15-MRSA}$.

The limitation of our work is that the gaps between the contigs, which are presumably caused by repeated sequence elements, could not be resolved since the average fragment size of the Illumina library was only about 250 nt. Also, we were unable to determine the $\text{spa}$ type reliably from our assembly since $\text{spa}$ is a highly repetitive locus of a variable number of imperfect repeats. This genomic arrangement typically provokes artifacts in read assembly.

Accession numbers

The raw read sequences have been deposited in the Sequence Read Archive database (Bioproject PRJNA386092) with accession numbers: SAMN06925301, SAMN06925302, SAMN06925303, SAMN06925304.

De-novo assembled contigs have been deposited at DDBJ/ENA/GenBank under the accession NHZV00000000, NHZW00000000, NHZX00000000. The version described in this paper is version NHZV01000000, NHZW01000000, NHZX01000000. The manually scaffolded sequences for $\text{hlb}_3\text{kb} \_\text{insert}$, $\text{vsaa}$, $\text{vsaf}$ and the 6 SCC element contigs have been submitted to the GenBank under the following accession numbers: MF185201, MF185202, MF185203, MF185204, MF185205, MF185206, MF185207, MF185208, MF185209

Conclusion

We provide the molecular characterization of a MRSA strain from a common lineage that until recently gave rise only to very few MRSA. The findings indicate that $\text{CC15-MRSA}$ has a novel SCC$mecV$/SCC$fus$ composite element. Changes in the $\text{hsdM}/\text{hsdS}$ system and the type II R-M locus probably played a role in the emergence of this rare MRSA strain. Close surveillance is needed, especially with regard to spread among humans and livestock in the Middle East and emergence of further $\text{CC15-MRSA}$ strains.

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Disclosure

The authors report no conflicts of interest in this work.
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## Table S1 Comparison of CC15-MRSA and CC15-MSSA

| Isolate name | RUH-2 | RUH-71 | RUH-98 | RUH-99 | VCU006 | MPROS1797 | 08-02,119 | ST2,01,30,938 | ST2,01,30,940 | ST2,01,30,941 |
|--------------|-------|--------|--------|--------|--------|-----------|-----------|--------------|--------------|--------------|
| Biosample accession | SAMN06925302 | SAMN06925301 | SAMN06925303 | SAMN06925304 | SAMN00138234 | SAMEA2664415 | SAMN04939716 | SAMN04166246 | SAMN04166494 | SAMN04166543 |
| Collection date | 07-Nov-2013 | 04-Apr-2014 | 26-Oct-2014 | 26-Oct-2014 | 26-Oct-2014 | 26-Oct-2014 | 26-Oct-2014 | 26-Oct-2014 | 26-Oct-2014 | 26-Oct-2014 |
| Collection place | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh | Saudi Arabia: Riyadh |
| Host | Homo sapiens | Homo sapiens | Camelus dromedarius | Camelus dromedarius | Camelus dromedarius | Camelus dromedarius | Camelus dromedarius | Camelus dromedarius | Camelus dromedarius | Camelus dromedarius |
| Host disease | Sepsis | Wound infection | Retail meat, neighborhood meat shop | Retail meat, neighborhood meat shop | Retail meat, neighborhood meat shop | Retail meat, neighborhood meat shop | Retail meat, neighborhood meat shop | Retail meat, neighborhood meat shop | Retail meat, neighborhood meat shop | Retail meat, neighborhood meat shop |
| Isolation source | | | | | | | | | | |
| MLST | 1535 | 1535 | 1535 | 1535 | 1535 | 1535 | 1535 | 1535 | 1535 | 1535 |
| Clonal complex | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| SCC element | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus | SCCmecV / SCCfus |
| Paired end sequencing | 2×51 | 2×51 | 2×51 | 2×51 | 2×51 | 2×51 | 2×51 | 2×51 | 2×51 | 2×51 |
| Average insert size | 300 | 290 | 290 | 290 | 290 | 290 | 290 | 290 | 290 | 290 |
| Fragments sequenced | 106707798 | 983979006 | 860190990 | 782081124 | 570533600 | 2852668 | 310 | 260 | 190 | 190 |
| Total number of bases | 10461449 | 9646853 | 8433245 | 7667462 | 70533600 | 2852668 | 310 | 260 | 190 | 190 |
| Estimated coverage | 73 contigs | 71 contigs | 72 contigs | 66 contigs | 300 | 300 | 300 | 300 | 300 | 300 |
| WGS accession | SAMN06925302 | SAMN06925301 | SAMN06925303 | SAMN06925304 | AGTZ00000000.1 | NHZU00000000.0 | NHZV00000000.0 | NHZW00000000.0 | NHZX00000000.0 | NHZ00000000.0 |
| Number of contigs | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| capsular genotype (assembly) | | | | | | | | | | |
| agr type (assembly) | II | II | II | II | II | II | II | II | II | II |
| RIDOM spa type (assembly) | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage | t328, uneven coverage |
| RIDOM spa profile | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 | 07–23:12:34–34 |
| RIDOM spa repeat count | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 |

(Continued)
Table S1 Comparison of CC15-MRSA and CC15-MSSA

| Isolate name | RUH-2  | RUH-71 | RUH-98 | RUH-99 | VCU006 | MPROS01797 | 08-02,119 | ST2,01,30,938 | ST2,01,30,940 | ST2,01,30,941 |
|--------------|--------|--------|--------|--------|--------|------------|-----------|---------------|---------------|---------------|
| cna (assembly) | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing |
| sarT/sarU (assembly) | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| sacC | Truncated | Truncated | Truncated | Truncated | Intact | Truncated | Intact | Intact | Intact | Intact | Intact | Intact |
| tetK | Missing | Present | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing |
| blaZ | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| fusC | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| mecA | Present | Present | Present | Present | Missing | Present | Missing | Missing | Missing | Missing | Missing | Missing |
| hlb | Truncated | Truncated | Truncated | Truncated | Truncated | Truncated | Truncated | Truncated | Truncated | Truncated | Truncated | Truncated |
| scn | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| chr | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| 3 kb hlb insert | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| sau3AI | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| sauUSI | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing | Missing |

Notes: Comparison genome properties of CC15-MRSA from this study with those of CC15-MSSA/MRSA from the NCBI GenBank.

Abbreviations: MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin susceptible S. aureus; MLST, multilocus sequence typing; WGS, whole genome shotgun.
CC15-MRSA genome sequencing