RESEARCH ARTICLE

Characterisation of a novel SCCmec VI element harbouring fusC in an emerging Staphylococcus aureus strain from the Arabian Gulf region

Abiola Senok1, Peter Slickers2,3, Helmut Hotzel4, Samar Boswihi5, Sascha D. Braun2,6, Darius Gawlik7, Elke Müller2,6, Anju Nabi8, Rania Nassar1, Hedda Nitschke9, Annett Reissig2,6, Antje Ruppelt-Lorz10, Joseph Mafofo11, Ali M. Somily12, Edet Udo5, Ralf Ehrlich2,6, Stefan Monecke2,6,10*

1 College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates, 2 InfectoGnostics Research Campus Jena, Jena, Germany, 3 Abbott (Alere Technologies GmbH), Jena, Germany, 4 Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Institute of Bacterial Infections and Zoonoses, Jena, Germany, 5 Department of Microbiology, Faculty of Medicine, Kuwait University, Jabiya, Kuwait, 6 Leibniz Institute of Photonic Technology (IPHT), Jena, Germany, 7 PTC - Phage Technology Center GmbH, Bönen, Germany, 8 Microbiology & Infection Control Unit, Pathology Department, Rashid Hospital, Dubai Health Authority, Dubai, United Arab Emirates, 9 Department of Laboratory Medicine, Hospital Dresden-Neustadt, Dresden, Germany, 10 Institute for Medical Microbiology and Hygiene, Medical Faculty "Carl Gustav Carus", Technische Universität Dresden, Dresden, Germany, 11 Agiomix FZ-LLC, Dubai Science Park Warehouse Complex, Dubai, United Arab Emirates, 12 Department of Pathology and Laboratory Medicine, College of Medicine, King Khalid University Hospital and King Saud University, Riyadh, Saudi Arabia

* monecke@rocketmail.com, stefan.monecke@leibniz-ipht.de

Abstract

Fusidic acid is a steroid antibiotic known since the 1960s. It is frequently used in topical preparations, i.e., ointments, for the treatment of skin and soft tissue infections caused by Staphylococcus aureus. There is an increasing number of methicillin-resistant S. aureus (MRSA) strains that harbour plasmid-borne fusB/far1 or fusC which is localised on SCC elements. In this study we examined a series of related CC30-MRSA isolates from the Arabian Gulf countries that presented with SCCmec elements and fusC, including a variant that—to the best of our knowledge—has not yet formally been described. It consisted of a class B mec complex and ccrA/B-4 genes. The fusidic acid resistance gene fusC was present, but contrary to the previously sequenced element of HDE288, it was not accompanied by tirS. This element was identified in CC30 MRSA from Kuwait, Saudi Arabia and the United Arab Emirates that usually also harbour the Panton-Valentin leukokidin (PVL) genes. It was also identified in CC8 and ST834 isolates. In addition, further CC30 MRSA strains with other SCCmec VI elements harbouring fusC were found to circulate in the Arabian Gulf region. It can be assumed that MRSA strains with SCCmec elements that include fusC have a selective advantage in both hospital and community settings warranting a review of the use of topical antibiotics and indicating the necessity of reducing over-the-counter sale of antibiotics, including fusidic acid, without prescription.
**Introduction**

Within a year after the introduction of penicillinase-resistant semi-synthetic penicillins such as methicillin, oxacillin and the first/second generation cephalosporins, methicillin-resistant *Staphylococcus aureus* (MRSA) was reported in the United Kingdom [1]. Beta-lactam resistance in MRSA is due to modified penicillin-binding proteins encoded by different mec genes, out of which mecA is by far the most common and most widespread [2, 3]. The mecA gene is located on potentially mobile, large and complex genetic elements, known as SCCmec (“staphylococcal cassette chromosome” or “staphylococcal chromosomal cassette” harbouring mecA). In addition to mecA or mecC, SCCmec elements include ccr recombinase genes, regulatory elements and, variably, additional genes encoding resistance to other antimicrobials, such as aminoglycosides or macrolides, and to heavy metal ions [4–11]. They also might contain the gene fusC encoding fusidic acid resistance [12, 13]. Fusidic acid [14, 15] is a steroid antibiotic known since the 1960s. It is frequently used in topical preparations, i.e., ointments, for the treatment of skin and soft tissue infections caused by *S. aureus*. In some countries, intravenous preparations are licensed that are administered in combination with other antimicrobials in order to treat staphylococcal bloodstream or orthopaedic infections. Resistance in staphylococci towards fusidic acid can essentially be attributed to five different genetic causes. One is caused by random mutation under selective pressure in the ubiquitous fusA or efg gene coding for elongation factor G [16]. Similarly, point mutations in fusE, or rplF, encoding riboprotein L6 can confer resistance [17, 18]. Another mechanism is related to the presence of the plasmid-borne gene fusB, also known as far1. Its gene product binds to elongation factor G (efg) and thereby protects efg from fusidic acid. Acquired resistance due to fusB/far1 is commonly observed in the community-acquired MRSA strain CC80-MRSA-IV that is common in Mediterranean and Middle Eastern countries [19–26]. A similar gene, fusC (Q6GD50) is localized on SCC elements. Such an element was first sequenced in a methicillin-susceptible strain, MSSA476 (GenBank BX571857.1) [27] where it is accompanied by ccrA/B1 genes. However, there are also various SCCmec elements that comprise both, mecA and fusC, together with various combinations of ccr genes and other markers [12, 13]. One of these markers is tirS, a putative virulence factor mimicking the human Toll/interleukin-1 receptor (TIR) resulting in attenuation of the inflammatory response [28]. Finally, there is a gene, fusD, that has been found in various coagulase-negative staphylococci (*S. arlettae*, *S. cohnii*, *S. microti*, *S. pettenkoferi* and *S. saprophyticus*) [17] but apparently not yet in *S. aureus*. A high consumption of fusidic acid at a population level has been shown to confer a clear selective advantage to strains carrying fusC and subsequently to their emergence and proliferation as it was well documented for New Zealand [29]. Much less is known on the situation in other geographic regions. However, a high prevalence of fusB/far1 and fusC and/or a high prevalence of fusidic acid resistance suggest a similar effect in Middle Eastern/Arabian Gulf countries. Indeed, fusidic acid is— or was until recently—available for purchase over-the-counter without prescription there. In the United Arab Emirates (UAE), prescriptions for the purchase of antibiotics became mandatory in late 2017. In this work we examine a series of related MRSA isolates from Arabian Gulf countries that presented with SCCmec VI elements and fusC. This included a variant that—to the best of our knowledge—has not yet formally been described and therefore it was characterised in detail.

**Material and methods**

**Isolates**

One CC30 MRSA isolate (RUH-32) obtained in September 2014 from a patient with septic arthritis at the King Khalid University Teaching Hospital in Riyadh, Saudi Arabia, yielded a
microarray hybridisation pattern that did not match hybridisation patterns of previously known SCC elements. This isolate was subjected to whole genome sequencing. The BioSample accession number for the isolate is SAMN06925305, the master accession number of the assembled contigs is SGWB00000000.1. This investigation prompted a search for additional CC30 isolates with both, SCC\textit{mec} VI elements and \textit{fusC} yielding one isolate from Dubai, UAE (2018), sixteen from Kuwait (2016/2017) [30] and two from Riyadh, Saudi Arabia (2014 and 2018). All were obtained from hospitalised patients. Finally, two archived non-CC30 isolates were retrospectively found by using an array for SCC\textit{mec} characterisation (see below and [9]) to harbour the same variant of a SCC\textit{mec} VI element as RUH-32. One was a ST834-MRSA isolated in 2011 from a cutaneous abscess of a 3 years old child from Riyadh [31]. The other one was a CC8-MRSA that was isolated in 2017 from a hospitalised patient in Dresden, Saxony, who had a history of travel to, or migration from, the Middle East.

**Microarray-based molecular characterisation**

All isolates were characterised using two different microarray-based assays (Alere Technologies GmbH/Abbott, Jena, Germany) designed for \textit{S. aureus} typing [32, 33] and for the characterisation of SCC elements [9]. This allowed a rapid detection of species markers, virulence genes, resistance genes and SCC-related markers as well as an assignment to strains and clonal complexes. Details on DNA preparation and hybridisation procedures, as well as on probe and primer sequences and on data analysis have been described previously [9, 32, 33].

**Genome sequencing**

Whole-genome sequencing of the RUH-32 isolate was carried out by a commercial service provider using the Illumina HiSeq-2500 platform. Raw reads were deposited in the Short Read Archive under accession SRR5520614. Sequencing reads were assembled de-novo using SPAdes version 3.10.1 ([http://bioinf.spbau.ru/spades](http://bioinf.spbau.ru/spades)). 51 Contigs were obtained with sizes larger than 200 nt and k-mer coverages greater than 10. Sequences from two reference strains, MRSA18 (European Nucleotide Archive accession number ERR108048) and 20121643 (ERR1595888), were downloaded from the European Nucleotide Archive and assembled with spades. In both cases, the complete SCC elements were found to be located on a single contig. The sequences of the SCC elements were excised from the respective contigs and then annotated.

**Sanger sequencing for closing the gap between the contigs**

Inspection of the contigs revealed that two contigs comprised typical SCC genes and that they may be linked by an IS431-like insertion element. Primers were designed for amplifying and capillary electrophoresis (CE) sequencing of the ambiguous linker region. Primer sequences are provided in Table 1. The process included PCR amplification using a thermal cycle program with an initial denaturation temperature of 96°C for 60s followed by 35 cycles of denaturation at 96°C for 15s, annealing at 70°C for 60s and extension at 72°C for 90s. The PCR product was fractionated by gel electrophoresis on a 1.5% agarose, the band with the main amplicon was excised and purified with the QIAamp Gel Extraction Kit (Qiagen, Hilden, Germany) according to the recommendations of the manufacturer. Cycle sequencing was carried out using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) on a ABI PRISM 3130 (Applied Biosystems). The first sequence obtained was then further extended by primer walking to a final size of 1133 nt (submitted to GenBank as “linker_RUH-32”). The final linker sequence (GenBank accession number MK991790) is overlapping with contig SGWB01000020.1 by 303 nt, and with contig SGWB0100002.1 by 490 nt.
These two contigs and the linker sequence were joined to a single contiguous sequence. For detailed sequence analysis, the SCC element and its flanking genes were extracted and deposited as a separate sequence entry in GenBank (accession number is MK991791). The SCC element was annotated by comparison to a database of genes which have previously been found in related SCC elements.

Results

A novel variant of a SCCmec VI element harbouring fusC

An overview on the gene content and the order of genes in the SCCmec VI (RUH-32) element is provided in Table 2 and a graphic representation is shown in Fig 1. In short, the element consists of a class B mec complex in which a mecA_{BA000018} (a N315-like allele of mecA [2]) is combined with ugpQ and delta mecR1. The fusidic acid resistance gene fusC is present, tirS is absent. In sequences of RUH-32, ccrA/B-4 are present, but ccrA-4 does not yield signals in array experiments. The reasons are polymorphisms in the probe binding site of ccrA-4 in RUH-32. Several other SCCmec elements that include fusC can be identified among previously published sequences. One is present in the CC5 strain HDE288 (AF411935; [13]) and can also be identified in CC8 (“UK-EMRSA-12/13”) sequences ASARH101 (SAMEA1565121), MPROS978 (SAMEA2041631) and MPROS1215 (SAMEA2663833). The SCCmec VI (RUH-32) element differed from the SCCmec VI (HDE288) element in an absence of tirS. Another difference is the presence of Q4LAG7 (“putative protein”; BX571857.1, position 55452 to 55880) in the RUH-32 sequence which was also detected by microarray. Differences to SCCmec VI element harbouring fusC of the CC8 strain MRSA18 (ERR10804/SAMEA1317993; [12]) include the presence of Q9XB68-dcs and SCCterm 3 (rather than SCCterm 7). Furthermore, MRSA18 has two copies of ccrA/B-4 genes. Strain AR466 (CP029080.1), a CC45-MRSA, has a SCCmec VI element harbouring fusC that could not be differentiated from the one in MRSA18 by array (hence, it is not shown in Table 3). However, it includes an additional hsdS/M/R (type I restriction-modification) operon and dfrA (dihydrofolate reductase), which usually is plasmid borne. Finally, there is another SCCmec VI-derived element in the CC8 strain 20121643 (ERR1595888/SAMEA3924203) that harbours fusC. It differs from RUH-32 in several markers (see Fig 1) including an absence of Q9XB68-dcs, presence of speG (spermidine N-acetyltransferase) and dfrA as well as a presence of three copies of ccrA/B-4 genes.

CC30-MRSA-[VI+fus] strains in the Arabian Gulf region

The novel SCCmec VI (RUH-32) element described herein was not present in all CC30-MRSA-[VI+fus] isolates. Instead, they could be categorized into three distinct clusters (Table 3). Isolates of one cluster carried the SCCmec VI (RUH-32) element. They harboured the PVL genes
Table 2. Genes in SCCmec VI (RUH-32).

| Gene      | Description/gene product                                      | Comments                                                                 | Orientation | Start position | End position |
|-----------|----------------------------------------------------------------|-------------------------------------------------------------------------|-------------|----------------|--------------|
| orfX      | 23S rRNA methyltransferase with the SCC integration site being located at the 3’ end of orfX | Identical (no mismatches) to GU235984.1[9:488]                           | FORWARD     | 1              | 480          |
| DR_SCC    | direct repeat of SCC                                           | Identical to BA000033.2 [34252:34270]                                    | FORWARD     | 462            | 480          |
| Q9XB68-acs | putative protein                                               | Identical to AFEF01000013.1 [388744:39039]                               | FORWARD     | 661            | 1,955        |
| Q7A213    | putative protein                                               | Identical to BA000033.2 [36062:36400]                                    | FORWARD     | 2,370          | 2,609        |
| tnpIS431-06 | transposase for IS431                                           | Identical to BA000018.3 [36435:37109]                                   | REVERSE     | 2,641          | 3,315        |
| mvaS-SCC  | truncated 3-hydroxy-3-methylglutaryl CoA synthase              | Identical to BA000033.2 [37179:37531]                                    | FORWARD     | 3,388          | 3,740        |
| Q9XB68    | putative protein                                               | Identical to AFEF01000013.1 [388744:39039]                               | FORWARD     | 3,838          | 4,068        |
| dru       | SCC direct repeat units                                        | dru repeats 0–2d-2d-2g-2n-3a-3b-4e-4i-5a-5b-7b-7b. No match identified in dru database. | FORWARD     | 3,978          | 4,415        |
| ugpQ      | glycerophosphoryl diester phosphodiesterase                   | Identical to BA000033.2 [38288:39031]                                    | FORWARD     | 4,617          | 5,360        |
| ydeM      | putative dehydrogenase                                         | Identical to BA000033.2 [39128:39556]                                    | FORWARD     | 5,457          | 5,885        |
| mecA      | penicillin binding protein 2a                                  | Identical to BA000033.2 [39602:41608]                                   | REVERSE     | 5,949          | 7,937        |
| Delta mecR1 | truncated mexitcin resistance operon repressor 1/           | Identical to BA000033.2 [41708:42682]                                   | FORWARD     | 8,037          | 9,011        |
| hsdR2-IS1272 | fragment of type I restriction-modification system endonuclease | Identical to BA000033.2 [42683:42916]                                   | FORWARD     | 9,012          | 9,245        |
| tnpIS1272 | transposase for IS1272 from isolate TCH                        | Identical to BA000033.2 [42917:44440]                                   | REVERSE     | 9,246          | 10,769       |
| Q9XB75-v1-NN1 | putative protein                                               | Identical to BA000033.2 [44576:45082]                                   | REVERSE     | 10,905         | 11,411       |
| Q7A207    | putative protein                                               | Identical to BA000033.2 [45097:45408]                                   | REVERSE     | 11,426         | 11,737       |
| Q7A206    | putative protein                                               | Identical to HF569097 [32048:32398]                                    | REVERSE     | 11,824         | 12,174       |
| UTR_ccrB  | highly conserved 3'-untranslated region of ccrB                | REVERSE 12,175 - 12,649                                                 |             |                |              |
| ccrB-4    | cassette chromosome recombinase B, type 4                      | REVERSE 12,677 - 14,305                                                |             |                |              |
| ccrA-4    | cassette chromosome recombinase A, type 4                      | REVERSE 14,302 - 15,663                                                |             |                |              |
| cch       | cassette chromosome helicase                                   | REVERSE 15,850 - 17,619                                                |             |                |              |
| DUF1413   | putative protein associated with cch                           | REVERSE 17,619 - 19,150                                                |             |                |              |
| Q83ZD5    | putative protein                                               | REVERSE 18,080 - 19,150                                                |             |                |              |
| helicaseM06 | DEAD/DEAH box helicase domain protein                           | FORWARD 19,244 - 21,184                                                |             |                |              |
| Q6GD51    | putative protein                                               | Related to BX571857.1[51926:52234]; six mismatches                      | FORWARD     | 21,441         | 21,749       |
| D3QF0     | putative lipase/protease                                       | Identical to BX571857.1[52281:52519]                                    | FORWARD     | 21,859         | 22,017       |
| fusC      | fusidic acid resistance protein C                             | Related to BX571857.1[52820:53458]; one mismatch                       | FORWARD     | 22,335         | 22,973       |
| sccterm03 | terminus of SCC towards orfX                                   | For a discussion of the SCC terminal regions, and its variability, see [9] | FORWARD     | 23,571         | 23,658       |
| Q6GD49    | putative protein within SCC                                    | FORWARD 23,659 - 24,288                                                |             |                |              |
| Q8CU43    | putative protein                                               | Identical to BX571857.1 [54788:55030]                                   | FORWARD     | 24303          | 24,545       |
| Q4L47G-45394F | putative protein                                               | Identical to BX571857.1 [55452:55880]                                   | REVERSE     | 24967          | 25,395       |
| yabV      | transcriptional regulator                                      | Related to BX571857.1[55861-56889]; two mismatches                    | FORWARD     | 25,475         | 26,404       |
| DR_SCC    | direct repeat of SCC                                           | FORWARD 26,501 - 26,519                                                |             |                |              |
| UTR_mcrB  | 5’-untranslated region of mcrB                                  | Identical to BX571856.1 [92902:93144]                                   | FORWARD     | 26,520         | 26,762       |
| mcrB      | type IV 5-methylcytosine-specific restriction enzyme subunit B | Identical to BX571856.1 [93145:94848]                                   | FORWARD     | 26,763         | 28,466       |
| mcrC      | type IV 5-methylcytosine-specific restriction enzyme subunit C | Identical to BX571856.1 [94841:95881]                                   | FORWARD     | 28,459         | 29,499       |

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although there was one exception. They also were positive for \( \text{cadD} \), \( \text{cadX} \), \( \text{blaZ} \), \( \text{linA/lnu(A)} \) (again, with one exception), \( \text{aadD} \), \( \text{dfrG} \) and \( \text{tet(K)} \). A second cluster carried another SCC \( \text{mecVI} \) element that yielded the same hybridisation signals as expected for a previously sequenced element from the Portuguese CC5 strain HDE288 [13]. One major difference to SCC \( \text{mecVI} \) (RUH-32) was the presence of \( \text{tirS} \). All isolates from this cluster were positive for the gene encoding the toxic shock syndrome toxin, \( \text{tst1} \). A majority of them also were positive for \( \text{pvl} \) genes (nine out of twelve) and the enterotoxin gene \( \text{sea} \) (eleven out of twelve). Other markers included the copper resistance genes \( \text{mco} \), \( \text{copA2} \), arsenic and cadmium resistance genes \( \text{arsB} \), \( \text{cadA} \), \( \text{cadC} \) as well as \( \text{blaZ} \) and (in some isolates) \( \text{erm(C)} \). A third cluster, comprising of a single isolate harboured another SCC \( \text{mec} \) element consistent to one previously described from a CC8 isolate "MRSA18" [12]. The Kuwaiti CC30 isolate was PVL-positive. It lacked \( \text{tirS} \) and \( \text{tst1} \). Heavy metal resistance genes were absent and \( \text{tetK} \) was the only antimicrobial resistance gene present in addition to \( \text{mecA} \).

Other MRSA strains with the same SCC\( \text{mecVI} \) element

One PVL-negative CC8 isolate was identified in a patient in Saxony who had links to the Middle East. Based on array hybridisation results, it also carried the SCC\( \text{mecVI} \) (RUH-32) element (Table 3). This observation prompted an extensive database search yielding four more CC8-MRSA sequences with SCC\( \text{mecVI} \) (RUH-32) in the NCBI Short Read Archive (SAMEA2385458, SAMEA2385540, SAMEA2664046, SAMEA2664096). We also identified the same element, SCC\( \text{mecVI} \) (RUH-32), retrospectively in a ST834 isolate (Riyadh_3497247, see Table 3).

Discussion

We describe a novel variant of a SCC element that harbours determinants for both, methicillin/beta-lactam and fusidic acid resistance. It was identified when performing DNA-microarray-based typing of clinical strains from Saudi Arabia because of a previously unseen...
Table 3. Hybridisation profiles (selected markers only) for CC30-MRSA-[VI+ fus], other isolates with the SCCmec VI (RUH-32) element and predicted hybridisation patterns for reference sequences. Column “A/S” indicates whether the isolate was characterised by array (A) or if a genome sequence was analysed (S).

| Isolate                  | A/S | CC | SCCmec type/ subtype | ugQ | mecR1 | mvaS | SCC Q4LAG7 (fus) | foxC | qacE and qacG | tleG | ACME and opp3 | speG | ccrA-4 | ccrB-4 | Q5R846 | dcs | SCCmec Term. 3 | SCCmec Term. 7 | mec (plasmidic) | copA2 (plasmidic) | cyaR (plasmidic) | cadA (TN554) | cadC (TN554) | cadD | cadK | cadL |
|--------------------------|-----|----|----------------------|-----|-------|------|-----------------|------|---------------|------|----------------|------|--------|--------|---------|-----|----------------|----------------|-----------------|------------------|----------------|-------------|-------------|------|------|------|
| Riyadh (RUH-32) 30       | S   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Riyadh (RUH-32) 30       | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Dubai-M25                | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_17412 30     | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_17555 30     | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_17749 30     | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_4415-1 30         | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Riyadh_52 30             | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Dresden-1041308.36 8     | A   | 8  | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Riyadh_3497247 834       | A   | 834| SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| HDE288 S 5               |     | 5 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_D703 30      | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_5250 30       | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_18841 30     | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_4924 30       | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_3145 30       | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_17941 30     | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_2017_18255 30      | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |
| Kuwait_4527 30           | A   | 30 | SCCmec VI avrV401(52) |     |       |      |                 |      |               |      |                |      |        |        |         |     | 1              | 4              |                 |                  |             |            |        |      |      |

(Continued)
| Isolate                          | A/S | CC | SCCmec type/ subtype | MacZ | emm | leuK/ leuW (A) | andD | dfrA | dfrG | tet(K) | tsa | abl | req | ace | aceReq | avr | eae | efg | lukF/S | PV | ace | rep (c = rep1913) | msrA | cdp | znu |
|---------------------------------|-----|----|----------------------|------|-----|---------------|------|------|------|--------|----|-----|-----|-----|--------|----|-----|-----|--------|----|-----|-----------------|------|-----|-----|
| Kuwait_5635                     | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | +  | +   | +   | +      | +  | +   | +              |      |     |     |
| Kuwait_5730–1                   | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Kuwait_5771                     | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Riyadh_39                      | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| MRRSA18                         | S   | 8  | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Kuwait_2017_3006                | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Strain 201216-43**              | S   | 8  | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Isolate                        | A/S | CC | SCCmec type/ subtype | MacZ | emm | leuK/ leuW (A) | andD | dfrA | dfrG | tet(K) | tsa | abl | req | ace | aceReq | avr | eae | efg | lukF/S | PV | ace | rep (c = rep1913) | msrA | cdp | znu |
| Riyadh (RUH-) 32 A              | S   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Riyadh (RUH-) 32 A              | S   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Dubai_M25                       | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Kuwait_2017_17412               | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Kuwait_2017_17555               | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Kuwait_2017_17749               | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Kuwait_4445-1                   | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Riyadh_52                       | A   | 30 | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Dresden-10436836                | A   | 8  | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Riyadh_3497247                  | A   | 834| SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| HD E288                         | S   | 5  | SCCmec VI             | +    | +   | +             | -    | +    | +    | +      | -  | +   | +   | +   | +      | -  | +   | +   | +      | -  | +   | +              |      |     |     |
| Kuwait_2017_4770                 | A   | 30 | SCCmec VI             | -    | -   | -             | -    | -    | -    | -      | -  | -   | -   | -   | -      | -  | -   | -   | -      | -  | -   | -              |      |     |     |

(Continued)
| Strain                  | SCCmec Type | 
|------------------------|-------------|
|                         | VI (HDE288) |
| Kuwait_2017_5250       | +           |
| Kuwait_2017_18848      | +           |
| Kuwait_2017_4924       | +           |
| Kuwait_2017_5145       | +           |
| Kuwait_2017_17841      | +           |
| Kuwait_2017_18255      | +           |
| Kuwait_5635            | +           |
| Kuwait_5750–1          | +           |
| Kuwait_5771            | +           |
| Riyadh_39              | +           |

**Characterisation of a novel SCCmec VI element harbouring fusC in MRSA from the Arabian Gulf region**

*arsB in HDE288, absent in SAMN0325541, but present in SAMN0325542.*

*harbours additionally genes ermA (A) and aminoglycoside resistance, which are not shown in the table.*

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hybridisation pattern and further characterised by sequencing. This element is related, but clearly distinguishable (see Results) from other such elements observed elsewhere as well as in the studied region. Furthermore, there is evidence for its horizontal transfer as we were able to detect it in two further, unrelated lineages of Staphylococcus aureus. On a practical level, the observations suggest that emerging fusidic acid resistance might hamper its use as topical treatment for staphylococcal skin and soft tissue infections. A replacement of this substance by mupirocin is not feasible as the usability of mupirocin itself is endangered by the spread of resistance [34, 35]. Other substances, such as betaisodona, polyhexanide or octenidine, should be considered.

On a more theoretical level, a co-localisation of genes encoding beta-lactam (mecA) and fusidic acid (fusC) resistance on one potentially mobile genetic element is an interesting example for coalescence of two “selfish replicators” [36] for mutual advantage. A community use of easily available topical fusidic acid could thus select for mecA methicillin resistance and a hospital use of systemic beta-lactam compounds could select for fusC, conferring benefit to MRSA with such combined elements in either biotope. Thus it can be expected that such strains emerge in hospital as well as in community settings and that it will be very complicated to contain or to eradicate them once they are established in a population. Indeed, there is a remarkable variety of different types and subtypes of SCCmec elements that additionally harbour fusC, indicating ongoing emergence and evolution. Such elements have been observed in as much as twenty-two different clonal complexes of Staphylococcus aureus, CC1, CC5, CC6, CC7, CC8, CC15, CC22, CC30, CC45 [agr I], CC45 [agr IV], CC50, CC59, ST72, CC88, CC97, CC121, CC152, CC361, CC779, ST834, CC913 and CC1153 from geographic regions as diverse as Germany, France, UK, Ireland, Portugal, Malta, Saudi Arabia, UAE, Kuwait, Australia and New Zealand. Regarding SCCmec VI elements that include fusC, there are at least five distinct variants (as represented by RUH-32, HDE288, MRSA18, AR466 and Strain 20121643; see above), and to the best of our knowledge, they have been identified in CC5, CC7, CC8 (“UK-EMRSA-12/13”), CC22, CC30, CC45, CC97, CC152, ST834 and CC913 from Germany, United Kingdom, Portugal, Kuwait and Saudi Arabia [9, 12, 13, 23, 29, 30, 32, 37–42]. Observations of SCCmec elements that harbour fusC geographically cluster in Western Europe, the Middle Eastern/Arabian Gulf countries, Australia and New Zealand. Whether this is caused by a sampling bias or related to formulations and usage of fusidic acid, or both, we currently cannot tell. However, given the current patterns of travel and migration, appearance and emergence of fusidic acid resistant MRSA cannot be ruled out anywhere. This phenotypic property cannot be seen any more as a surrogate marker for the presumptive identification of PVL-positive CC80-MRSA-IV (“European”/Mediterranean clone) but it should prompt further investigation. As mentioned above, co-evolution and co-selection of resistance traits that favour resistant pathogens in hospitals as well as in the community outside pose a public health hazard. This should prompt a review of the use of topical antibiotics such as fusidic acid (or mupirocin) including restrictions to uncontrolled and unlimited over-the-counter sale of such compounds.

Supporting information
S1 File. Full sequence of SCCmec VI (RUH-32) as well as of assembled sequences of MRSA18 (ERR10804/SAMEA1317993) and strain 20121643 (ERR1595888/SAMEA3924203). (PDF)

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Author Contributions

**Conceptualization:** Abiola Senok, Ralf Ehricht, Stefan Monecke.

**Formal analysis:** Peter Slickers, Joseph Mafofo, Stefan Monecke.

**Funding acquisition:** Abiola Senok.

**Investigation:** Abiola Senok, Helmut Hotzel, Samar Boswihi, Sascha D. Braun, Darius Gawlik, Elke Müller, Anju Nabi, Rania Nassar, Hedda Nitschke, Annett Reissig, Antje Ruppelt-Lorz, Ali M. Somily, Edet Udo, Ralf Ehricht.

**Methodology:** Ralf Ehricht.

**Project administration:** Ralf Ehricht, Stefan Monecke.

**Resources:** Abiola Senok.

**Supervision:** Ralf Ehricht, Stefan Monecke.

**Visualization:** Peter Slickers, Stefan Monecke.

**Writing – original draft:** Abiola Senok, Ralf Ehricht, Stefan Monecke.

**Writing – review & editing:** Abiola Senok, Peter Slickers, Helmut Hotzel, Sascha D. Braun, Darius Gawlik, Elke Müller, Edet Udo, Ralf Ehricht, Stefan Monecke.

References

1. Jevons MP. "Celbenin"-resistant Staphylococci. British Medical Journal. 1961; 1(5219):1924–5.

2. Monecke S, Muller E, Schwarz S, Hotzel H, Ehricht R. Rapid microarray based identification of different mecA alleles in Staphylococci. Antimicrob Agents Chemother. 2012; 56(11):5547–54. Epub 2012/08/15. https://doi.org/10.1128/AAC.00574-12 PMID: 22890767.

3. Becker K, Ballhausen B, Köck R, Kriegeskorte A. Methicillin resistance in Staphylococcus isolates: The "mec alphabet" with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. Int J Med Microbiol. 2014; 304(7):794–804. https://doi.org/10.1016/j.ijmm.2014.06.007 PMID: 25034857.

4. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2001; 45(5):1323–36. https://doi.org/10.1128/AAC.45.5.1323-1336.2001 PMID: 11302791.

5. Ito T, Katayama Y, Hiramatsu K. Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant Staphylococcus aureus N315. Antimicrob Agents Chemother. 1999; 43(6):1449–58. PMID: 10348769.

6. Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. Antimicrob Agents Chemother. 2004; 48(7):2637–51. https://doi.org/10.1128/AAC.48.7.2637-2651.2004 PMID: 15215121.

7. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. Insights on antibiotic resistance of Staphylococcus aureus from its whole genome: genomic island SCC. Drug Resist Updat. 2003; 6(1):41–52. PMID: 12654286.

8. IWG-SCC. Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother. 2009; 53(12):4961–7. https://doi.org/10.1128/AAC.00579-09 PMID: 19721075.

9. Monecke S, Jatzwauk L, Muller E, Nitschke H, Pfohl K, Slickers P, et al. Diversity of SCCmec elements in Staphylococcus aureus as observed in South-Eastern Germany. PLoS ONE. 2016; 11(9):e0162654. Epub 2016/09/21. https://doi.org/10.1371/journal.pone.0162654 PMID: 27648947.

10. Shore A, Rossney AS, Keane CT, Enright MC, Coleman DC. Seven novel variants of the staphylococcal chromosomal cassette mec in methicillin-resistant Staphylococcus aureus isolates from Ireland. Antimicrob Agents Chemother. 2005; 49(5):2070–83. https://doi.org/10.1128/AAC.49.5.2070-2083.2005 PMID: 15855533.
11. Shore AC, Coleman DC. Staphylococcal cassette chromosome mec: recent advances and new insights. Int J Med Microbiol. 2013; 303(6–7):350–9. https://doi.org/10.1016/j.ijmm.2013.02.002 PMID: 23499303

12. Ellington MJ, Reuter S, Harris SR, Holden MT, Cartwright EJ, Greaves D, et al. Emergent and evolving antimicrobial resistance cassettes in community-associated fusidic acid and meticillin-resistant Staphylococcus aureus. Int J Antimicrob Agents. 2015; 45(5):477–84. Epub 2015/03/15. https://doi.org/10.1016/j.ijantimicag.2015.01.009 PMID: 25769787.

13. Oliveira DC, Milheiro JS, de Lencastre H. Redefining a structural variant of staphylococcal cassette chromosome mec, SCCmec type VI. Antimicrob Agents Chemother. 2006; 50(10):3457–9. https://doi.org/10.1128/AAC.00629-06 PMID: 17005831

14. Williamson DA, Carter GP, Howden BP. Current and Emerging Topical Antibacterials and Antiseptics: Agents, Action, and Resistance Patterns. Clinical microbiology reviews. 2017; 30(3):827–60. Epub 2017/06/07. https://doi.org/10.1128/CMR.00112-16 PMID: 28592405.

15. Schofer H, Simonsen L. Fusidic acid in dermatology: an updated review. Eur J Dermatol. 2010; 20(1):6–15. Epub 2009/12/17. https://doi.org/10.1684/ejd.2010.0833 PMID: 20007058.

16. Turnidge J, Collignon P. Resistance to fusidic acid. Int J Antimicrob Agents. 1999; 12 Suppl 2:S35–44. Epub 1999/10/21. https://doi.org/10.1016/s0924-8579(99)00072-7 PMID: 10528785.

17. Farrell DJ, Castanheira M, Chopra I. Characterization of Global Patterns and the Genetics of Fusidic Acid Resistance. Clinical Infectious Diseases. 2011; 52(suppl_7):S487–S92. https://doi.org/10.1093/cid/cir164 PMID: 21546656

18. Lannergård J, Cao S, Norström T, Delgado A, Gustafson JE, Hughes D. Genetic Complexity of Fusidic Acid-Resistant Small Colony Variants (SCV) in Staphylococcus aureus. PLOS ONE. 2011; 6(11):e28366. https://doi.org/10.1371/journal.pone.0028366 PMID: 22140579

19. Monecke S, Slickers P, Hotzel H, Richter-Huhn G, Pohle M, Weber S, et al. Microarray-based characterisation of a Panton-Valentine leukocidin-positive community-acquired strain of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect. 2006; 12(8):718–28. https://doi.org/10.1111/j.1469-0691.2006.01420.x PMID: 16842566

20. Aires de Sousa M, Bartzavali C, Spiliopoulos I, Sanches IS, Crisostomo MI, de Lencastre H. Two intergenic regions Lies near Resistance Genes in Staphylococcus aureus. PLOS Pathogens. 2011; 1016420.x PMID: 16842566

21. Basset P, Amhis W, Blanc DS. Changing molecular epidemiology of methicillin-resistant Staphylococcus aureus in an Algerian hospital. J Infect Dev Ctries. 2015; 9(2):206–9. Epub 2015/02/24. https://doi.org/10.3855/jidc.4620 PMID: 25699496.

22. Ben Slama K, Gharsa H, Klibi N, Jouini A, Lozano C, Gomez-Sanz E, et al. Nasal carriage of Staphylococcus aureus in healthy humans with different levels of contact with animals in Tunisia: genetic lineages, methicillin resistance, and virulence factors. Eur J Clin Microbiol Infect Dis. 2011; 30(4):499–508. Epub 2010/11/16. https://doi.org/10.1007/s10096-010-1109-6 PMID: 21076928.

23. Boswini SS, Udo EE, Al-Sweih N. Shifts in the Clonal Distribution of Methicillin-Resistant Staphylococcus aureus in Kuwait Hospitals: 1992–2010. PLoS ONE. 2016; 11(9):e0162744. https://doi.org/10.1371/journal.pone.0162744 PMID: 27531623

24. Tokajian ST, Khalil PA, Jabbour D, Rizk M, Farah MJ, Hashwa FA, et al. Molecular characterization of Staphylococcus aureus in Lebanon. Epidemiol Infect. 2010; 138(5):707–12. Epub 2010/06/18. https://doi.org/10.1017/S0950268810000440 PMID: 20202283.

25. Udo EE, Sarkhoo E. Genetic analysis of high-level mupirocin resistance in the ST80 clone of community-associated meticillin-resistant Staphylococcus aureus. J Med Microbiol. 2010; 59(Pt 2):193–9. https://doi.org/10.1099/jmm.0.013268-0 PMID: 19833783.

26. Yovuli S, Perimeni D, Makri A, Polemis M, Voyiatzi A, Vatopoulos A. Community acquired MRSA infections in a paediatric population in Greece. Euro Surveill. 2005; 10(5):78–9. PMID: 16077207.

27. Holden MTG, Feil EJ, Lindsay JA, Peacock SJ, Day NPJ, Enright MC, et al. Complete genomes of two clinical Staphylococcus aureus strains: Evidence for the rapid evolution of virulence and drug resistance. PNAS. 2004; 101(26):9786–91. https://doi.org/10.1073/pnas.0402521101 PMID: 15213324

28. Patot S, Re Imbert P, Baude J, Martins Simões P, Campergue J-B, Louche A, et al. The TIR Homologue Lies near Resistance Genes in Staphylococcus aureus, Coupling Modulation of Virulence and Antimicrobial Susceptibility. PLOS Pathogens. 2017; 13(1):e1006092. https://doi.org/10.1371/journal.ppat.1006092 PMID: 28060920

29. Williamson DA, Monecke S, Hefferman H, Ritchie SR, Roberts SA, Upton A, et al. High usage of topical fusidic acid and rapid clonal expansion of fusidic acid-resistant Staphylococcus aureus: a cautionary tale. Clin Infect Dis. 2014; 59(10):1451–4. Epub 2014/08/21. https://doi.org/10.1093/cid/ciu858 PMID: 25139961.
30. Boswili SS, Udo EE, Monecke S, Mathew B, Noronha B, Verghese T, et al. Emerging variants of methicillin-resistant *Staphylococcus aureus* genotypes in Kuwait hospitals. PLoS One. 2018; 13(4): e0195933. Epub 2018/04/19. https://doi.org/10.1371/journal.pone.0195933 PMID: 29668723.

31. Monecke S, Muller E, Buechler J, Rejman J, Stieber B, Akpaka PE, et al. Rapid detection of Panton-Valentine leukocidin in *Staphylococcus aureus* cultures by use of a lateral flow assay based on monoclonal antibodies. J Clin Microbiol. 2013; 51(2):487–95. Epub 2012/11/24. https://doi.org/10.1128/JCM.02285-12 PMID: 23175260.

32. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al. A field guide to pandemic, epidemic and sporadic resistant methicillin-resistant *Staphylococcus aureus*. PLoS One. 2011; 6(4): e17936. https://doi.org/10.1371/journal.pone.0017936 PMID: 21494333.

33. Monecke S, Slickers P, Ehrcht R. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol Med Microbiol. 2008; 53:237–51. https://doi.org/10.1111/j.1574-695X.2008.00426.x PMID: 18507678.

34. Monecke S, Nitschke H, Slickers P, Ehrcht R, Swanston W, Manjunath M, et al. Molecular epidemiology and characterisation of MRSA isolates from Trinidad and Tobago. Eur J Clin Microbiol Infect Dis. 2012; 31(7):1497–500. Epub 2011/11/10. https://doi.org/10.1007/s10096-011-1469-6 PMID: 22068275.

35. Monecke S, Ruppelt-Lorz A, Muller E, Reissig A, Thurmer A, Shore AC, et al. Dissemination of high-level mupirocin-resistant CC22-MRSA-IV in Saxony. GMS Hyg Infect Control. 2017; 12:Doc 19. Epub 2017/12/01. https://doi.org/10.3205/dgkh000304 PMID: 29184755.

36. Dawkins R. Das egoistische Gen (The selfish gene, German ed.). Heidelberg: Spektrum Verlag; 1994.

37. Dauwalder O, Lina G, Durand G, Bes M, Meugnier H, Jarlier V, et al. Epidemiology of invasive methicillin-resistant *Staphylococcus aureus* clones collected in France in 2006 and 2007. J Clin Microbiol. 2008; 46(10):3454–8. https://doi.org/10.1128/JCM.01050-08 PMID: 18667599.

38. Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ, Ehricht R, Monecke S, et al. Emergence of sequence type 779 methicillin-resistant *Staphylococcus aureus* harboring a novel pseudo staphylococcal cassette chromosome mec (SCCmec)-SCC-SCCCRISPR composite element in Irish hospitals. Antimicrob Agents Chemother. 2013; 57(1):524–31. Epub 2012/11/14. https://doi.org/10.1128/AAC.01689-12 PMID: 23147725.

39. Monecke S, Skakni L, Hasan R, Ruppelt A, Ghazal SS, Hakawi A, et al. Characterisation of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. BMC Microbiol. 2012; 12(1):146. Epub 2012/07/25. https://doi.org/10.1186/1471-2180-12-146 PMID: 22823982.

40. Scerri J, Monecke S, Borg MA. Prevalence and characteristics of community carriage of methicillin-resistant *Staphylococcus aureus* in Malta. J Epidemiol Glob Health. 2013; 3(3):165–73. Epub 2013/08/13. https://doi.org/10.1016/j.jegh.2013.05.003 PMID: 23932059.

41. Senok A, Ehrcht R, Monecke S, Al-Saeedan R, Somily A. Molecular characterization of methicillin-resistant *Staphylococcus aureus* in nosocomial infections in a tertiary-care facility: emergence of new clonal complexes in Saudi Arabia. New Microbes New Infect. 2016; 14:13–8. Epub 2016/09/14. https://doi.org/10.1016/j.nmni.2016.07.009 PMID: 27621823.

42. Senok AC, Somily AM, Slickers P, Raji MA, Garaweem G, Shibil A, et al. Investigating a rare methicillin-resistant *Staphylococcus aureus* strain: first description of genome sequencing and molecular characterization of CC15-MRSA. Infect Drug Resist. 2017; 10:307–15. Epub 2017/10/19. https://doi.org/10.2147/IDR.S145394 PMID: 29042801.