Characterisation of S. aureus/ MRSA CC1153 and review of mobile genetic elements carrying the fusidic acid resistance gene fusC

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While many data on molecular epidemiology of MRSA are available for North America, Western Europe and Australia, much less is known on the distribution of MRSA clones elsewhere. Here, we describe a poorly known lineage from the Middle East, CC1153, to which several strains from humans and livestock belong. Isolates were characterised using DNA microarrays and one isolate from the United Arab Emirates was sequenced using Nanopore technology. CC1153 carries agr II and capsule type 5 genes. Enterotoxin genes are rarely present, but PVL is common. Associated spa types include t504, t903 and t13507. PVL-positive CC1153-MSSA were found in Egyptian cattle suffering from mastitis. It was also identified among humans with skin and soft tissue infections in Saudi Arabia, France and Germany. CC1153-MRSA were mainly observed in Arabian Gulf countries. Some isolates presented with a previously unknown SCCmec/SCCFus chimeric element in which a mec B complex was found together with the fusidic acid resistance gene fusC and accompanying genes including ccrA/B-1 recombinase genes. Other isolates carried SCCmec V elements that usually also included fusC. Distribution and emergence of CC1153-MRSA show the necessity of molecular characterization of MRSA that are resistant to fusidic acid. These strains pose a public health threat as they combine resistance to beta-lactams used in hospitals as well as to fusidic acid used in the community. Because of the high prevalence of fusC-positive MRSA in the Middle East, sequences and descriptions of SCC elements harbouring fusC and/or mecA are reviewed. When comparing fusC and its surrounding regions from the CC1153 strain to available published sequences, it became obvious that there are four fusC alleles and five distinct types of fusC gene complexes reminiscent to the mec complexes in SCCmec elements. Likewise, they are associated with different sets of ccrA/B recombinase genes and additional payload that might include entire mec complexes or SCCmec elements.

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Staphylococcus aureus (S. aureus) is a common coloniser, or pathogen, among humans as well as among wild and domestic animals. It can cause a broad variety of infections that include not only superficial skin and soft tissue infections (SSTI) but also life-threatening conditions such as sepsis, infective endocarditis and pneumonia. While beta-lactams are crucial for treatment, resistant strains, so-called methicillin-resistant Staphylococcus aureus (MRSA), were first reported nearly 60 years ago. Beta-lactam resistance in MRSA is caused by alternative penicillin-binding proteins encoded by different mec genes/alleles, out of which mecA is the most common and widespread one. The mecA gene is located on large and complex genetic elements, known as SCCmec ("staphylococcal cassette chromosome" or "staphylococcal chromosomosomal cassette" harbouring mecA) in which it is linked to ccr recombinase genes and, variably, to additional genes encoding antimicrobial or heavy metal resistance. Originally, MRSA was restricted to healthcare settings, but from the mid-1990s on, infections with community-acquired MRSA (CA-MRSA) were observed. Many, but not all, CA-MRSA strains carry emerging SCCmec types IV or V, as well as Panton-Valentine leukocidin (PVL; encoded by lukSF-PV genes). This is a cytotoxic, pore forming toxin localized on prophages. It is associated with recurrent, chronic and/or severe SSTI as well as with rapidly progressing necrotising pneumonia. The emergence and spread of PVL-positive CA-MRSA has extensively been studied in the United States and Australia, where they are common, as well as in Western Europe, where they pose a comparatively minor problem. Less data is available for other parts of the world, but during recent years it became obvious that PVL-positive CA-MRSA are an important public health issue in Mediterranean countries, the greater Middle East, Pakistan and India. The Arabian Gulf countries are of special interest because they are a major destination for migrants, expatriate workers, tourists and pilgrims from all over the world. This might result in importation, exchange and exportation of MRSA strains epidemic to other regions of the world. Indeed, in these countries, a high degree of diversity of MRSA strains has been observed with several strains being linked to other parts of the world.

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Since PVL is associated with clinically relevant skin conditions, topical treatments are frequently used. One option is fusidic acid, a steroid antibiotic known since the 1960s. Unfortunately, an excessive consumption of fusidic acid might quickly lead to an emergence of resistance, as it is well documented from New Zealand.

Fusidic acid resistance is common in Middle Eastern/Arabian Gulf states, usually being due to plasmid-borne fusB/far1 or SCC-associated fusO1,2,23,32.

Here, we describe a poorly known S. aureus lineage from the Middle East, CC1153, to which several distinct strains from humans and from livestock belong. Most isolates identified are PVL-positive, and many are MRSA that additionally harbour fusC. One CC1153 strain harboured a previously undescribed SCCmec/fus composite element. This observation prompted Nanopore sequencing and subsequent analysis of its genome. SCCmec/fus are reviewed and five distinct gene clusters associated with fusC are defined.

Results

Description of the clonal complex. CC1153 included sequence types (ST) 1153 (1–13–1–1–124–5–3) and ST2482 (1–141–1–1–124–8–3). Possible RIMDR spa types are t504 (26–17–20–17–12), t903 (26–22–19–17–20–17–12) and t13507 (26–22–19–17–20–17–12). Isolates belonged to agr group II and capsule type 5. The sasG gene was present but cna and the enterotoxin homologue ORF CM14 are uniformly absent. CC1153 isolates did not harbour egc locus and leukocidin genes lukD/E were variably present.

When analysing 154 core genomic markers (Supplement 3), CC1153 appeared to be most related to CC6, CC7 and CC1290. When comparing these 154 markers, all together consisting of 124,248 nucleotides, to CC6 strain PFESA1528, (GenBank FKTB), CC7 strain TCI959 (GenBank AAB) and CC1290 strain 015H (GenBank FMMV), differences of respectively, 0.38%, 0.37% and 0.38% were noted. As comparison, for CC1 (MW2, BA000033), CC5 (N315, BA000018), CC8 (TCH1516, CP000730) and S. argenteus (Bank FMMV), differences of respectively, 0.38%, 0.37% and 0.38% were noted. As comparison, for CC1 (MW2, BA000033), CC5 (N315, BA000018), CC8 (TCH1516, CP000730) and S. argenteus (Bank FMMV), differences of respectively, 0.38%, 0.37% and 0.38% were noted. As comparison, for CC1 (MW2, BA000033), CC5 (N315, BA000018), CC8 (TCH1516, CP000730) and S. argenteus (Bank FMMV), differences of respectively, 0.38%, 0.37% and 0.38% were noted. As comparison, for CC1 (MW2, BA000033), CC5 (N315, BA000018), CC8 (TCH1516, CP000730) and S. argenteus (Bank FMMV), differences of respectively, 0.38%, 0.37% and 0.38% were noted.

CC1153-MSSA strains. Twenty-three isolates of CC1153-MSSA were characterised (see Supplemental File 1). They originated from France (11/23), Egypt (6/23), Saudi Arabia (5/23) and Germany (1/23). The Egyptian isolates originated from cattle with subclinical mastitis, all other isolates were of human origin. CC1153-MSSA isolates, including those from cattle, are usually PVL-positive with lukSF-PV being detected in 21 out of 23 isolates. Some isolates, mainly Saudi Arabian (n = 4) and French (n = 3) ones, harboured enterotoxin genes sek and seq. The staphylokinase gene sak and scn (staphylococcal complement inhibitor) were always present while only one isolate was positive for cph (chemotaxis-inhibiting protein). All isolates carried the penicillinase operon (blaZ/blaI/blaR) while other resistance genes were only sporadically found (erm(C), msrA, mph(1), aadD and fusB/far1; each once in 23 isolates).

CC1153-MRSA strains. Twenty-six isolates of CC1153-MRSA were identified and characterised (see Supplemental File 1). Nine isolates were assigned to different variants of SCCmec V or VT elements. Five of these isolates came from Kuwait, one from Riyadh, Saudi Arabia, and two from the UAE (one each from Dubai and Umm-al-Qwain) and one from an Egyptian child living in Germany. All isolates, except the oldest one (isolated in 2009) also carried the fusC gene. In two isolates, the SCCmec/fusC composite element was further characterised using a second microarray assigning them to SCCmec V + fusC (rather than to SCCmec VT + fusC). One isolate (from Kuwait) yielded the same pattern (with signals for mecA, qpxQ, fusC, mvaSCC, Q4LAG7, ccaaA, cccrC, SCCerm3, SCCerm10) as observed
in a possibly livestock- (i.e., camel-) associated CC15 strain from Saudi Arabia. The other one (from Germany/ Egypt) yielded signals for mecA, uppQ, fusC, mvaS-SCC, Q4LAG7, ccrA, ccrC and SCCerm11 possibly indicating a difference affecting the SCCmec/fusX junction site and/or another SCCmec/fus subtype. Seven of these isolates harboured PVL genes. Enterotoxin genes and cipA were not identified, but sak and scn were always present. Eight out of the nine isolates harboured fusC. All were positive for blaZ/blaI/blaR. The gentamicin resistance gene aacA-aphD was found in eight isolates, the tetracycline resistance marker tet(K) in four isolates.

Seventeen CC1153-MRSA isolates belonged to a strain which, to the best of our knowledge, carried an unknown SCCmec/fusC chimeric element. Three of these isolates were investigated with the second array yielding signals with mecA, uppQ, Delta mecR1, fusC, Q4LAG7 (MSSA476), mvaS-SCC, ccrA-1, ccrB-1 and dcs. This prompted genome sequencing of one isolate, henceforth designated M58 (see below). Isolates with the new chimeric element originated from Kuwait (n = 14), UAE (n = 2) and France (n = 1). All but one were positive for lukF/S-PV genes. Genes sak and scn were always present while enterotoxin genes were not detectable. Sixteen isolates of this strain harboured blaZ/blaI/blaR, and erm(C) was found once.

Description of the SCCmec element in M58.

One of the seventeen isolates with an apparent unknown SCCmec/fusC chimeric element was subjected to genome sequencing (Nanopore) to characterise this element (see GenBank CP065857.1). Its gene content of the SCCmec/fusC element is summarised in Table 1, and Fig. 1 provides a graphical overview as well as a comparison to other, previously published, reference sequences.

In short, the element comprised a mec complex B, ccrA/B-1 recombinase genes and fusC, while tirS (that commonly accompanies fusC) was absent. The gene pls-SCC, which normally is part of SCCmec I, was also absent. This constellation raises the question whether the element was derived from a SCCmec I element truncated by an insertion of fusC, or if it was a mec complex B element from a SCCmec I or IV element supplemented by fusC and accompanying ccrA/B-1 recombinase genes.

The actual mecA allele was identical to one which is widespread in SCCmec IV strains including, for instance, MW2 but differing from the one in COL. The mec complex B was followed by some genes encoding “putative proteins” and by ccrB-1 and ccrA-1 recombinase genes, as it was also the case in SCCmec I. A closer inspection of the sequence of the genes encoding “putative proteins” and of ccrB-1 revealed differences compared to the corresponding sequences in SCCmec I. The ccrB-1 allele from SCCmec I in COL differed by 7.2% of its nucleotides while ccrA-1 was too conserved to allow a meaningful analysis.

This prompted a search for possible donors of recombinase and fusC-associated genes. While fusC itself was identical to MSSA Sanger 476, GenBank BX571857.1, the surrounding region was different in both gene content (most notably, in absence of tirS) as well as in gene sequences (8.4% difference in ccrB-1). Most closely related sequences of fusC-associated genes were identified in the S. aureus CC5 strain 06BA18369, GenBank ARXY, and in the Staphylococcus hominis subsp. hominis strain NTUH-3390, GenBank KY643657.1. These strains carry Q8CU82, tarF-SCC, A9UFT0, Q9KX75, Q7A207, Q7A206, ccrB-1, ccrA-1, ccrC-1, DUF1413, Q83ZD5, helice M06, Q6GD51, D3QFP0-scc, D3JCW9, tnpIS150, tnp_A8YYY6, Q4LAG7-SCCfus and yobV (for explanations and GenBank entries of the genes discussed, see Table 1 and Supplemental File 4). However, Q8CU82, tarF-SCC-I and A9UFT0 were absent in M58. The gene encoding the putative protein Q9KX75 in M58 was virtually identical to the ones in COL and MW2 but differed from the one in 06BA18369 and NTUH-3390, GenBank KY643657.1. From Q9KX75 on downstream, however, 06BA18369 and NTUH-3390 sequences were virtually identical to the ones in M58.

The PVL prophage in M58.

The sequence of the PVL prophage in the genome of M58 (CP065857.1) was identical to the one in the S. aureus CC1153 strain 3688STDY6124889, GenBank FQHT01000001. It was also identical to the PVL prophage in USA300-TCH1516, CP000730.1.

M58 was shown by a lateral flow assay to secrete detectable amounts of PVL. This was also the case for two other CC1153-MRSA-PseudoSCCmec [class B + fusC + ccrAB1] isolates as well as for three of the CC1153-MSSA isolates.

Discussion

CC1153 and the SCCmec/fusC element in isolate M58.

We describe a clonal complex of S. aureus that we identified in several Middle Eastern countries. A couple of publicly available genomes, deposited in GenBank (GenBank FQHT01000000) and/or the Short Read Archive (SAMEA2661948, SAMEA2661956, SAMEA2662240, SAMEA2662319, SAMEA2710354, SAMEA2710468, SAMEA2814613, SAMEA3448866, SAMEA3448996, SAMEA457522, SAMN03289718) belong to it, but to the best of our knowledge, this clonal complex has not yet been reviewed. Three of these sequences originated from Thailand (GenBank FQHT01000001 as well as BioSamples SAMEA3448866 and SAMEA3448996), and one from the United Kingdom (SAMN03289718) while for the others, no locations were reported. An additional observation of CC1153 isolates originated from Myanmar. Our isolates were collected in the greater Middle East (Egypt and Arabian Gulf countries) and Western Europe although at least one of the European cases had connections to Egypt. There are no data confirming or explaining a discontinuous distribution in the Middle East and in South-East Asia. However, the presence of millions of expatriate South-East Asians in the Gulf countries could easily explain a transmission of a S. aureus lineage into either direction.

An interesting observation is the presence of CC1153 in Egyptian bovines. The detection of PVL (rather than of lukM/lukF-P83) and of haemolysin-beta-converting prophages in these isolates indicates a human provenance of these isolates so that the cattle probably served as sentinels for an unrecognised epidemiological situation among humans in the Nile Delta.

The majority of CC1153, including MRSA and MSSA, is PVL-positive harbouring the same prophage (in M58 and FQHT01000001.1) as other pandemic strains such as USA300.
| Gene ID | Explanation | Position in SCC (nt) | Position in genome (nt) | Length (nt) | Direction | Sequence identical to |
|---------|-------------|----------------------|-------------------------|-------------|-----------|----------------------|
| orfX    | (23S rRNA methyltransferase) | 33,698–34,177 | 34,178–34,161 | 480 | Forward | MW2, BA0000033 [34169:34450] |
| sRNA6   | (Antisense RNA associated with orfX) | 34,178–34,161 | 480 | Forward | |
| DR_SCC  | Direct repeat of SCC | 1–19 | 34,159–34,177 | 19 | |
| dcs-L1  | Downstream constant segment, locus 1 | 20–301 | 34,178–34,459 | 282 | |
| Q9X686-dcs | Putative protein | 302–1596 | 34,460–34,459 | 1295 | Forward | COL, CP000046 [37058:37091] and MW2, BA0000033 [37137:37170] |
| Q7A213  | Putative protein | 2011–2250 | 36,169–36,408 | 240 | Forward | COL, CP000046 [38329:39072] and MW2, BA0000033 [38288:39031] |
| IR_IS431 | Inverted repeat of IS431 | 2233–2238 | 36,381–36,396 | 16 | |
| tmplIS431 | Transposase for IS431 | 2282–2956 | 36,440–37,114 | 675 | Reverse (trunc.) | COL, CP000046 [37550:37859] |
| Teg143  | Trans-encoded RNA associated with tmplIS431 | 2987–3020 | 37,145–37,178 | 34 | |
| IR_IS431 | Inverted repeat of IS431 | 2997–3012 | 37,155–37,170 | 16 | |
| mvaS-SCC | Truncated 3-hydroxy-3-methylglutaryl CoA synthase | 3029–3381 | 37,187–37,539 | 353 | Forward | COL, CP000046 [37100:37452] and MW2, BA0000033 [37179:37531] |
| Q5HJW6  | Putative protein | 3479–3709 | 37,637–37,867 | 231 | Forward | COL, CP000046 [37690:38127] |
| dru     | SCC direct repeat units | 3619–4056 | 37,777–38,214 | 438 | Forward | COL, CP000046 [37690:38127] |
| ugpQ    | Glycerolphosphoryl diester phosphodiesterase | 4258–5001 | 38,116–39,159 | 744 | Forward | COL, CP000046 [38329:39072] and MW2, BA0000033 [38288:39031] |
| ydeM    | Putative dehydratase | 5098–5526 | 39,256–39,684 | 429 | Forward | COL, CP000046 [39169:39597] and MW2, BA0000033 [39128:39556] |
| txbi_mecA | Bidirectional rho-independent terminator of mecA | 5517–5581 | 39,675–39,739 | 65 | |
| mecA    | Penicillin binding protein 2a | 5572–7578 | 39,730–41,736 | 2007 | Reverse | MW2, BA0000033.2 [39602:41608] |
| mecR1-trunc | Methicillin resistance operon repressor | 7678–8652 | 41,836–42,810 | 975 | Forward (trunc.) | COL, CP000046 [42724:42957] and MW2, BA0000033 [42917:44440] |
| hsdR2-IS1272 | Fragment of type I restriction-modification system endonuclease | 8653–8886 | 42,811–43,044 | 234 | Truncated | COL, CP000046 [42724:42957] and MW2, BA0000033 [42917:44440] |
| tmplIS1272 | Transposase for IS1272 | 8887–10,410 | 43,045–44,568 | 1524 | Reverse | COL, CP000046 [42958:44481] and MW2, BA0000033 [42917:44440] |
| Q9KX75  | Putative protein | 10,546–11,052 | 44,704–45,210 | 507 | Reverse | COL, CP000046 [44724:45297] and MW2, BA0000033 [44724:45297] |
| Q7A207  | Putative protein | 11,068–11,379 | 45,226–45,537 | 312 | Reverse | 06BA18369, ARXY01000001 [13883:13519] |
| Q7A206  | Putative protein | 11,466–11,816 | 45,624–45,974 | 351 | Reverse | 06BA18369, ARXY01000001 [135281:135631] |
| ccrB-1  | Cassette chromosome recombinase B, type 1 | 12,282–13,907 | 46,440–48,065 | 1626 | Reverse | COL, CP000046 [137744:139093] |
| ccrA-1  | Cassette chromosome recombinase A, type 1 | 13,929–15,278 | 48,087–49,436 | 1350 | Reverse | 06BA18369, ARXY01000001 [137744:139093] |
| cch-1   | Cassette chromosome helicase | 15,466–17,235 | 49,624–51,393 | 1770 | Reverse | CMFT532, HF569114 [8453:10222] |
| DUF1413-06BA18369 | Putative protein associated with cch | 17,235–17,525 | 51,393–51,683 | 291 | Reverse | 06BA18369, ARXY01000001 [141050:141340] |
| Q83ZD5  | Putative protein | 17,696–18,769 | 51,854–52,927 | 1074 | Forward | 06BA18369, ARXY01000001 [141511:142584] |
| Helicase M06 | DEAD/DEAH box helicase domain protein | 18,863–20,803 | 53,021–54,961 | 1941 | Forward | 06BA18369, ARXY01000001 [142678:144618] |
| Q6GD51  | Putative protein | 21,060–21,368 | 55,218–55,526 | 309 | Forward | 06BA18369, ARXY01000001 [144875:145183] |
| D3QFP0-SCC | Putative lipase/protease | 21,415–21,653 | 55,573–55,811 | 239 | Reverse (trunc.) | 06BA18369, ARXY01000001 [145230:145468] and MSSA476, BX571857 [52281:52519] |
CC1153-MRSA were mostly identified in Kuwait and the UAE. The clear majority, i.e. all isolates except the oldest one, harboured SCCmec/SCCfus chimeric elements and the most common variant that could be described either as SCCmec I + fusC element or as a pseudoSCCmec class B + fusC + ccrA/B-1 element was sequenced. Sequence analysis also revealed (see above and Table 1) that ccrB-1 and accompanying genes are more related to alleles from other SCCfus elements rather than to the ones from SCCmec. Thus, a description as a pseudoSCCmec class B + ccrA/B-1 + fusC element should be regarded as the correct one. The mec complex B could have been derived from either a SCCmec I or SCCmec IV element. However, the latter one was more probable based on of the MW2-like allele of mecA.

The entire region associated with fusC (encompassing Q7A207, Q7A206, ccrB-1, ccrA-1, DUF1413, Q83ZD5, helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, fusC, tnpIS150, tnp_A8YYY6, Q4LAG7-SCCfus and yobV) could be seen as one mobile genetic element that got introduced into a CC1153-MRSA replacing Q7A207, Q7A206 and the ccr recombinase genes that previously belonged to its SCCmec element. This set of genes was also

| Gene ID         | Explanation                  | Position in SCC (nt) | Position in genome (nt) | Length (nt) | Direction | Sequence identical to                                                                 |
|-----------------|------------------------------|----------------------|-------------------------|-------------|-----------|--------------------------------------------------------------------------------------|
| D3JCW9         | Putative protein             | 21,478–21,636        | 55,636–55,794           | 159         | Forward   | 06BA18369, ARXY01000001 [145293:145451] and MSSA476, BX571857 [52344:52502]         |
| fusC           | Fusidic acid resistance protein C | 21,954–22,592      | 56,112–56,750           | 639         | Forward   | 06BA18369, ARXY01000001 [145769:146407] and MSSA476, BX571857 [52820:53458]         |
| tnpIS150       | Transposase of IS150         | 23,068–23,382        | 57,226–57,540           | 315         | Forward   | CMFT463, H569110 [16043:16357]                                                      |
| tnp A8YYY6     | Transposase                  | 23,394–24,209        | 57,552–58,367           | 816         | Forward (trunc.) |                                                                                     |
| Q4LAG7-SCCfus  | Putative protein             | 24,356–24,784        | 58,514–58,942           | 429         | Reverse   | 06BA18369, ARXY01000001 [148171:148599]                                               |
| yobV           | Transcriptional regulator    | 24,864–25,793        | 59,022–59,951           | 930         | Forward   | 06BA18369, ARXY01000001 [148679:149608]                                              |
| DR_SCC         | Direct repeat of SCC         | 25,890–25,908        | 60,048–60,066           | 19          | Reverse   |                                                                                     |

Table 1. The SCCmec/SCCfus composite element in M58.
found in a *Staphylococcus hominis* subsp. *hominis* strain and a CC5-MRSA from Canada (06BA18369 GenBank ARXY00000000.1) as described above. Furthermore, MRSA strains from Saudi Arabia25 (as represented by isolates CMFT492, HF569112.1 and CMFT532, GenBank HF569114.1; see Tables 2, 3, 4 and 5) also carried the same region associated with fusC (differing from the one in MSS only in minor random deletions) as part of complex chimeric SCCmec II elements. In CMFT492, this cluster was inserted between orfX and a truncated SCCmec II element that lacked the *ldp* locus, *cstA/B/R* and the transposons introducing *ble/taadD* and *erm(A)/ant9*. In CMFT532 and other strains (see Tables 2, 3, 4 and 5), the region associated with fusC was inserted between orfX and a normal SCCmec II element. In these strains, additional markers (sccterm13, Q8CU82, tarF-SCC, A9UFT0, Q9KX75) were also associated with the fusC element that were absent in M58. Furthermore, there were also strains such as FORC 090, GenBank CP029198.1 or AR466, CP029080.1 in which fusC and its immediate neighbours (Q83ZD5, helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, tpnIS150, tsp A8YY86, Q4LAG7-SCCfus and yobV) were accompanied by other *ccr* recombinase genes and other genes upstream, towards orfX. This prompted us to review published sequences and to compare them with the CC1153 strain described herein to sort and to classify the different gene clusters accompanying fusC.

**Review of fusC elements.** When comparing the region around fusC from M58 to published sequences, it became obvious that 64 published *S. aureus* sequences (plus three *S. hominis* sequences and nine un-assembled *S. aureus* sequences from the Short Read Archive) cluster into 31 different SCCfus or SCCmec/fusC chimeric or composite elements (Tables 2, 3, 4, 5, Supplemental File 5). None of these fully matched the M58 sequence. When considering only the immediate region around fusC, strains were identified in which the same gene cluster as M58 was present and it was observed that there are only four, possibly five, different sets of genes directly accompanying this resistance gene (Tables 2, 3, 4, 5, Supplemental File 5 and Fig. 2).

These sets could be regarded as fixed gene complexes in analogy to the mec complexes A (in SCCmec II and III), B (in SCCmec I and IV) and C (in SCCmec V). Likewise, they are also associated with different sets of SCC-recombinase genes including alleles of *ccrA-1/ccrB-1, ccrA-1/ccrB-3, ccrA-3/ccrB-3, ccrA/ccrB* (FORC 90) and *ccrA/ccrC*. Resulting "SCCfus" elements can, besides fusC-complexes and recombinase genes, also carry additional payload including *tarF* (teichoic acid biosynthesis protein F), *speG* (spermidine N-acetyltransferase), various variants of type I restriction-modification systems or mec complexes. They also can be linked to entire SCCmec elements resulting in complex genomic islands sometimes even including multiple sets of recombinase genes. These additional components can be localized upstream (towards orfX) or downstream (see Tables 2, 3, 4, 5, Supplemental File 5).

The longest known of the fusC-complexes ("A", Table 2, see also Supplemental File 5 and Fig. 2), as in MSSA476, BX571857.1, comprises Q6GD54 (putative protein), Q6GD53 (putative protein), *tirS* (staphylococcal Toll/interleukin-1 receptor domain mimic), Q6GD51, D3QFP0-SCC, D3JCW9, fusC, sccterm03, Q6GD49 (putative protein), Q8CU43 (putative protein), Q4LAG7-SCCfus and yobV (for explanations and GenBank entries of the genes discussed, see Supplemental File 4). It can be found in MSSA, such as the prototypical CC1-MSSA sequence Sanger MSSA476, as well as in MRSA. It appears in MRSA strains (CC1 and CC5) with SCCmec IV elements, mainly from the Middle East25, 28, 30, 31, Australia and New Zealand26 as well as in SCCmec V strains from the Middle East.

A second fusC-complex ("B", Table 3, see also Supplemental File 5 and Fig. 2) comprises Q6GD54, Q6GD53, *tirS*, Q6GD51, D3QFP0-SCC, D3JCW9 and fusC. Besides gene content, it also differs from all others in five characteristic single nucleotide polymorphisms (SNP) within the fusC gene (14A > C, 150T > G, 537A > T; 632T > C). This complex has apparently not yet been observed in MSSA but there are several MRSA strains harbcing it connected to various SCCmec elements. One is HDE288, as prototypical sequence the "New Paediatric" strain from Portugal48. Here, the fusC-complex B, a combination also referred to as SCCmec VI. Another CC5 (ST149) strain, known from Malta46, the Middle East14, 25 and UK26 harbours the same fusC-complex, together with *ccrA/B-3* alleles and a SCCmec IV element. It also appears, although Q6GD54 is absent, in a SCCmec I MRSA strain from France (CC5, "Geraldine Clone")49.

A third fusC-complex ("C", Table 4, see also Supplemental File 5 and Fig. 2) consists of Q83ZD5, helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, fusC, sccterm150, tsp A8YY86, Q4LAG7-SCCfus and yobV. The fusC sequence is identical to the one in MSSA476, BX571857.1. This is the variant found in M58 and the other strains discussed above. In these strains, it is accompanied by a largely identical set of recombinase-associated genes. The carriage of sccterm13, Q8CU82, *tarF-SCC, A9UFT0, Q9KX75* as additional payload is variable; in M58 these genes are replaced by the mec complex, while in the CMFT492, CMFT535 etc., the SCCmec element is located downstream away from orfX25. Strains FORC090 (CP029198.1), AR466 (CP029080.1), MRSA18 (SAMEA1317993)30 and 20121643 (ERR159588/SAMEA3924203) harbour the same fusC-complex but in these sequences, it is accompanied by other recombinase alleles.

A fourth fusC-complex ("D1", Table 5, see also Supplemental File 5 and Fig. 2) consists of Helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, fusC, sccterm03, Q6GD49, Q8CU43, Q4LAG7-SCCfus and yobV. Its fusC gene has one characteristic SNP (309G > A). It was not yet found in MSSA, but in MRSA belonging to CC8, CC30 and ST83425. In these strains, it is accompanied by a mec complex B and a set of *ccrA/B-4* genes. It has been found neither in any other context, nor in MSSA strains.

A CC15 SCCmec V MRSA strain harbours a fusC-complex consisting of helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, fusC, sccterm03, Q6GD49 and Q8CU43. The fusC sequences contain a specific SNP (486T > C), but otherwise, gene content (apart from the lack of Q4LAG7-SCCfus and yobV) and the order of genes are the same as in the fourth complex (hence, "D2", Table 5, see also Supplemental File 5 and Fig. 2). The fusC-complex itself is in all these sequences localised on one contiguous, but other associated markers such as SCCmec, orfX etc. are split across several contigs. One ST72 sequence (HST-084, AZTF00000000.1) has a SCCmecV/SCCfus chimeric
Table 2. Staphylococcal Cassette Chromosomes with a \textit{fusC}-associated complex A consisting of \textit{Q6GD54}, \textit{Q6GD53}, \textit{tirS}, \textit{Q6GD51}, \textit{D3QFP0-SCC}, \textit{D3JCW9}, \textit{fusC}, \textit{sccterm03}, \textit{Q6GD49}, \textit{Q8CU43}, \textit{Q4L47G7-SCC} and \textit{yobV}. Please note that this is an abridged version (with genes encoding “putative proteins” omitted); for a more detailed version, see Supplement 5. References 25, 26, 35, 38–46. *Variably present in different sequences of the respective type. **Split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined.

| Reference Sequence: Strain (Clonal Complex), GenBank accession | Other SCC markers upstream/towards from \textit{orfX} | \textit{fusC}-associated recombinases | \textit{fusC}-associated complex | Other SCC markers downstream/away from \textit{orfX} | Other genome sequences harbouring the same SCC/\textit{fusC} element | Other references or descriptions and geographic background |
|---------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------------------------|
| **MSSA49** (CC4), BX217353 | \textit{sccterm06} (terminus of SCC towards \textit{orfX}), \textit{AoCH} (type I restriction-modification system; TIRMS, endonuclease), \textit{hsdM} (TIRMS site-specificity determinant), \textit{hsdR} (TIRMS DNA methylase) | \textit{ccrB-1, ccrD-1} | A | N/A | \textit{SCC} IV | \textit{MSSA} 25, 28, 29 (from the USA) |
| **MSSA50** (CC1), BX217355 | \textit{sccterm06} (terminus of SCC towards \textit{orfX}), \textit{hsdM} (TIRMS, endonuclease), \textit{hsdR} (TIRMS DNA methylase) | \textit{ccrB-1, ccrD-1} | A | N/A | N/A | \textit{MSSA} 25, 28, 29 (from the USA) |
| **MSSA64** (CC7), LT671859 | \textit{sccterm06} (terminus of SCC towards \textit{orfX}), \textit{hsdR}, \textit{hsdS}, \textit{speG} \textit{ccrB-1} | \textit{ccrB-1, ccrD-1} | A | N/A | N/A | \textit{MSSA} 25, 28, 29 (from the USA) |
| **MSSA65** (CC7), LT671860 | \textit{sccterm06}, \textit{hsdR}, \textit{hsdS}, \textit{speG} \textit{ccrB-1} | \textit{ccrB-1, ccrD-1} | A | N/A | N/A | \textit{MSSA} 25, 28, 29 (from the USA) |

Table 3. Staphylococcal Cassette Chromosomes with a \textit{fusC}-associated complex B consisting of \textit{Q6GD54}, \textit{Q6GD53}, \textit{tirS}, \textit{Q6GD51}, \textit{D3QFP0-SCC}, \textit{D3JCW9} and \textit{fusC} (14A > C; 150 T > G; 290G > C; 537A > T; 632 T > C). Please note that this is an abridged version; for a more detailed version, see Supplement 5. References 14, 25, 26, 35, 47–51.
Table 4. Staphylococcal Cassette Chromosomes with a fusC-associated complex C consisting of Q83ZD5, helicase M06, Q6GD51, D3QFPO-SCC, D3CJW9, fusC, tnpIS150, tnpA8YYY6, Q4LAG7-SCCfus and yobV. Please note that this is an abridged version; for a more detailed version, see Supplement 5. References25, 26, 45, 52, 53.

| Reference Sequence: Strain (Clonal Complex), GenBank accession | Other SCC markers upstream/towards from seqX | fusC-associated recombinases | fusC-associated complex | Other SCC markers downstream/away from seqY | Other genome sequences harbouring the same SCCfus element | Other references or descriptions and geographic background |
|---------------------------------------------------------------|---------------------------------------------|-----------------------------|-------------------------|-----------------------------------------------|---------------------------------------------------------------|
| RUH-52 (CC30), MK91791.3                                      | mcr complex B                              | ccrB-4, ccrC-4              | D1                      | N/A                                           | CC-MRSA from Germany/Middle East15, CC-MRSA from Kuwait, Saudi Arabia and UAE17, ST34-MRSA from Saudi Arabia 18, Human and livestock CC13-MRSA from Saudi Arabia 17, S. hominis subsp. hominis from Lebanon |
| RUH-62 (CC30), MF182004.1 to -09.1**                          | mcr complex C                              | ccrB-4, ccrC-4              | D2                      | SCCmec V with integrated plasmid harbouring aux-de-uptake** | RUH-71 (CC15), NIZW, RUH-98 (CC15), NIZW, RUH-99 (CC15), NIZW |
| H30-004-CC75, AL21**                                          | SCCmec V1**                                | ccrB-4                      | N/A                     | N/A                                           | CC13-MRSA from Lebanon |

Table 5. Staphylococcal Cassette Chromosomes with a fusC-associated complex D consisting of Helicase M06, Q6GD51, D3QFPO-SCC, D3CJW9, fusC (309G > A), and yobV (D1) or HelicaseM06, Q6GD51, D3QFPO-SCC, D3CJW9, fusC (486T > C), tnpIS150, tnpA8YYY6, and yobV. Please note that this is an abridged version; for a more detailed version, see Supplement 5. References25, 26, 30, 34. **Split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined.

| Reference Sequence: Strain (Clonal Complex), GenBank accession | Other SCC markers upstream/towards from seqX | fusC-associated recombinases | fusC-associated complex | Other SCC markers downstream/away from seqY | Other genome sequences harbouring the same SCCfus element | Other references or descriptions and geographic background |
|---------------------------------------------------------------|---------------------------------------------|-----------------------------|-------------------------|-----------------------------------------------|---------------------------------------------------------------|
| RUH-52 (CC30), MK91791.3                                      | mcr complex B                              | ccrB-4, ccrC-4              | D1                      | N/A                                           | CC-MRSA from Germany/Middle East15, CC-MRSA from Kuwait, Saudi Arabia and UAE17, ST34-MRSA from Saudi Arabia 18, Human and livestock CC13-MRSA from Saudi Arabia 17, S. hominis subsp. hominis from Lebanon |
| RUH-62 (CC30), MF182004.1 to -09.1**                          | mcr complex C                              | ccrB-4, ccrC-4              | D2                      | SCCmec V with integrated plasmid harbouring aux-de-uptake** | RUH-71 (CC15), NIZW, RUH-98 (CC15), NIZW, RUH-99 (CC15), NIZW |
| H30-004-CC75, AL21**                                          | SCCmec V1**                                | ccrB-4                      | N/A                     | N/A                                           | CC13-MRSA from Lebanon |

*split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined.

**split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined.

The fusC-associated complex D contains Helicase M06, Q6GD51, D3QFPO-SCC, D3CJW9, fusC (309G > A), and yobV. This complex is known to be associated with SCCmec V and SCCmec V+.

Further investigations on fusC-complexes associated with SCCmec V+V7 are warranted as such isolates from diverse clonal complexes including CC5, CC97, CC121 and CC1153 (see above) have been observed, especially in the Arabian Gulf region.

Finally, truncated fusC-complexes were also observed as part of a very complex composite SCCmec element in a CC779 isolate M06/0171, HE980450.132 and of a composite “pseudo-SCC” element (i.e., without ccr genes) in Staphylococcus hominis subsp. hominis TFGsh5-1, AB930128.1. As the third and the fourth complex, it encompasses genes for helicase M06, Q6GD51, D3QFPO-SCC, D3CJW9 and fusC; but its fusC allele indicates relation to the third one (Table 4, see also Supplemental File 5).
Figure 2. The different sets of genes directly accompanying fusC in M58 and in previously published SCCfus or SCCmec/fus elements.
There was no obvious phenotypical correlation of fusC-complexes to fusidic acid MICs, all tested strains (Supplemental File 6) were highly resistant regardless of their actual type of fusC-complex.

Further open questions are the timeframe of the evolution of SCCfus elements as well as their geographical origins. A wide variety of fusC-positive strains has not yet been sequenced. It would be interesting to know whether additional elements exist, and whether there are MSSA strains harbouring those fusC-complexes yet observed in MRSA only. The origin and evolutionary history of tirS is another open question. This virulence factor\(^ {33} \) is present in two out of five fusC-complexes but to the best of our knowledge, it has never been observed in another context.

As previously discussed\(^ {33,48} \), fusC was detected in as much as twenty-two different clonal complexes of \textit{S. 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 essentially all parts of the world. This and the emergence of fusC-MRSA especially in the Middle East indicate a selective advantage associated with its presence. Fusidic acid can be administered intravenously, but this is not commonly done, and the intravenous formulation is not available everywhere. It is also used topically, as ointment for presumably staphylococcal skin conditions. Observations from New Zealand suggest a quick emergence of fusC-positive \textit{S. aureus} in parallel to an increasing use of this compound\(^ {34,49} \). A co-evolution of SCCmec and SCCfus elements might result in a public health hazard, as strains with composite or chimeric elements are selected for both, in the hospital by beta-lactam administration as well as in the community by topical use of fusidic acid. Thus, antibiotic stewardship and infection control measures targeting MRSA in the hospital must be accompanied by restrictions to an uncontrolled over-the-counter sale of fusidic acid in outpatient settings as well as by a prudent use in outpatient settings.

Materials and methods

Isolates. A list of the isolates studied is provided in Supplement 1. Isolates were selected out of various typing and epidemiological projects based on array hybridisation profiles indicative for an affiliation to CC1153. The CC1153-MRSA from the UAE were isolated from skin and wound infections. The CC1153-MRSA from Saudi Arabia also originated from a wound infection. One CC1153-MRSA from Saudi Arabia was a nasal colonizer from a healthcare worker, the others originated from skin and soft tissue infections (with three of them being identified during an earlier study\(^ {49} \)). The first CC1153-MRSA reported in Kuwait was cultured from a wound inflicted by a dog bite in 2009\(^ {33} \). The other isolates were obtained between 2017 and 2020 mostly from wound infections of patients located in six hospitals. One isolate was isolated from a gynaecological swab, one from a nasal swab and one was obtained from blood culture. Egyptian isolates were identified from rural small-holder dairy cattle that showed sub-clinical mastitis, i.e., somatic cell counts >200,000 cell/mL and positive results of California Mastitis Test. The isolates were collected from six different cows in a herd, consisting of 25 crossbred dairy cows, located at Dakahilia Governorate in the northeast of Cairo, Egypt. The milking procedure was performed manually in the examined cows, while the medical records of the farm revealed the usage of a wide spectrum of antibacterial agents\(^ {37} \). French isolates (11 MSSA, 1 MRSA) had been isolated during infections (cutaneous (n = 8), respiratory (n = 3), blood culture (n = 1)) in ten different hospitals between 2010 and 2017. One German MRSA isolate was cultured from an abscess of an approximately half-year-old Egyptian child whose family lives in Germany. One German MSSA isolate originated from an abscess.

PVL detection was performed on six isolates by an experimental lateral flow test\(^ {54} \). Fusidic acid MICs were determined by agar gradient dilution tests with commercially available strips (01B10122 Fusidinsäure MIC Test Strip 0.016—256 µg/mL, Bestbion dx GmbH, Cologne, Germany).

Microarray-based molecular characterization. Genotyping of all strains was performed using the \textit{S. aureus} Genotyping Kit 2.0 system (Abbott [Alere Technologies GmbH, Jena, Germany]) microarray-based assay. The array covers 333 different targets related to approximately 170 different genes and their allelic variants. The list of target genes as well as sequences of probes and primers has previously been published along all relevant protocols\(^ {33,39,48} \).

\textit{Staphylococcus aureus} was cultivated on Colombia blood agar. The DNA extraction was performed using lytic enzymes (lysozyme, lysozyme, RNAse) and buffer from the \textit{S. aureus} Genotyping Kit 2.0 kit and Qiagen DNA extraction columns (Qiagen, Hilden, Germany) according to manufacturers’ instructions. Then, a linear amplification was performed using one primer for each target sequence. During the linear multiplex-amplification, biotin-16-dUTP was incorporated into the amplicons, which were then stringently hybridised to the microarrays. Microarrays were photographed and analysed with a designated reader and software (IconoClust, Abbott [Alere Technologies]). Analysis allowed detecting presence or absence of certain genes or alleles, as well as assignment to the clonal complex, strains, and SCCmec types.

Whole-genome sequencing. Genomic DNA was isolated from an overnight culture grown at 37 °C on Columbia blood agar using a Macherey and Nagel NucleoSpin Microbial DNA kit (MACHEREY–NAGEL GmbH & Co. KG, Düren, Germany).

The Nanopore Oxford MinION platform was used for sequencing the whole genome of the CC1153 isolate M58 from the UAE. Briefly, size selection was performed using AMPure beads in a ratio 1:1 (v/v) with the DNA sample. The DNA library was generated using the nanopore sequencing kit SQK-LSK109 and the native barcoding expansion kit EXP-NBD103 (Oxford Nanopore Technologies, Oxford, UK) according to manufacturer’s instructions. The used flowcell FLO-MIN106 (R9-Version) was primed by the flow cell priming kit EXP-FLP9001 (Oxford Nanopore, Oxford, UK). The protocol named “1D Native barcoding genomic DNA” was used in version
Phylogenetic analysis. A panel of 154 non-motile, core genomic markers was selected. Inclusion criteria were presence in all S. aureus clonal complexes analysed as well as uniform length in all genomes. The used genes as well as the genome sequences considered are listed in Supplemental File 3. Sequences were concatenated and analysed using SplitsTree 4.06 using default settings (Supplemental File 3).

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Author contributions

S.M., E.M., S.D.B. and R.E. wrote the manuscript drafts, M.A.P., M.B., S.B., M.E.A., M.G., R.N., H.H., A.S., A.M.S. and E.U. provided isolates and their initial identifications as well as background information. E.M., M.B., S.B., D.G., A.R., R.N. and A.R.L. did array experiments, S.D.B. and I.E. performed sequencing and initial analysis. S.M. analysed sequences and reviewed published sequences. All authors read, revised and approved the manuscript.
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Competing interests
IE is employee of BLINK AG, Jena, Germany. She provided crucial advice and supervision regarding Nanopore sequencing, as an experienced user of that technology but without having commercial affiliations or connections to Oxford Nanopore. DG is employee of PTC—Phage Technology Center GmbH, Hönen, Germany. He performed experiments for this study before being employed by this company. Thus, both commercial entities had no influence on the decision to publish and on the content of the study. All other authors declare no competing interests.

Additional information
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