mecC MRSA in Israel—genomic analysis, prevalence and global perspective

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Background: MRSA is a major global healthcare problem. In 2011, a new mec variant designated mecC was described, presenting partial identity at the DNA level, thus undetectable by routine mecA PCR.

Objectives: Until now, no reliable information regarding mecC MRSA prevalence was available in Israel. In this study, to the best of our knowledge, we describe the first case of mecC MRSA in Israel, with focus on genomic analysis and global context.

Methods: The mecC MRSA isolate was analysed by WGS with focus on phylogeny, global contextualization, virulence and resistance genes. The strain was characterized by antibiotic susceptibility testing, spa typing and presence of mecA/C and pvl genes.

Results: An MRSA strain (SA10610), isolated from a urine sample of an 83-year old patient, was found negative for the mecA and pvl genes. The MLST and spa type were ST130 and t1736, respectively. SA10610 presented resistance to oxacillin, penicillin and cefoxitin, and susceptibility to all non-β-lactam agents tested. Phylogenetic comparison with a global dataset of 586 mecC MRSA genomes revealed substantial genomic divergence. The nearest genomic relatives were human and animal isolates from Denmark. A screen of 12 761 S. aureus isolates collected during 2011–18 in Israel indicated this is the only mecC-positive strain.

Conclusions: A high degree of genetic variability was found between the SA10610 strain and previously sequenced mecC MRSA isolated worldwide. The genomic and phylogenetic analysis suggest that mecC MRSA isolates have evolved independently rather than from a common ancestor.

Introduction

MRSA is a major bacterial human pathogen involved in a wide variety of diseases, ranging from relatively minor superficial skin infections to serious and life-threatening invasive infections. In addition to infections in humans, MRSA can cause diseases in a wide range of hosts including livestock, wildlife and companion animals.1

Resistance to β-lactams in Staphylococcus aureus is mediated by the mec genes, including mecA and mecC, which encodes an alternative PBP with a lower affinity for virtually all β-lactam antibiotics. The mec genes reside within a mobile genetic element named the staphylococcal cassette chromosome mec (SCCmec) and resistance to β-lactams is conferred by the acquisition of this cassette.2 Based on their genetic content and their structural organization, SCCmec elements have been classified into types and subtypes and to date 14 types (I–XIV) have been described.3–4

In 2011, during an epidemiological study of bovine mastitis, a new mec variant was described.5 This variant, named mecC (formerly mecC_{LA251}) exhibits only 70% nucleotide sequence homology with the classical mecA gene5 and 63% identity at the amino acid level.5 The new mec gene is located on a novel SCCmec element, designated type –XI SCCmec. As a consequence of the limited mecA-mecC sequence homology and their respective proteins, mecA PCR and immunological tests targeting PBP2a fail to detect mecC MRSA, posing a diagnostic challenge for clinical microbiology laboratories.5–7 S. aureus can be found in the normal flora of healthy humans and animals. However, it can cause diseases in both hosts as an opportunistic pathogen. During the last few years a new type of MRSA has emerged, livestock-associated (LA) MRSA and infections with this type of MRSA have been increasingly reported worldwide especially in people with occupational livestock exposure.8–11 Clonal complex (CC) 130 is the most prevalent LA MRSA lineage in Europe although other lineages such as CC1 and CC7
also have been found to colonize and cause infections in livestock.

In the last decade, MRSA clones with a meC gene have been detected in different animal species and humans, mainly in European countries but also on other continents, with isolates mainly belonging to CC130, CC1943 and CC425. Zoonotic transmission of meC MRSA has been previously reported, although data on the prevalence, animal reservoir and epidemiology of meC MRSA are still limited.

This study represents, to the best of our knowledge, the first report of meC-positive MRSA isolation in Israel, with focus on genomic and phenotypic characterization. Phylogeny and global context were analysed by genomic comparison with meC-positive MRSA genomes isolated from humans and animals.

Materials and methods

Bacterial isolates, media and lysates

S. aureus SA10610 was isolated from a urine sample of an 83-year-old male patient in October 2017. The sample had been submitted to the national S. aureus reference centre for bacteriological characterization. All MRSA and MSSA isolates from bacteraemia as well as MRSA from wound infections are referred to the national centre for further analysis. Between 2011 and 2018, 12,761 S. aureus isolates were analysed by in-depth strain characterization and typing. All isolates were stored in a deep freeze in our strain bank. spa types known to be prevalent in meC S. aureus strains: t843, t1773, t978, t1535, t1899, t6293, t7947, t7485, t7946, t7734, t11702, t6220, t9280, t373, 528, t1048, t1532, t3218, t3256, t3570, t5970, t6300, t6292, t6386, t742, t11706, t978, t1945, t2345, t1391, t8835, t529. NCTC 13552 strain, meC MRSA, was used as a control in meC PCR reaction. Strain SA104 (mecA and pvl positive, spa type 1008) was used as a positive control in mecA-PVL PCR and spa PCR. ATCC strain 43300 and 29213 were used as controls for Etest assay. All strains were grown at 37°C for 24–26 h.

The strains were cultured on 4S agar and were used as controls for Etest assay. All strains were cultured at 37°C for 18–24 h before conducting susceptibility tests. For Etest, several colonies were suspended in saline to a turbidity of 0.5 McFarland. The suspension was seeded on a Mueller-Hinton agar plate (Hy Laboratories Ltd, Rehovot, Israel) and then Etest strips (bioMérieux) were put on each agar plate for oxacillin and ceftoxitin. Plates were incubated at 35 ± 1°C for 24 h (oxacillin) and 18–24 h (ceftoxitin). MIC values were determined according to CLSI guidelines: resistance to oxacillin is defined at MIC values ≥ 4 mg/L; resistance to cefotaxin is defined at MIC values ≥ 8 mg/L.

Detection of resistance genes was carried out with the PATRIC tool using the Comprehensive Antimicrobial Resistance Database (CARD). Detection of virulence factors was carried out using the S. aureus functional genotyping tool.

Antimicrobial susceptibility testing (AST)

All MRSA strains were grown at 37 ± 1°C for 18–24 h before conducting susceptibility tests. For Etest, several colonies were suspended in saline to a turbidity of 0.5 McFarland. The suspension was seeded on a Mueller-Hinton agar plate (Hy Laboratories Ltd, Rehovot, Israel) and then Etest strips (bioMérieux) were put on each agar plate for oxacillin and cefoxitin. Plates were incubated at 35 ± 1°C for 24 h (oxacillin) and 18–24 h (cefoxitin). MIC values were determined according to CLSI guidelines: resistance to oxacillin is defined at MIC values ≥ 4 mg/L; resistance to cefoxitin is defined at MIC values ≥ 8 mg/L.

Brot microdilution was performed with Sensititre susceptibility plates (Gram-positive GPALL1F AST Plate) according to manufacturer’s instructions. Briefly, ~5 colonies were suspended in DDW to a turbidity of 0.5 McFarland. Then, 10 μL of the suspension were transferred into 1 mL of CAMHB (cat. Number T3462). The plate was inoculated using the Sensititre Autonoculator/AIM. Following incubation for 24 h, results were read using the VIZION platform (Sensititre). MICs were determined according to CLSI guidelines (M100 2020).

Vitek AST was done with the Vitek 2 automated AST system using an AST-P649 card (bioMérieux, Marcy-l’Étoile, France).

Data availability

The following genomes were used for WGS analysis comparison: meC_5417136 (accession: FR821779); M10/0061 (accession: FR823292); Patient A (accession: ERR084771); Cow A (accession: ERR144792); Patient B (accession: ERR144788); Sheep B (accession: ERR144749); OFVD (accession: OFVD01000000); OFUF (accession: OFUF01000000); meC MRSA isolated in Spain (accession: ERR403511); meC MRSA isolated in Brazil (accession: GCA_009763195.1); meC MRSA isolated in New Zealand (accession: ERR4517136); meC MRSA isolated in England (accession: ERR3595448); and meC MRSA isolated in Australia (accession: LLJG00000000). WGS of strain SA10610 was deposited in the pubMLST database under the ID 34000.

Results

Strain SA10610 was isolated from a urine sample from an 83-year-old prostate carcinoma patient referred to the HMO due to a urinary tract infection. Vitek analysis (GP CARD) at the regional HMO laboratory identified the isolate as MRSA (positive cefoxitin screen and oxacillin MIC ≥ 4 mg/L), and the patient was treated with oxacillin (Oflloxacin). In order to broaden the antibiotic resistance...
profile of the SA10610 isolate, broth microdilution testing was performed. SA10610 was tested for phenotypic susceptibility to common antimicrobial compounds indicating resistance to oxacillin, penicillin and cefoxitin and susceptibility to all other non-β-lactam agents tested (chloramphenicol, ciprofloxacin, levofloxacin, minocycline, daptoxin, erythromycin, gentamicin, linezolid, rifampicin, tetracycline, trimethoprim/sulfamethoxazole, vancomycin and nitrofurantoin).

**Molecular characterization**

Further analysis at the *S. aureus* national reference centre revealed that this strain is seemingly MSSA genotypically, negative for the mecA gene by PCR. The strain was phenotypically resistant to oxacillin and cefoxitin as determined by Etest (MIC values 24 mg/L and 32 mg/L, respectively), and Vitek 2 (0.5 mg/L and ≥6 mg/L, respectively). This observation was surprising and motivated us to check for the presence of the mecC gene. In PCR analysis for the mecC gene, SA10610 was found positive. The isolate was negative by PCR for the Panton–Valentine leukocidin (PVL) genes. MLST and spa typing assigned the isolate to ST130 and spa type t1736. PCR analysis for the detection of SCCmec type XI was positive.

We applied WGS in order to investigate the genomic context of the mecC gene, and the phylogenetic lineage of the strain. WGS of strain SA10610 revealed 100% identity to the mecC gene sequence of *S. aureus* strain TRN6234, isolated from a patient in a German hospital with a wound infection, and 99.9% identity to SCCmec type XI of strain M10/0061. The SCCmec sequence of strain SA10610 showed 99.9% identity to SCCmec type XI of strain M10/0061.

### Table 1. WGS analysis for the presence of mobile genetic elements (MGE) conferring antibiotic resistance

| Gene | Mobile genetic element | SA10610 mecAGA251 | Cow A | Patient A | Patient B | Sheep B | New Zealand | England | Spain | Australia | Brazil |
|------|------------------------|--------------------|-------|-----------|-----------|---------|--------------|---------|-------|-----------|--------|
| blaZ | Tn552                  | −                  | −     | −         | −         | −       | −            | +       | −     | −         | −      |
| mecA | SCCmec                | −                  | −     | −         | −         | −       | −            | +       | −     | −         | −      |
| mecC | SCCmec                | +                  | +     | +         | +         | +       | +            | +       | +     | +         | +      |
| tet(K) | pT181              | −                  | −     | −         | −         | −       | −            | +       | −     | −         | −      |
| tet(M) | Tn916              | −                  | −     | −         | −         | −       | −            | −       | −     | −         | −      |

### Table 2. Data available for all strains analysed or mentioned in current article

| Strain     | Accession number | Source     | spa type | ST  | mecA | mecC | pvl | scn | chp | ϕ3 |
|------------|------------------|------------|----------|-----|------|------|-----|-----|-----|----|
| SA10610    | 34000 (pubMLST)  | human      | t1736    | 130 | −    | +    | −   | −   | −   | +  |
| LSA25      | NA               | cattle     | t529     |     |      |      |     |     |     | −  |
| LSA57      | NA               | cattle     | t529     |     |      |      |     |     |     | −  |
| LSA63      | NA               | cattle     | t529     |     |      |      |     |     |     | −  |
| LSA50      | NA               | cattle     | t529     |     |      |      |     |     |     | −  |
| LSA52      | NA               | cattle     | t529     |     |      |      |     |     |     | −  |
| mecAGA251  | FR821779 (NCBI)  | milk container | t6300  | 425 | −    | +    | −   | −   | −   | NA |
| Patient A  | ERR08477 (NCBI)  | human      | t843     | 130 | −    | +    | −   | −   | −   | NA |
| Cow A      | ERR144792 (NCBI) | cattle     | t843     | 130 | −    | +    | −   | −   | −   | NA |
| Patient B  | ERR144788 (NCBI) | human      | t843     | 130 | −    | +    | −   | −   | −   | NA |
| Sheep B    | ERR144749 (NCBI) | sheep      | t843     | 130 | −    | +    | −   | −   | −   | NA |
| Australia  | LUG0000000000    | cot        | t6292    | 425 | −    | +    | −   | −   | −   | NA |
| Spain      | ERR403511        | deer       | NA       | 425 | −    | +    | −   | −   | −   | NA |
| England    | ERR3595448       | hedgehog   | t15289   | 6460| −    | +    | −   | −   | −   | NA |
| New Zealand| ERR54717136      | hedgehog   | NA       | 6432| −    | +    | −   | −   | −   | NA |
| Brazil     | GCA_009763195.1  | cow        | t605     | 126 | −    | +    | −   | −   | −   | NA |

*mecC* gene of this strain is not located in SCCmec type XI.

NA, not applicable.

NA, not applicable.
In addition, we compared the WGS-derived antimicrobial resistance profile of SA10610 isolate to that of the prototype mecA_LGA251, along with genomes of S. aureus isolates isolated from human and animal origin worldwide. The results presented in Table 1 show agreement between all isolates tested except from mecC MRSA strain isolated from Sheep B and mecC MRSA isolated in Brazil, which were positive for pT181, a tet(K)-carrying plasmid, and Tn552, a β-lactamase-carrying transposon, respectively.

We investigated the prevalence of mecC among a national strain bank maintained by the national reference centre. The fact that specific spa types were associated with mecC-positive S. aureus isolates motivated us to screen our strain bank database for common spa types, known to be prevalent in mecC S. aureus strains. None of the mecC-related spa types were found in our human-origin strains database. However, five S. aureus strains of animal origin (LSA25, LSA50, LSA52, LSA57 and LSA63) that belonged to spa type t529 were found in our database. Further analysis revealed that those strains are negative for mecA, mecC and pvl (Table 2).

**WGS analysis**

mecC MRSA strains have been isolated from a vast range of countries and some of the genomes were deposited in public databases. We aimed to compare the SA10610 sequence to globally reported mecC-positive MRSA. Using the BioNumerics software, we generated a minimum spanning tree based on wgMLST data of 586 mecC-positive MRSA isolates whose genomic assemblies were deposited in the pubMLST database. The tree presented in Figure 1 shows that SA10610 is closely related to CC130. This large clade consists of 443 isolates that belong to CC130; of them, 327 belong to ST130 and the rest to closely related STs. We further assessed the diversity between SA10610 genome and the genome of the prototype mecA_LGA251 along with other sequenced genomes of well-characterized mecC MRSA isolates by generating a minimum spanning tree on wgMLST data. The minimum spanning tree presented in Figure 2 shows that SA10610 is closest to isolates from Denmark and belongs to ST130. There is a clear distinction between SA10610 and mecA_LGA251, mecC MRSA isolates isolated in Brazil, England and New Zealand. On the other hand, the genomes of strains Cow A, Patient A, Sheep B, Patient B, OFVD and OFUF clustered into one clade which is differentiated by 273 alleles from the SA10610 genome.

**mecC MRSA isolates have been globally isolated from a wide range of diseases in humans and animals.**

We further investigated the virulence determinants and their relation to the cgMLST of the SA10610 isolate by analysis of the whole genome.
sequence, and compared it with mecC MRSA genomes including mecA LGA251 and mecC MRSA strains isolated worldwide. The results presented in Figure 3 show correlation between cgMLST and virulence profile analysis, the virulence profile of SA10610 is similar to CC130 strains. In addition, the similarity level of virulence profile decreases as the level of cgMLST variability increases.

**Livestock**

The fact that mecC MRSA was first isolated from mastitis in a cow, and in addition that most of the patients from which mecC was isolated were in proximity to animals, stimulated us to try to determine its origin. In addition, the fact that five animal origin S. aureus strains of spa type t529, linked to CC130, were found in our database enabled us comparison between SA10610 isolate and animal origin strains. The results presented in Table 2 show that all isolates tested were negative for scn, chp and φ3 PCR except from strains LSA25 and SA10610, which were positive for φ3 only. LSA63 was positive for both φ3 and scn. In addition these results are reinforced by the WGS analysis of the sequenced genome of strain SA10610, showing that strain SA10610 is negative for the human immune evasion genes sak, chp and scn. These genes are carried on the βC-ΦS bacteriophage alongside sea genes, which encode the immune evasion molecule staphylokinase and enterotoxin A, respectively, which are also absent from the SA10610 genome. On the other hand, tet(M), an animal-origin marker, is also absent from the SA10610 genome.

**Discussion**

The first discovery of mecC MRSA was as a result of epidemiological study of bovine mastitis in 2007, subsequently, more mecC-positive isolates were identified, both of human and animal origin, in other countries. As far as we know, this is the first report of mecC MRSA in Israel, which is assigned spa type t1736 and ST130 and belongs to clonal complex CC130, which is the major lineage responsible for the vast majority of mecC isolates to date. The antibiotic susceptibility pattern of strain SA10610, as reported for most mecC MRSA isolates, is characterized by susceptibility to the majority of all non-β-lactam antibiotics.

In addition, all the genomes tested in this analysis, except from the isolate isolated in Brazil, carry the same SCCmec and have very similar horizontally transmissible accessory genomes. The patient from whom SA10610 was isolated lives in an urban environment and data regarding animal exposure was unavailable. Human-associated isolates carry phages encoding human innate immune modulators that are rare in livestock-associated isolates and therefore may be used as markers.
In this report we are trying to consider our findings. The fact that the distance between the clades is substantial in the cgMLST, resistance and virulence profiles may reflect a weakly clonal lineage.

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Transparency declarations
None to declare.

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