Report of meCC-carrying MRSA in domestic swine

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Introduction

MRSA carrying a new variant of the mecA gene, now named mecc, was first described in 2011 from humans and cattle in the UK, Ireland and Denmark.1,2 García-Álvarez et al.3 demonstrated that the mecc gene is encoded by the type XI staphylococcal cassette chromosome mec (SCCmec) element, which is known to be present in at least four Staphylococcus aureus lineages: CC130, CC325, CC705 and CC1943. mecc was retrospectively detected in a human MRSA isolate from Denmark isolated in 1975.3

Petersen et al.4 found that mecc-MRSA CC130 and CC2361 account for ~2% of the annual number of MRSA cases in Denmark. They also found that some human mecc-MRSA CC130 isolates with spa type t843 were related to isolates from sheep and cows. Farm transmission of mecc-MRSA between humans and ruminants has also been demonstrated previously by WGS.5 mecc-MRSA has been isolated from a number of livestock species, such as cattle, sheep and horses, as well as from companion animals and a large number of wild animal species, including a single report from wild boar in Spain.6,7 To the best of our knowledge, this is the first report of mecc-MRSA isolated from domestic swine.

Materials and methods

Nasal swabs were initially collected from 10 weaners in a Danish swine farm in connection with an ongoing MRSA study. The farm was situated in eastern Zealand and contained ~600 sows and 100 beef cattle. The cattle were occasionally located in the same buildings as the swine and occasionally in pens that had been used by swine. The workers at the farm did not change clothes or boots when moving between the buildings or animal species.

The samples were cultivated on Brilliance MRSA 2 agar plates (Oxoid) with and without pre-enrichment in tryptic soy broth (Sigma–Aldrich) containing 6.5% NaCl, and denim blue colonies were subcultured for verification. Two out of 10 weaner samples were found to be positive for MRSA. The isolates were found to be positive for mecc by PCR.8 As this was, to the best of our knowledge, the first identification of mecc-MRSA from domestic swine, additional samples were collected. Nasal samples were collected from 10 sows in the farrowing unit, 10 piglets in the weaning unit and 10 slaughter pigs. In addition, nasal swabs from 20 beef cattle, 2 workers and the farm dog were tested. Approval from the National Committee on Health Research Ethics was not necessary for this investigation (Decision No. 16035690).

The mecc-MRSA were analysed by WGS. For comparative purposes we reviewed the national MRSA database to identify and extract information on persons colonized or infected with mecc-MRSA between 2013 and
mecC-MRSA in domestic swine

A total of 34 mecC positive isolates from Zealand were included, of which five originated in the same municipality as the swine farm. Samples and isolates included in the study can be found in Table 1. The methods used for WGS, phylogenetic analysis of the core genome, analysis of the accessory genome, MLST, SCCmec typing, and searching for resistance genes are available as Supplementary data at JAC Online. All sequences can be obtained using the ENA Study accession number PRJEB15105.

Antibiotic susceptibility testing was performed by MIC determination using a custom-made panel (DKSSP2, TREK Diagnostics). Antimicrobials tested, including ranges and breakpoints, are described in Table S1 (available as Supplementary data at JAC Online). spa typing was performed as described previously.

Results

From the farrowing unit, 9 out of 10 samples were found to be mecC-MRSA positive. In the weaning unit, five samples were found to be mecC-MRSA positive, in addition to the two isolates from the initial screening. All slaughter pigs and beef cattle as well as the farm dog were found to be MRSA negative. One MRSA isolate from each pig was further characterized. Fifteen swine isolates belonged to spa type t843 (repeat succession: 04–82–17–25–17–25–16–17), whereas the remaining swine isolate harboured spa type t11205 (repeat succession: 04–16–17). One of the two farm workers was MRSA positive, and in order to assess the genomic variation present within this individual, three isolates from this sample were whole-genome sequenced and spa typed; all three isolates belonged to spa type t1535 (repeat succession: 04–82–17–25–17–25–16–17).

All 19 mecC-MRSA isolates were phenotypically resistant to penicillin and cefoxitin, but susceptible to all other antimicrobials tested. All 19 mecC-MRSA isolates belonged to CC130 and did not carry mecA or any other resistance genes. The isolates were carrying the mecC gene on the mobile element SCCmec XI. All isolates carried the entire SCCmec XI element excluding strain H35, which did not contain three hypothetical genes downstream from the ars operon.

The phylogenetic analysis was based on 2530 variable SNPs identified within 84% of the reference genome and found to be conserved within the strain collection, and showed that the 19 farm isolates (16 swine and 3 human isolates) were found on a separate branch on the maximum-likelihood tree (Figure 1) with a pairwise distance between 0 and 30 SNPs (mean = 18). The three isolates from the farm worker (H1a–c) were found to branch out from the basal part of the group and differed by 5–12 SNPs. In comparison, between 11 and 24 SNP differences were observed between the human and swine isolates (W1–6 and F1–9). There were no canonical SNPs separating the human and swine farm isolates. The most closely related human isolates from the MRSA database originated from five persons living in the same municipality as the swine farm (H2–6) and all had spa type t843. The remaining human isolates originated from other parts of Zealand and were found to be diverse and only distantly related to the farm and municipality isolates.

Analysis of the accessory genome confirmed a very close affiliation between the farm isolates and the five human isolates from the same municipality (Figure S1, available as Supplementary data at JAC Online). A cluster of 48 genes was consistently present in the 48 genes are available as Supplementary data at JAC Online. All sequences can be obtained using the ENA Study accession number PRJEB15105. some of the identified genes comprise a novel entire or partial phage; however, using PHAST (http://phast.wishartlab.com), some of the identified genes are associated to an incomplete phage found in S. aureus strain LGA251 (data not shown).

Discussion

To the best of our knowledge, this is the first report of mecC-carrying S. aureus isolated from domestic swine, emphasizing that this variant of S. aureus has a broad host range. This report also underlines the importance of characterizing MRSA isolates for the presence of both mecA and mecC. In the herd, the genome sequences of the pig isolates did not support any clear differentiation between isolates from the weaning unit (W1–7) and the farrowing unit (F1–9). This was not unexpected due to the lack of restriction in moving animals between the different units. The observed SNP variation in the core genome also supports that the isolates have been present in the

Table 1. Samples and isolates included in the study

| Sampling site                      | Sampling date | Number of samples | mecC-positive samples | spa types (number of isolates) |
|------------------------------------|---------------|-------------------|-----------------------|--------------------------------|
| Farm isolates (H1a–c, F1–9, W1–7) |               |                   |                       |                                |
| weaning unit                       | Sep 2015      | 10 (W1–2)         | 2                     | t843 (2)                       |
| farrowing unit                     | Oct 2015      | 10 (F1–9)         | 9                     | t843 (8), t11205 (1)           |
| weaning unit                       | Nov 2015      | 10 (W3–7)         | 5                     | t843 (5)                       |
| slaughter unit                     | Nov 2015      | 10                | 0                     |                                |
| cattle                             | Nov 2015      | 20                | 0                     |                                |
| dog                                | Jan 2016      | 1                 | 0                     |                                |
| human workers                      | Jan 2016      | 2 (H1a–c)         | 1 (3 isolates)         | t1535 (3)                      |
| Isolates from Zealand (H2–35)     |               |                   |                       |                                |
| human isolates                     |               |                   | 5                     | t843 (5)                       |
| 2014                               |               |                   | 9                     | t843 (9)                       |
| 2015                               |               |                   | 19                   | t843 (16), t1535 (3)           |
| 2016                               |               |                   | 1                     | t15667 (1)                      |

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farm for an extended period of time. Previous reports have indicated that mecC-carrying MRSA show a preference for cattle.\textsuperscript{1,8} Surprisingly, at this farm no mecC-MRSA was detected in the cattle. The mecC strains did not carry any tetracycline resistance genes, unlike the more commonly occurring mecA-MRSA isolates circulating in Danish swine herds.\textsuperscript{9} Tetracycline was used regularly on this farm for individual treatment of sows and nursery pigs; however, the total antibiotic usage was lower than the Danish average for swine herds. Therefore, antibiotic usage alone cannot explain the occurrence of mecC-carrying MRSA in the case herd. The introduction and survival of new MRSA clones such as this mecC-carrying MRSA into Danish pig production may complicate attempts to lower MRSA frequencies by decreasing the use of antimicrobials such as tetracycline.

Figure 1. Phylogenetic analysis of mecC-carrying S. aureus from Zealand. The phylogenetic relationship of 53 isolates was inferred using maximum likelihood based on 2530 SNPs, contained within 84% of the reference chromosome (2.35 Mb). W1 – 7, isolates from weaning pigs; F1 – 9, isolates from farrowing unit; H1a–c, isolates from farm worker; H2–6, human isolates from the same municipality as the farm; H7–35, human isolates from other parts of Zealand. The bar indicates substitutions per site, and is equivalent to \( \approx 50 \) SNPs.
Three different spa types were found among the isolates from the swine farm. The spa types t843 and t1535 differ by only a single repeat, emphasizing the close affiliation between the isolates from the farmer and the pigs (Table 1). One of the pig isolates belonged to spa type t11205, which differs from t843 by a loss of a group of six adjacent repeats and most probably represents a single genetic event.

The swine isolates were closely affiliated to the human isolates from the same farm, indicating a transmission between the farmer and the swine. Zoonotic transmission of mecC-carrying MRSA has also been previously reported. Concluding on clonal transmission based on single isolates can, however, be problematic due to the diversity present among MRSA isolates in a single host. Genetic diversity was also observed among the three isolates obtained from the farm worker. The clustering of the multiple isolates from the farmer suggests that the farmer could have been the source of introduction into the herd. This is further supported by the adjacent clustering of human isolates recently obtained from the same geographical area, based on analysis of both the core genomes and the accessory genomes. A high diversity was observed between the human mecC-carrying isolates from Zealand in 2013–16, indicating multiple introductions or a genetic diversity that has been accumulating over many years. This corresponds well with the fact that mecC-carrying MRSA has been present in Denmark consistently from 2003 onwards.

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**Transparency declarations**

None to declare.

**Supplementary data**

Supplementary methods, Table S1 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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