Diversity of MRSA CC9/CC398 genomes and the phylogenetic relationship between food and human clinical isolates

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Background

*Staphylococcus (S.) aureus* - Methicillin-resistant *S. aureus* (MRSA)

- *S. aureus* colonizer of skin / mucosa of humans / animals
- MRSA *from farm to fork*:
  - highly prevalent, worldwide
  - in livestock and food
  - one clone (CC398 (spa t011, t034), laMRSA) predominates
- high occupational exposure risk (livestock professionals)
- Food as source of humans (?)
Risk Assessment LA-MRSA and food

...since 2009

“Food may be contaminated by MRSA (including CC398): eating and handling contaminated food is a potential vehicle for transmission. There is currently no evidence for increased risk of human colonisation or infection following contact or consumption of food contaminated by CC398 both in the community and in hospital.”
MRSA prevalence in food in Germany, 2009-2015

- Up to 43% in raw meat from turkey

Dr. A. Fetsch, ECCMID 2017, 2017-04-25, Vienna, Austria
CC9/CC398 MRSA: a new hybrid clone in humans in DK

- Human cases with CC9/CC398 MRSA
- sporadic
- urban area
- no (direct) livestock link
- no reservoir of CC9/CC398 MRSA in livestock in DK

- Source and possible transmission pathways?
  - poultry/poultry meat as source?
  - strains showing adaption to poultry and humans

Larsen et al. 2016 CID
„CC9/CC398 MRSA“: own analysis

- Collection of > 14,000 strains
- Mainly along the different food value chains
- 37 additional CC9/CC398 MRSA
- 31 CC9/CC398: sak/scn/chp positive

NGS 4x CC9/CC398 MRSA:
- 12S01032
- 13-ST00660
- 14-ST01012
- 14-ST00667

Fetsch et al. 2016 CID
Generation of reference genomes

- PureLink gDNA isolation (Invitrogen) and library preparation
- PacBio RSII (<2 Mio. raw reads, 7-15 kb in size) and Illumina MiSeq sequencing (<4 Mio. raw reads, 200-300 nt in size)

- Filtering, trimming and mapping of raw reads
Bioinformatic analysis of the WGS-Daten

- PacBio RSII sequencing
- HGAP3 assembly of raw reads
- Manual curation of the genome sequence (e.g. trimming of genome ends)
- de novo assembling of MiSeq raw reads against the PacBio genome (increase of sequence depth)
- Annotation of the genome (identification of genes, CDSs, RNAs, antimicrobial resistances, prophages and plasmids)
- Phylogenetic tree calculation (SNP analysis) against selected genome sequences
Genome characteristics CC9/CC398 MRSA

<table>
<thead>
<tr>
<th>Feature</th>
<th>14-ST0067</th>
<th>13-ST00660</th>
<th>12S01032</th>
<th>14-ST01012</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Predicted prophages (no.)</strong></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>intact</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>incomplete</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>questionable</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Plasmids (no.)</strong></td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
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**Antibiotic resistances**

<table>
<thead>
<tr>
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<th>14-ST01012</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactam</td>
<td>meca (100%)</td>
<td>meca (100%)</td>
<td>meca (100%)</td>
<td>meca (100%)</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>norA (92.03%)</td>
<td>norA (92.03%)</td>
<td>norA (92.03%)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Streptogramin B</td>
<td>vga(A) (83.13%)</td>
<td>vga(A) (83.20%)</td>
<td>vga(A) (83.20%)</td>
<td>vga(A) (83.20%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>tet(M) (100%)</td>
<td>tet(M) (100%)</td>
<td>tet(M) (100%)</td>
<td>tet(M) (100%)</td>
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**Plasmid resistances (Contig no.)**

<table>
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<th>14-ST01012</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactam</td>
<td>blaZ (100%) C1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>blaZ (100%) C1</td>
</tr>
<tr>
<td>Phenicol</td>
<td>n.d.</td>
<td>n.d.</td>
<td>cat(p221) (97.69%) C7</td>
<td>n.d.</td>
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<tr>
<td>Aminoglycoside</td>
<td>n.d.</td>
<td>n.d.</td>
<td>aph(2′)-Ih (82.99%) C4</td>
<td>n.d.</td>
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**Virulence genes**

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<th>Exoenzymes</th>
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</thead>
<tbody>
<tr>
<td>Aureolysin</td>
<td>aur (100%)</td>
<td>aur (100%)</td>
<td>aur (100%)</td>
<td>aur (100%)</td>
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**Host immune evasion**

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<tbody>
<tr>
<td>Staphylolysin</td>
<td>sak (99.80%)</td>
<td>sak (99.80%)</td>
<td>sak (99.80%)</td>
<td>sak (99.80%)</td>
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<tr>
<td>Staphylococcal complement inhibitor</td>
<td>scn (100%)</td>
<td>scn (100%)</td>
<td>scn (100%)</td>
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<tr>
<td>Chemotaxis inhibitor protein precursor</td>
<td>chp (100%)</td>
<td>chp (100%)</td>
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**Other**

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</thead>
<tbody>
<tr>
<td>Gamma-hemolysin component B precursor</td>
<td>hlgB (100%)</td>
<td>hlgB (100%)</td>
<td>hlgB (100%)</td>
<td>hlgB (100%)</td>
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<tr>
<td>Gamma-hemolysin component C</td>
<td>hlgC (100%)</td>
<td>hlgC (100%)</td>
<td>hlgC (100%)</td>
<td>hlgC (100%)</td>
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<tr>
<td>Gamma-hemolysin chain II precursor</td>
<td>hlgA (99.28%)</td>
<td>hlgA (99.28%)</td>
<td>hlgA (99.28%)</td>
<td>hlgA (99.28%)</td>
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<tr>
<td>Beta-hemolysin</td>
<td>hlb (100%)</td>
<td>hlb (100%)</td>
<td>hlb (100%)</td>
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**GenBank accession**

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</tbody>
</table>

- Differences mainly in mobile genetic elements (plasmids, prophages)
Poultry meat: source of human CC9/CC398 cases?

Phylogenetic analysis

- Single Nucleotide Polymorphism (SNP) analysis ("Variant calling") against reference genome (Larsen et al., 2016)

- 3 Cluster (A, B, C)

- Sub-cluster of cluster A
  - A1: isolates from turkey
  - A2: human isolates and turkey isolates
  - A3 & A4: isolates from poultry
  - A5: turkey isolates and human isolates

- Genetic relationship of human isolates and from turkey
Summary & Conclusions

- Generation of reliable reference genomes
- Dissection of the genomic features of all genomes
- Identification of mobile genetic elements, antimicrobial resistances and virulence factors
- Identification of infection sources based on the phylogenetic relationship of the genomes

Food, mainly turkey meat as source of MRSA in humans?!
Acknowledgement

- Katja Drache
- Ylanna Kelner-Burgos
- Daniel Leeser
- Units 44 „Microbial Toxins“
- Unit 43 „Epidemiology, Zoonoses and Antimicrobial Resistance“

Funding body

- Britta Kraushaar
- Annemarie Käsbohrer
- Jens Hammerl
- Jesper Larsen, Robert Skov (SSI)

Coordinator:
Robin Köck, Klinikum Oldenburg
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