FEDERAL INSTITUTE FOR RISK ASSESSMENT

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

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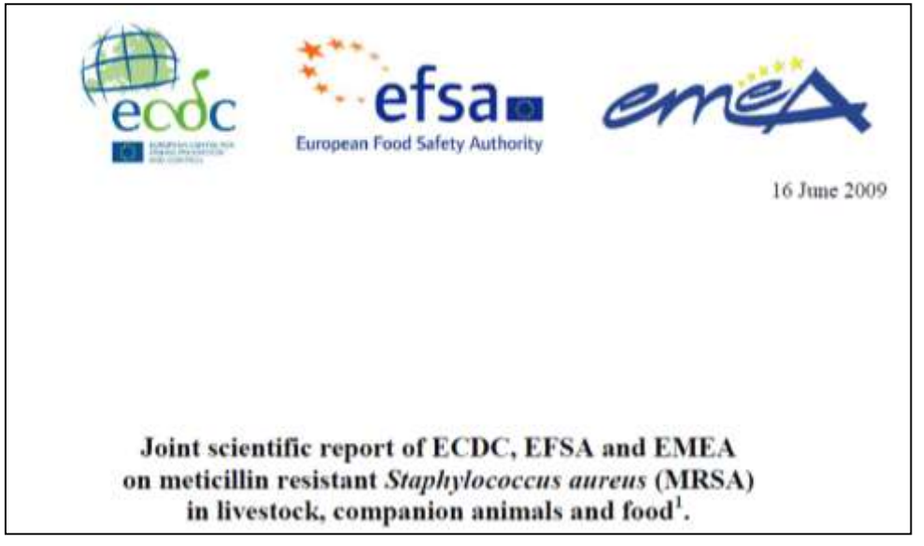
Background

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 (?)

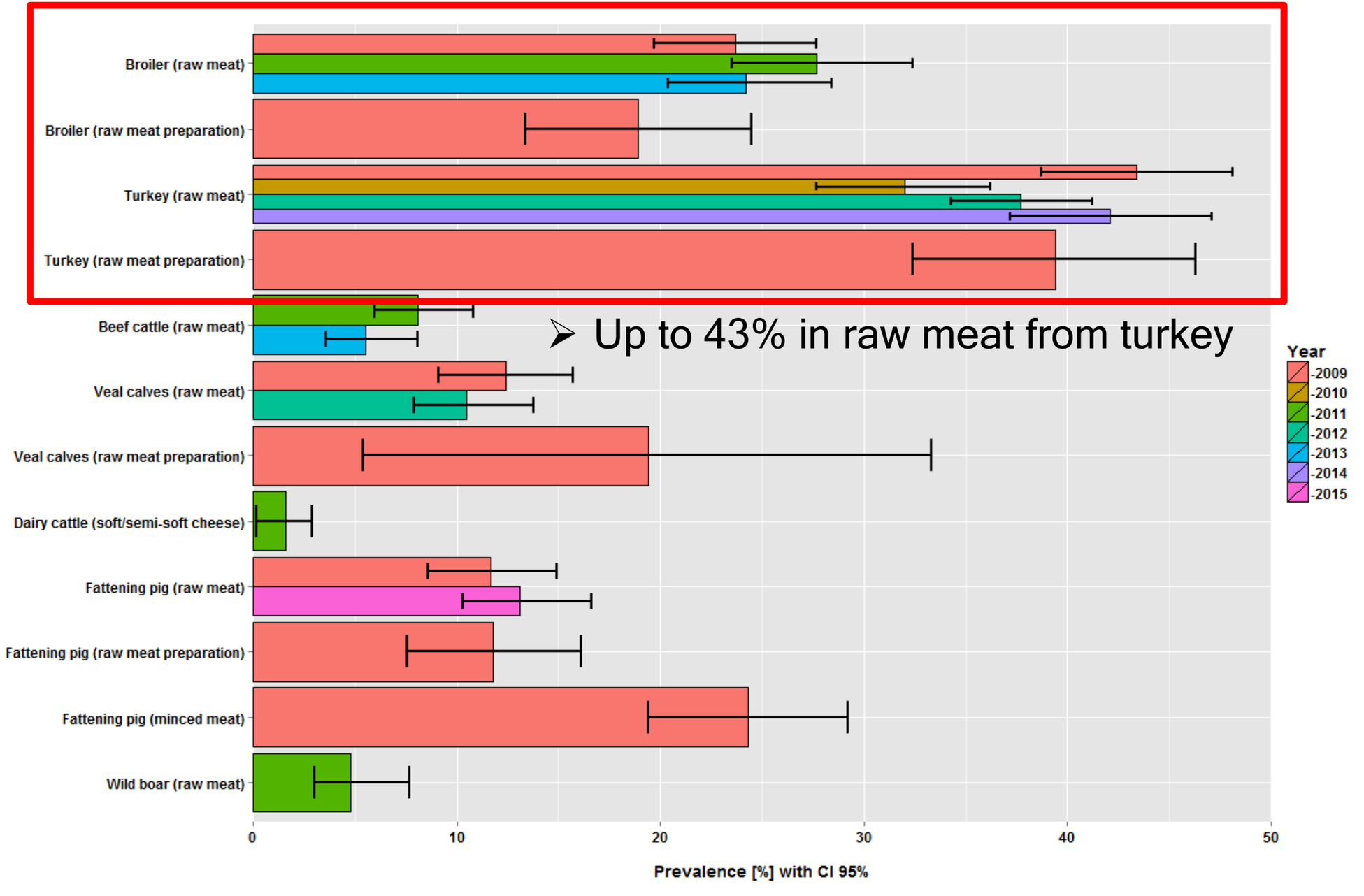
Risk Assessment LA-MRSA and food

…since 2009



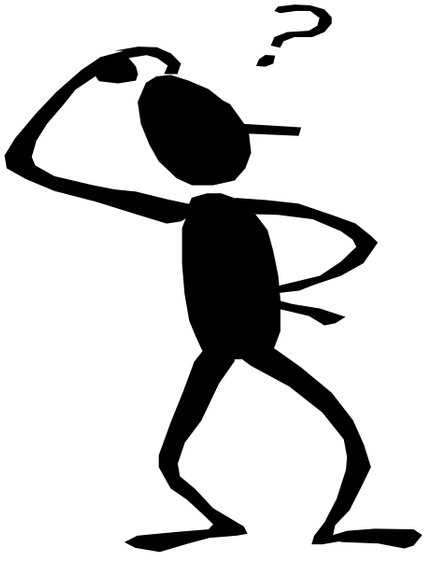
"Food may be contaminated by MRSA (including CC398): eating and handling contaminated food is a potential vehicle for transmission. There is currently ***no evidence for increased risk of human colonisation or infection following contact or consumption of food contaminated by CC398*** *both in the community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

MRSA prevalence in food in Germany, 2009-2015



➢ Up to 43% in raw meat from turkey

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

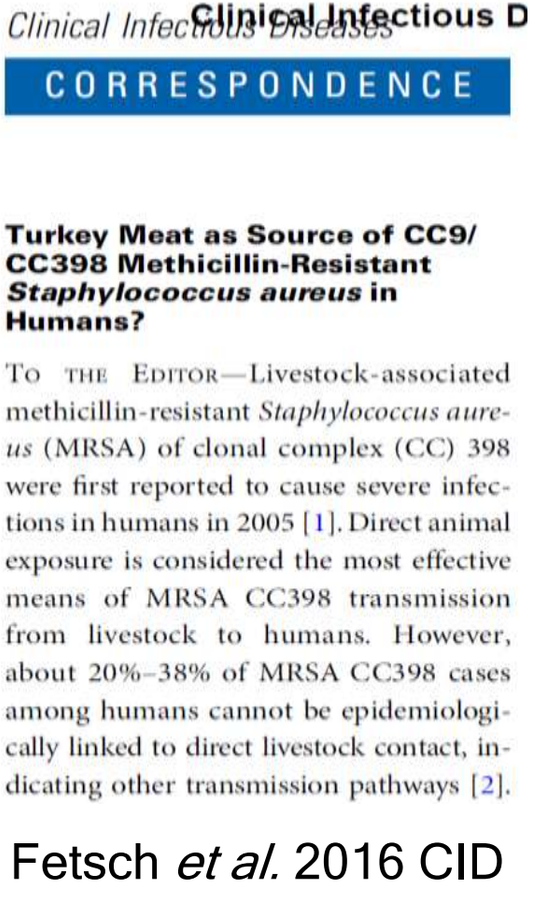
Larsen *et al.* 2016 CID

„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
- 8/31 CC9/CC398: SAAV_2008/2009 (ϕAvβ+)

➢ **NGS 4x CC9/CC398 MRSA:**

- 12S01032
- 13-ST00660
- 14-ST01012
- 14-ST00667



Clinical Infectious Diseases Clinical Infectious D

CORRESPONDENCE

Turkey Meat as Source of CC9/CC398 Methicillin-Resistant *Staphylococcus aureus* in Humans?

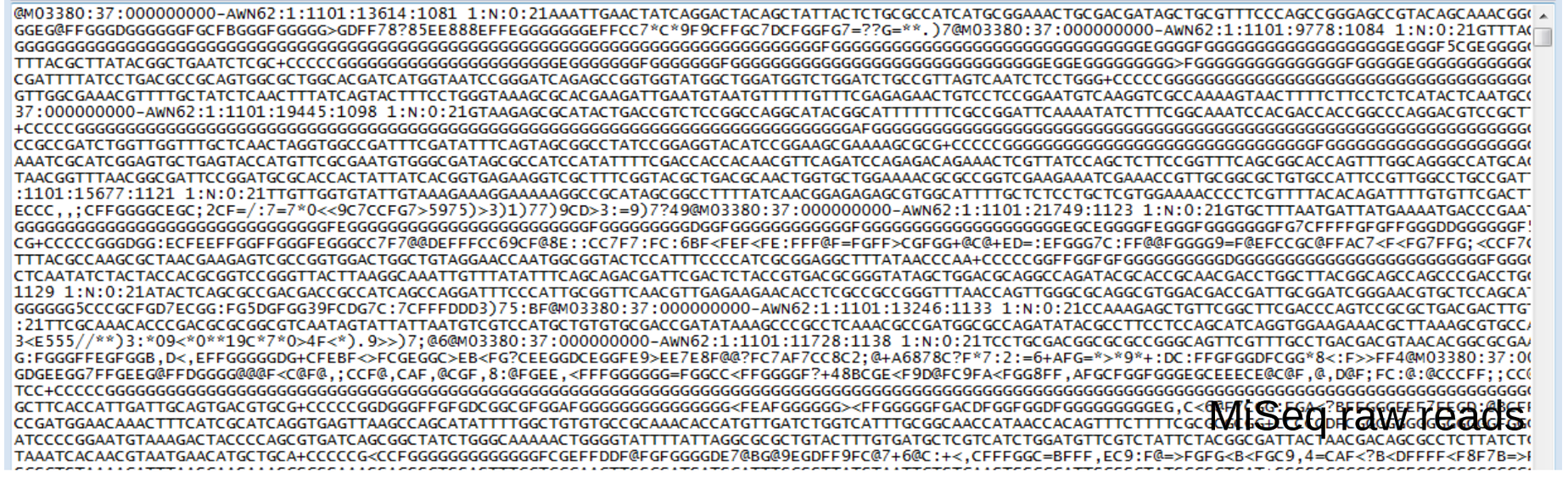
To the Editor—Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) of clonal complex (CC) 398 were first reported to cause severe infections in humans in 2005 [1]. Direct animal exposure is considered the most effective means of MRSA CC398 transmission from livestock to humans. However, about 20%–38% of MRSA CC398 cases among humans cannot be epidemiologically linked to direct livestock contact, indicating other transmission pathways [2].

Fetsch *et al.* 2016 CID

Generation of reference genomes

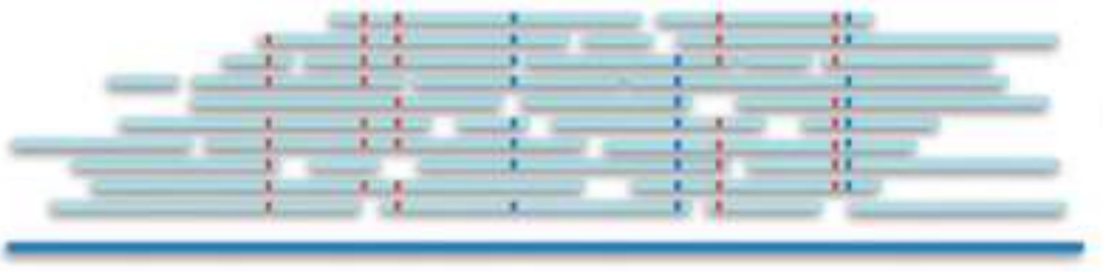
- PureLink gDNA isolation (Invitrogen) and library preparation
- PacBio RSII (<2 Mio. raw reads, 7-15 kb in size) and

  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

Feature	14-ST0067	13-ST00660	12S01032	14-ST01012
Predicted prophages (no.)	**4**	**4**	**4**	**6**
intact	2	2	2	4
incomplete	2	2	2	2
questionable	0	2	0	0
Plasmids (no.)	**1**	**2**	**4**	**1**
Antibiotic resistances				
β-lactam	*mecA* (100%) *blaZ* (98.94%)	*mecA* (100%) *blaZ* (99.29%)	*mecA* (100%) *blaZ* (99.29%)	*mecA* (100%) n.d.
Fluoroquinolones	*norA* (92.03%)	*norA* (92.03%)	*norA* (92.03%)	*norA* (92.03%)
Streptogramin B	*vga(A)* (83.13%)	*vga(A)* (83.20%)	*vga(A)* (83.20%)	*vga(A)* (83.20%)
Tetracycline	*tet(M)* (100%)	*tet(M)* (100%)	*tet(M)* (100%)	*tet(M)* (100%)
Plasmid resistances (Contig no.)				
β-lactam	*blaZ* (100%) C1	n.d.	*blaZ* (100%) C3	*blaZ* (100%) C1
phenicol	n.d.	n.d.	*cat(pC221)* (97.69%) C7	n.d.
aminoglycoside	n.d.	n.d.	*aph(2")-Ih* (82.99%) C4	n.d.
Virulence genes				
Exoenzymes				
aureolysin	*aur* (100%)	*aur* (100%)	*aur* (100%)	*aur* (100%)
Host immune evasion				
staphylokinase	*sak* (99.80%)	*sak* (99.80%)	*sak* (99.80%)	*sak* (99.80%)
staphylococcal complement inhibitor	*scn* (100%)	*scn* (100%)	*scn* (100%)	*scn* (100%)
chemotaxis inhibitor protein precursor	*chp* (100%)	*chp* (100%)	*chp* (100%)	*chp* (100%)
Other				
gamma-hemolysin component B precursor	*hlgB* (100%)	*hlgB* (100%)	*hlgB* (100%)	*hlgB* (100%)
gamma-hemolysin component C	*hlgC* (100%)	*hlgC* (100%)	*hlgC* (100%)	*hlgC* (100%)
gamma-hemolysin chain II precursor	*hlgA* (99.28%)	*hlgA* (99.28%)	*hlgA* (99.28%)	*hlgA* (99.28%)
beta-hemolysin	*hlb* (100%)	*hlb* (100%)	*hlb* (100%)	*hlb* (100%)
GenBank accession				
Bioproject no.	PRJNA287148	PRJNA287147	PRJNA287146	PRJNA287149
Biosample no.	SAMN03776869	SAMN03776868	SAMN03776867	SAMN03776870
Accession no.	CP011872-CP011873	CP011874-CP011876	CP011877-CP011881	CP011870-CP011871

no. number; n.d. not detected

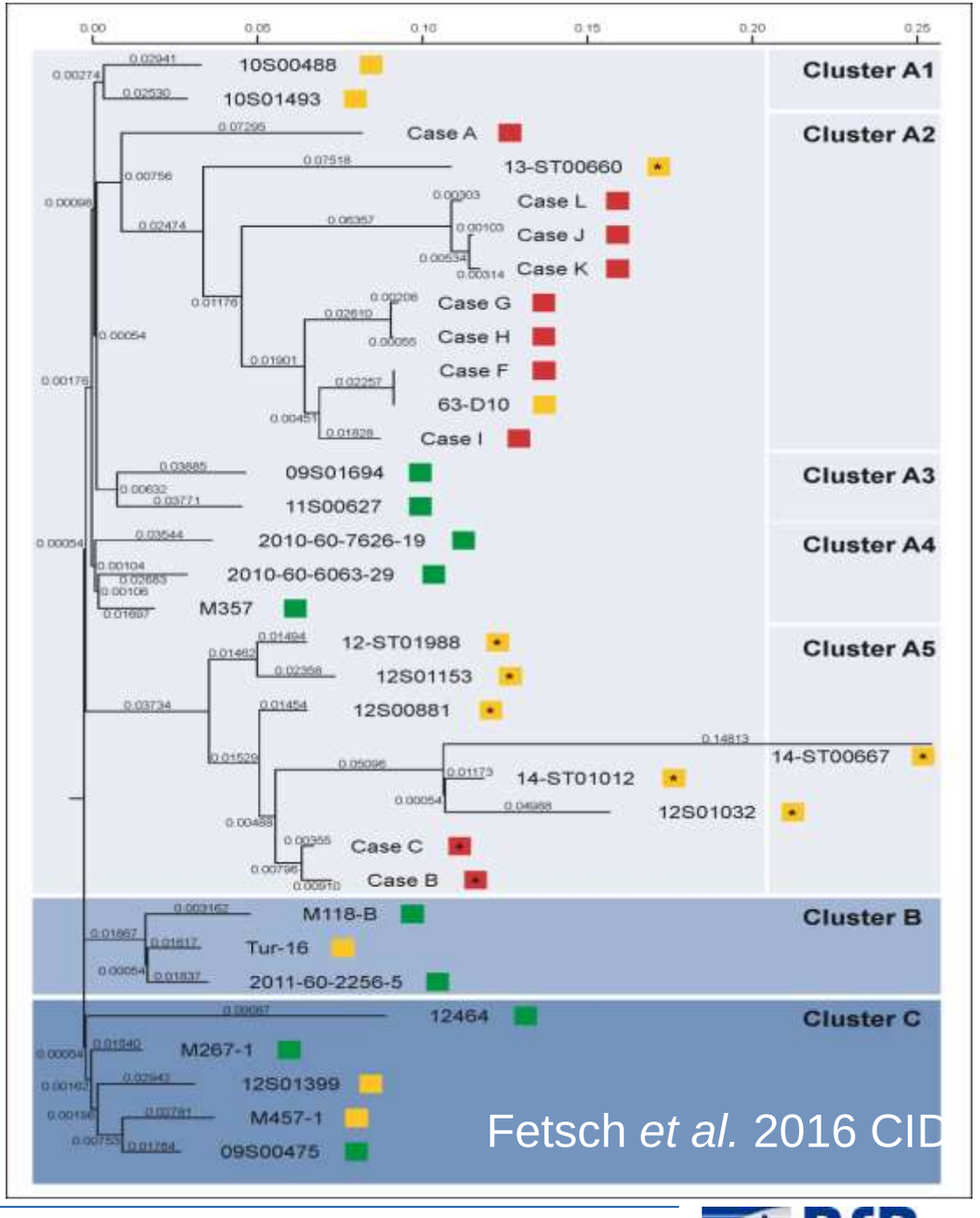
- Differences mainly in mobile genetic elements (plasmids, prophages)

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?!**

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... for your attention!

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