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Horse-adapted MRSA CC398 t011 clone in Denmark

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Summary of findings. Danish horses are potential reservoirs of MRSA. Whole-genome phylogenetic analysis showed that one of the isolated genotypes, MRSA CC398 t011, belonged to the Central European horse clone. Importantly, these horse-adapted strains carried the phage Φ Sa3 and associated IEC genes, which facilitate adaptation to horses and humans and may enhance the zoonotic potential of this genotype. Danish horses also carried MRSA CC398 t034, which clustered with isolates of pig origin suggesting a spillover from pigs; and *mecC*-MRSA CC130 t528, a genotype found in multiple hosts.

Background. In Denmark, livestock-associated (LA-) MRSA CC398 *spa* types t034 and t011 are together with MRSA CC5 t002 the most prevalent lineages in humans. Most cases are associated to contact with pigs, but a constant proportion every year (10-15%) reports no exposure to livestock, suggesting other unidentified transmission routes or reservoirs. We investigated horses as a possible source of LA-MRSA CC398 in Denmark.

Methods. Nasal swabs were collected from 401 horses from 74 farms. Horses had not visited a horse clinic within at least 30 days. Laboratory analyses are shown in Figure 1. Whole genome sequencing was performed in: 1) all horse MRSA CC398 isolates 2) all Danish human isolates with the same genotype as the most prevalent horse clone (Danish MRSA registry) 3) a collection of Danish MRSA CC398 pig isolates. Phylogenetic analyses was performed including a well known MRSA CC398 collection (Price et. al 2012). Information on animal contact was retrieved from all humans (see Table).

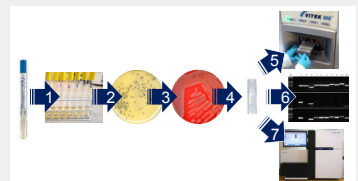


Figure 1. Laboratory methods for the isolation, identification and sequencing of MRSA in Danish horses. 1) Enrichment of nasal swabs in Mueller-Hinton broth 6.5% NaCl, 2) plating overnight culture on Brilliance MRSA2 agar (Oxoid), 3) subculture one blue colony, 4) freeze pure culture at -80°C, 5) species confirmation by MALDI-TOF (Biomerieux), 6) PCRs for *spa*, *mecA*, *mecC*, *scn*, CC398-specific *sauI-hsdS1*, PVL-marker *lukF-PV*, and *spa* typing and 7) whole-genome sequencing of confirmed MRSA CC398 isolates.

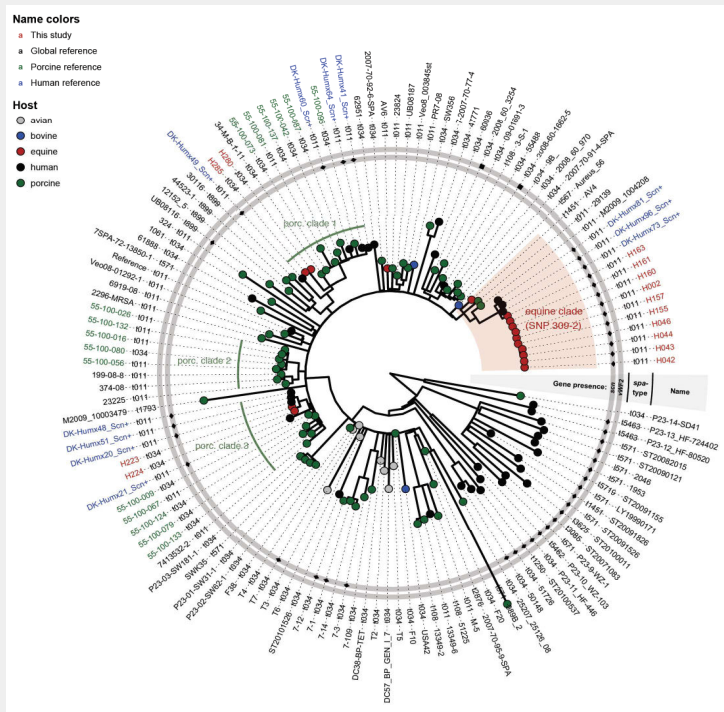


Figure 2. Phylogenetic analysis of 130 fully sequenced MRSA CC398 strains, including 14 horse isolates from this study (ten t011 *scn+* and four t034 *scn-*), 11 t011 *scn+* human isolates from the national MRSA collection, 16 Danish pig-associated isolates and the 89 strains sequenced by Price et al in another study.

Results. MRSA belonging to three lineages was found in 17 horses (4.2%) located at seven farms (9.5%):

- MRSA CC398 t011 *scn+* (11 horses)
- MRSA CC398 t034 *scn-* (4 horses)
- *mecC*-MRSA CC130 t528 *scn-* (2 horses)

Strains of MRSA CC398 t011 *scn+* from all horses and 3/11 humans belonged to a previously identified horse-specific clone of MRSA CC398 (equine clade). People carrying the horse-adapted clone were horse veterinarians. The remaining eight MRSA CC398 t011 *scn+* human isolates belonged to the porcine clades 1 and 3, together with the MRSA CC398 t034 *scn-* isolates from horses. Most people carrying the porcine-adapted strains reported contact with pigs (6/8). The genetic marker of human adaptation, *scn*, was widespread in horse isolates belonging to horse-adapted clone.

Table. Description of Danish persons colonized or infected with MRSA CC398 *scn+* t011.

Isolate	Sex	Age (y)	Isolated in	Specimen	Contact with animals	Cluster
DK-Humx41_Scn+	F	38	2010	Screening (nares)	Wife of pig farmer (family A)	Porc. clade 1
DK-Humx64_Scn+	F	0	2010	Screening (nares)	Child of pig farmer (family A)	Porc. clade 1
DK-Humx60_Scn+	M	50	2011	Screening (nares)	Pig farmer	Porc. clade 1
DK-Humx96_Scn+	M	27	2014	Wound swab	Horse veterinarian (family B)	Equine clade
DK-Humx81_Scn+	M	61	2014	Screening (nares)	Horse veterinarian (family B)	Equine clade
DK-Humx48_Scn+	M	24	2014	Abscess	Pig farmer (family C)	Equine clade
DK-Humx49_Scn+	F	32	2014	Wound swab	Horse contact	Porc. clade 1
DK-Humx51_Scn+	F	4	2014	Screening (perineum)	Niece of pig farmer	Porc. clade 2
DK-Humx73_Scn+	F	44	2014	Wound swab	Horse veterinarian	Equine clade
DK-Humx21_Scn+	F	73	2015	Wound swab	No livestock contact	Porc. clade 2
DK-Humx20_Scn+	M	58	2015	Screening (nares)	Father of pig farmer (family C)	Porc. clade 2