

Whole genome sequencing and CGE servers in public health microbiology, analyses of *mecC* gene positive, methicillin-resistant *Staphylococcus aureus* isolated in Finland 2003 - 2014

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Objectives. Bacterial whole genome sequencing (WGS) is becoming increasingly accessible for routine use in public health microbiology. During this year we have tested WGS in different microbiological applications. Aim of this study was to use WGS and publicly available sequence analyses tools for typing of *mecC* positive *S. aureus* strains and also for resistance and virulence gene detection.

Methods. Thirteen *mecC* PCR positive *S. aureus* isolates of human origin, submitted to THL between January 2003 and October 2014 and two *mecC* positive strains isolated from a cow and a dog were included in the study.

Antimicrobial susceptibility testing was done using E-test and microbroth dilution methods. Whole genome sequence libraries were prepared using Nextera XT kit. Libraries were sequenced using the Illumina Miseq platform generating paired-end reads.

Illumina paired end reads were analysed using publicly available web servers ResFinder, VirulenceFinder and MLST of the Center for Genomic Epidemiology (CGE, www.genomicepidemiology.org) to detect resistance and virulence genes and the sequence types. In order to confirm the presence of *mec* genes sequences in draft genomes, the assembly of the Illumina paired end reads and subsequent sequence analysis were done using CLC Genomic workbench (7.5). Rapid local Ridom SeqSphere Ad hoc MLST+ schema was also created and it was used to analyze the data assembled with the Velvet assembler integrated in the Ridom SeqSphere+ software.

Results. MLST-types were obtained for all strains. These types included ST1245, ST130, ST2616, ST425 and ST1943. All strains were cefoxitin resistant and tetracycline susceptible. Using ResFinder *mec* gene sequence was detected only in genome sequences of thirteen strains. However, using CLC software *mec* genes were detected also in those draft genomes that were negative in ResFinder analysis (Fig.1)

The sequence of Tet(38) efflux transporter gene, a gene that confers resistance to tetracycline and fitness in abscesses, was detected in genome sequences of all strains.



Conclusion.

- Nextera XT kit and Illumina Miseq platform offers a robust and fast system for whole genome sequencing of bacteria.
- CGE servers are easy-to-use www-based services for typing, resistance and virulence gene detection. MLST server performed very well and all strains were typed. However, ResFinder was not able to detect *mec* genes in all WGS sequences despite of that all strains were *mecC* positive and the *mec* gene sequences were present in the draft genomes assembled using CLC Genomic workbench.
- An unexpected finding was that all strains were phenotypically tetracycline susceptible although Tet(38) gene was detected. However, in abscesses and on skin where its natural fatty acid inducers are present Tet(38) gene may be expressed and contribute the tetracycline resistance and survival of these strains over time.
- To summarize, Illumina platform and CGE servers are promising tools to be used in public health microbiology.

Year of Isolation	Strain	spa-type	spa-repeat	ST	Oxacillin MIC (mg/L)	Tetracyclin MIC (mg/L)	Resistance (disc diffusion)
2010	797	t7229	04-82-17-25-16-16-17	1245	>4	0,75	cefoxitin
2003	641	t843	04-82-17-25-17-25-25-16-17	1245	4	0,25	cefoxitin
2003	684	t843	04-82-17-25-17-25-25-16-17	1245	4	0,19	cefoxitin
2005	1645	t843	04-82-17-25-17-25-25-16-17	1245	>4	0,19	cefoxitin
2014	368	t843	04-82-17-25-17-25-25-16-17	1245	4	0,19	cefoxitin
2013	744	t843	04-82-17-25-17-25-25-16-17	1245	2	0,5	cefoxitin
2013	1220	t843	04-82-17-25-17-25-25-16-17	130	0,5	0,19	-
2014	1140	t843	04-82-17-25-17-25-25-16-17	1245	2	0,38	cefoxitin
2014	650	t3256	04-82-17-25-17-25-25-25-16-17	130	4	1	cefoxitin
2012	IH254532	t3256	04-82-17-25-17-25-25-25-16-17	130	>4	0,25	cefoxitin
2006	1198	t10471	121-21-16-34-16-34-17-34-02-24	2616	1	0,5	cefoxitin
2014	391	t10471	121-21-16-34-16-34-17-34-02-24	2616	1	0,38	cefoxitin
2014	498	t10471	121-21-16-34-16-34-17-34-02-24	2616	2	0,19	cefoxitin
2013	692	t742	14-44-12-17-23-18-17-17-17-23-24	425	1	0,5	cefoxitin
2014	IH267107	t8835	14-44-16-12-17-17-23-24	1943	2	0,125	cefoxitin

Antibiotics tested according to EUCAST methodology: cefoxitin, linezolid, levofloxacin, gentamycin, tobramycin, erythromycin, clindamycin, ciprofloxacin, tetracyclin, cloramphenicol, rifampisin, trimethoprim/sulfamethoxazole, fusidic acid and mupirocin

Table 1. Characteristics of the *mecC* –positive MRSA strains from this study

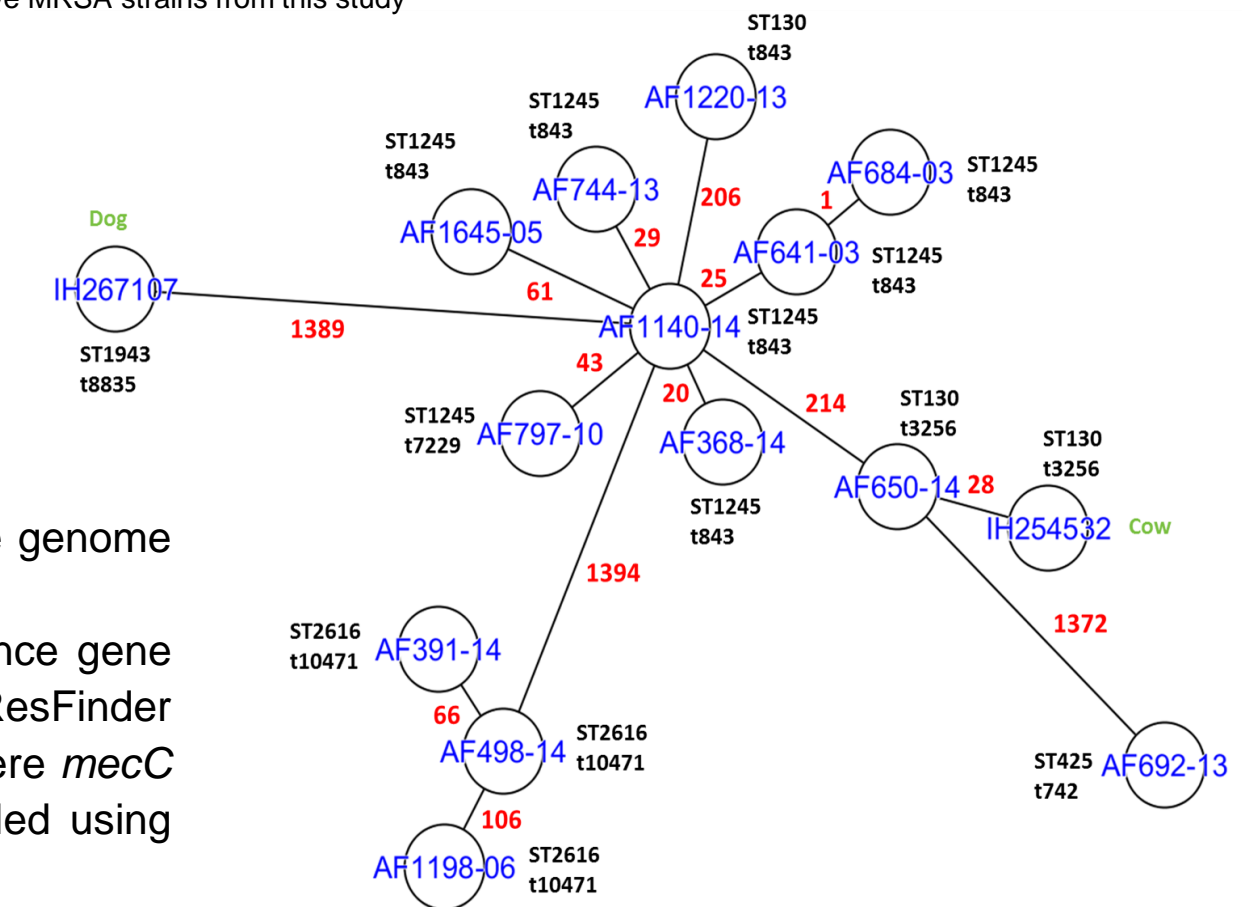


Figure 2. Minimum spanning tree for *MecC* strains isolated in Finland based on the allele diversity of 1650 genes using Ridom SeqSphere+ Ad hoc MLST+ schema.

