Staphylococcus aureus: the prince of pathogens strikes back

The first 1500+ consecutive whole genome sequenced MRSA isolates in Copenhagen, Denmark: 2013-2014

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Objectives: We have typed MRSA by spa-tying since 2003. In the Capital Region of Denmark whole genome sequencing (WGS) has been routinely used for all MRSA isolates, since 2013. In Denmark MRSA is a reportable disease and typing has been used for epidemiologic and outbreak investigations.

Methods: All MRSA from the Capital Region of Denmark (1.7 M inhabitants) are submitted to Hvidovre Hospital for WGS. All isolates were sequenced on the Illumina MiSeq platform. 2 x 150 bp paired-end-reads were produced. Reads were mapped with Stampy to a USA_300 reference genome. Single nucleotide polymorphism (SNP) calling was performed using Samtools. The pipeline used, was developed at Oxford Centre for Gene Function. Phylogeny was inferred by neighbor-joining (NJ) analysis and bootstrapping in SplitsTree4. In house developed software was used to identify, spa-type, Multilocus Sequence Type (MLST), meca or mecc, presence of Panton-Valentine Leukocidin (PVL) and arginine catabolic mobile element (ACME), ccr genes, dru type and nuc gene.

Results: 1593 genomes were sequenced from January 1, 2013 to October 1, 2014. This was reduced to 1435 individual genomes by removing results obtained by re-sequencing poorly sequenced isolates without spa-type (108 isolates twice and 8 isolates 3 times) and removing 34 isolates that were not MRSA. mecC was found in 12 isolates. We identified 225 spa-types, of which 48 % belonged to 7 spa-types; t002, t008, t304, t019, t127, t024, t044 and t437. We identified 86 MLST types of which 49 % were; ST8, ST5, ST30, ST22 and ST6. 2.7 % of isolates had no MLST, because an allele was on two contigs. PVL was found in 36.8 % of all isolates, in 33 MLST types and 71 spa-types. In spa-type t008; 55 isolates had ACME and PVL corresponding to USA300, 57 isolates had PVL and no ACME and 20 isolates were PVL and ACME negative. CC22 isolates were very polyclonal based on SNP typing. CC22 had 4 ST, 31 spa-types and PVL was found in 19 % of the isolates. 1.9 % of isolates were Pig-MRSA, ST398. The SNP tree of 114 isolates of t304, will show how we use this tool to elucidate a very large neonatal MRSA outbreak.

Conclusion: Genomics has revealed a polyclonal structure of MRSA in the Capital Region of Denmark. This is mainly due to import associated with travel and hospitalization abroad. Outbreaks have been seen in nursing homes and neonatal wards, but are uncommon in other hospital wards. WGS has in our laboratory a reagent cost of about €100 and has been used for typing of more than 10 other bacterial species.