Application of Next Generation Sequencing (NGS) for outbreak investigations at the German National Reference Centre for Salmonella and other bacterial enteric pathogens

Sandra Simon[1], Erhard Tietze[1], Burkhard Malorny[2], Martin Mielke[3]

1 Robert Koch Institute, Division for Enteropathogenic Bacteria and Legionella, Burgstr. 37, Wernigerode, Germany

2 Federal Institute for Risk Assessment, National Reference Laboratory for Salmonella, Diedersdorfer Weg 1, Berlin, Germany

3 Robert Koch Institute, Head of the Dept. for Infectious Diseases, Nordufer, Berlin, Germany

The investigation of outbreaks with foodborne infections are generally conducted by the microbiologists and epidemiologists from the Robert Koch Institute (RKI) in close collaboration with the colleagues from the Federal Institute for Risk Assessment (BfR), which supports the RKI with the respective food and veterinary isolates as well as with relevant background information. Furthermore, effective outbreak investigation / source attribution largely depends on the close cooperation with the local and state health and food safety authorities.

Recently, next generation sequencing was established for the identification and high-resolution characterization of bacterial isolates in the context of outbreak investigations at the Division for Bacterial enteric pathogens and *Legionella* and the associated National Reference Centre for *Salmonella* and other bacterial enteric pathogens of the RKI. In the following examples sequencing was performed using an Illumina MiSeq Benchtop sequencer. For subsequent mapping / variant calling an in-house pipeline based on publicly available tools was used. SNP-based phylogenetic analyses were done with Geneious (Biomatters Ltd., Auckland, New Zealand).

We here describe exemplarily our experiences with NGS in the context of two *Salmonella* outbreaks in Germany:

1. Salmonella Derby outbreak Dec. 2013 / Jan. 2014 – retrospective analysis

- 116 confirmed cases in a defined geographical region
- suspected source (case-control study; questionnaire): raw pork sausage
- samples of a raw fermented pork sausage positive for S. Derby
- two workers of the meat-processing company were asymptomatic carriers of S. Derby
- outbreak investigation with classical methods (phage typing, PFGE, MLVA) revealed that isolates from patients, carriers and food samples were indistinguishable with all three methods
- retrospective WGS analysis of 33 outbreak strains (patients, carrier, food) and 22 control strains (random collection of S. Derby from different sources from 2004 – 2014; 12x same phage type as the outbreak strain, 10x other phage types)
- index case PacBio-sequenced to serve as the reference for subsequent mapping

- SNP-based phylogeny confirmed results of classical methods but was of higher discriminatory power: questionable isolates (same phage type, temporal but no spatial correlation) could either be confirmed (n=3) or excluded (n=5) from the scenario

2. Salmonella Enteritidis outbreak Sept. 2015 – real time analysis

- about 20 cases of a distinct phage type (PT 4/6 based on the London and Budapest typing schemes) in a defined geographical region
- suspected source (questionnaire conducted by the local health authorities): pastries from a local bakery
- three positive food samples (two from the local bakery, one from another source)
- resolving power of classical methods (phage typing, PFGE, MLVA, Ribotyping) often not sufficient for discrimination of S. Enteritidis strains
- realtime NGS analysis of 16 outbreak (patients, food) and 5 control strains (4x same phage type, 1x other phage type)
- *S*. Enteritidis str. P125109 (AM933172.1) was used as reference genome for mapping
- SNP-based phylogeny clearly indicated the products from the local bakery as the probable outbreak source whilst the food sample from the other source could be excluded
- furthermore, two questionable strains (same phage type, temporal but no spatial correlation) could be distinguished from the outbreak strains

We conclude that NGS is a highly suitable typing method for *Salmonella* outbreak investigation. Its discriminatory power exceeds that of the classical typing methods what is particularly important for strains with epidemiologically uncertain attribution. Nevertheless, sequencing costs are still quite high and especially for cross-country and cross-sector investigations standards for data analysis and interpretation need to be established. It remains to be seen, whether NGS will proof as a suitable frontline method for bacterial subtyping in the context of general food safety management.