NGS based analysis of persistent *Listeria monocytogenes* isolates

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Background: The Austrian National Reference Laboratory for Listeria (NRLL) receives about 1300 food associated isolates per year. These strains are routinely typed by molecular serotyping and pulsed-field gel electrophoresis (PFGE). In 2015 whole genome based typing (1) was established. A set of 90 isolates (gathered from 2011-2015) showing identical serotype and PFGE pattern was considered for core genome MLST (cgMLST) analysis (1).

Results: The 90 isolates belonging to the molecular serotype IIb, with identical *AscI/ApaI* PFGE pattern, could be differentiated by cgMLST into cluster types (CT) CT48, CT53 and CT2784, all belonging to classical MLST type 5. Whole genome sequencing revealed a close relatedness of the isolates. 82 isolates could be assigned to CT48 with a maximum of 3 allelic differences, however the majority (n=59) had 0 allelic differences within the CT48 cluster. Seven isolates could be assigned to CT53 with a maximum of 6 allelic differences within the cluster. Between CT48 and CT53 only 8 allelic differences could be found. One isolate could be assigned to CT2784 with 8 alleles difference to the CT53 cluster (figure). Eighty-two isolates with CT48 originated from food processing plant (FPP) AT01, seven isolates with CT53 and one isolate with CT2784 from FPP AT02. The six sequences from the reference strains with classical MLST ST5 could be assigned to CT48.

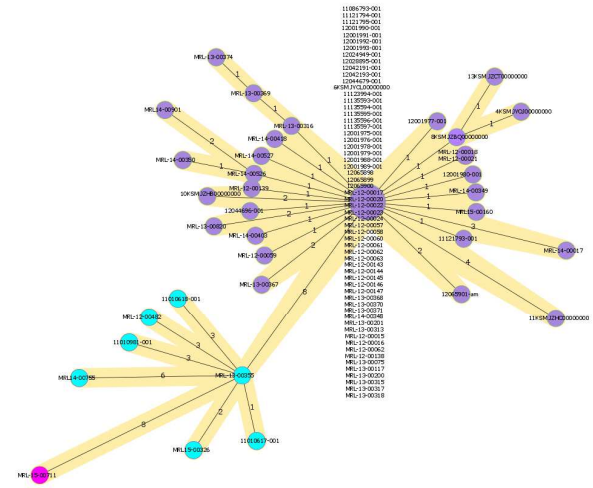

Figure legend: Minimum spanning tree of 96 *Listeria monocytogenes* isolates.

Violet CT48: 82 isolates from producer AT01 and 6 reference strains
Turquoise CT53: 7 isolates from producer AT02
Pink CT2784: one isolate from producer AT02

Material/methods: PFGE was performed according to PulseNet recommendations (2) and molecular serotyping according to Doumith et al. (3). Data analysis was carried out with Bionumerics version 7.5 (Applied Maths).
NGS was performed using Illumina's NexteraXT kit and paired end sequencing (2x300 basepairs) on a MiSeq platform (Illumina Inc.). Raw reads were de-novo assembled using Velvet version 1.1.04. Contigs were filtered for a minimum coverage of 5 and a minimum length of 200bp. A core genome (cg)MLST scheme comprising 1701 target genes was used for NGS data interpretation using SeqSphere+ (Ridom) as described previously (1). Whole genome sequences of six isolates with classical MLST ST5 were downloaded from the NCBI nucleotide database for comparison.

Conclusions: Whole genome based typing confirmed the close relatedness of the isolates showing identical serotype, classical MLST type and PFGE pattern. Our investigations revealed that the 90 isolates originated from only two food processing plants (FPP) showing these persistent strains over a five year time period. All CT48 isolates (n=82) were from FPP AT01, the CT2784 isolate and the CT53 isolates were from FPP AT02.

References:
1. Ruppitsch W et al. 2015 J Clin Microbiol 53:2869-2876.
2. http://www.pulsenetinternational.org
3. Doumith M et al. 2004 J Clin Microbiol 42:3819–3822.