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Whole genome sequence based virulence gene profiling of the 2009/10 listeriosis outbreak isolates and genetically related non-outbreak isolates

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Background: A multinational listeriosis outbreak of 2009 and 2010 was caused by contaminated Austrian acid curd cheese (ACCO). Two different *Listeria monocytogenes* strains were responsible for 34 clinical cases. Strain type 1 caused more clinical cases of listeriosis than type 2 [1]. In this study, the genetic background of all related isolates was investigated, focusing on known virulence factors.

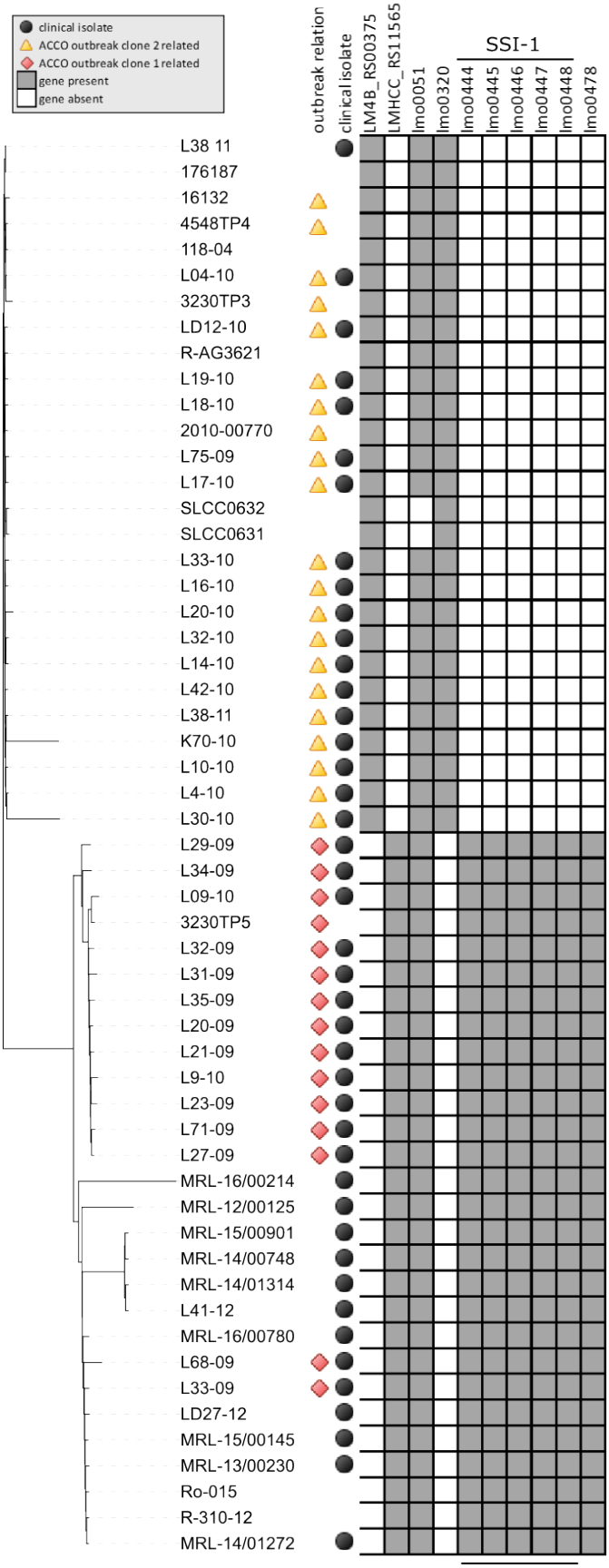
Material/methods: Genomes of 39 isolates of the ACCO study were downloaded from public databases. Sixteen genome sequences of closely related isolates were provided by the National Reference Laboratory for *Listeria monocytogenes* at the Austrian Agency for Health and Food Safety. Genomes were assembled using Velvet 1.1.04 and annotated using prodigal 2.6.3. Protein sequences were searched for homologues using orthofinder 0.7.1. Genome alignment and tree calculation was performed with progressiveMauve 2.4.0. Visualisation was performed using iTOL v3 on itol.embl.de and InkScape 0.91.

Results: A phylogenetic tree was created from the whole genome alignment of 55 strains, related to one of the outbreak strains with less than 30 alleles different in cgMLST. Of 169 known virulence related genes [2], 132 genes were present in strain type 2, while 137 genes were present in strain type 1. A distinct virulence profile comprising ten targets was obtained for the different outbreak cluster types (Figure). Genes present in strain type 1 but not in strain type 2 were: *lmo0444-lmo0448*, (stress-survival islet) *lmo0478*, a gene involved in adapting to environmental niches, and *LMHCC_RS11565* an internalin-like protein. Genetic homologues found in strain type 2, but not in strain type 1 were

LM4B_RS00375, an *EsaC* protein analog and *lmo0320* (vip), a surface protein, which is positively regulated by *PrfA*.

Conclusions: Although the genetic difference between both strain types is relatively large, there are only few differences in the virulence gene profile. Our results show, that the first outbreak clone harbours genetic homologues to *lmo0444-lmo0448*, which was described as the stress-survival-islet (SSI-1) [3]. SSI-1 absence decreases a strains ability to survive at low pH values and high salt concentrations. This could serve as explanation for the higher virulence compared to its abundance in the food product causing the outbreak. Here we show, that the ability to determine the virulence potential of strains is another valuable benefit of NGS based typing, for both consumers and food industry.

Figure:


clinical isolate
ACCO outbreak clone 2 related
ACCO outbreak clone 1 related
gene present
gene absent
outbreak relation
clinical isolate
LM4B_RS00375
LMHCC_RS11565
lmo0051
lmo0320
SSI-1
lmo0444
lmo0445
lmo0446
lmo0447
lmo0448
lmo0478
L38 11
176187
16132
4548TP4
118-04
L04-10
3230TP3
LD12-10
R-AG3621
L19-10
L18-10
2010-00770
L75-09
L17-10
SLCC0632
SLCC0631
L33-10
L16-10
L20-10
L32-10
L14-10
L42-10
L38-11
K70-10
L10-10
L4-10
L30-10
L29-09
L34-09
L09-10
3230TP5
L32-09
L31-09
L35-09
L20-09
L21-09
L9-10
L23-09
L71-09
L27-09
MRL-16/00214
MRL-12/00125
MRL-15/00901
MRL-14/00748
MRL-14/01314
L41-12
MRL-16/00780
L68-09
L33-09
LD27-12
MRL-15/00145
MRL-13/00230
Ro-015
R-310-12
MRL-14/01272

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