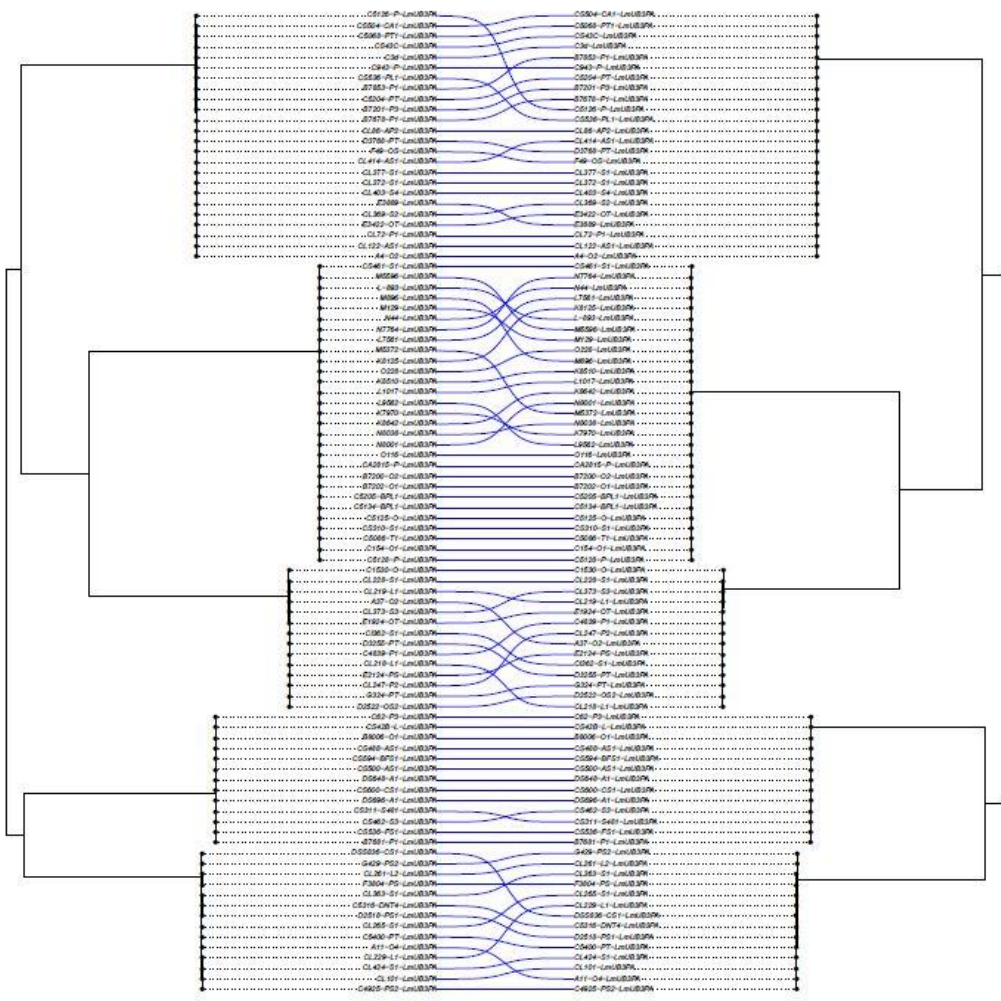


1 **Additional file 5. Topology and distance comparison between phylogenomic reconstructions based**
2 **on iVARCall2 and Snippy pipelines**

3 Concatenated core variants from the reference alignments of short reads of 94 *L. monocytogenes*
4 isolates were generated using iVARCall2 and Snippy pipelines, including respectively GATK-
5 HaplotypeCaller and Freebayes, two of the most widely used variant callers for the identification of
6 SNPs and InDels in microbial genomics study. Then, the phylogenomic trees built with the iVARCall2-
7 and Snippy-based concatenated SNPs (50,349 and 66,142 SNPs respectively) were compared in terms
8 of tree topologies (i.e. patterns of branching) and distances (i.e. branch lengths). The topology
9 variations and indexes for distance were calculated using different R packages in accordance to Henri
10 et al. (2017) [1]. In particular, the Fowlkes-Mallows index (FMI) [2] for the measure of similarity
11 between two clusterings was computed. Moreover, the correlation between the cophenetic distance
12 matrices of the two cluster trees was computed using the cophenetic correlation coefficient (CCC) [3]
13 implemented in the “cor_cophenetic” function of R “dendextend” package. To better visualize the
14 comparison between the two phylogenies reconstructions, data were plotted connecting the same
15 strains between leaves (on the left iVARCall2- based and on the right Snippy-based trees). The
16 comparison of trees topology, calculated calibrating the trees by correlating sampling date to the tip
17 distance from root, revealed that the two variant calling pipelines lead to highly similar results showing
18 no clustering differences in the shapes of the trees. Based on the FMI and CCC estimation, the
19 quantification of similarity between the iVARCall2- and Snippy-based phylogenies resulted in
20 extremely high values, FMI = 1 and CCC = 0.9985065. These statistical results strongly support the
21 conclusion that these two variant calling methods lead to the same phylogenomic reconstruction, even
22 though the number of analysed variant sites differed. Therefore, results from iVARCall2 pipeline were
23 used for further analyses in the present study.



24

25 **References**

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