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Title

Microevolution and persistence of *Listeria monocytogenes* in the environment and links to a foodborne outbreak

Keywords

Listeria monocytogenes, outbreak, microevolution, discrimination power, persistence

Background

Listeria monocytogenes is a foodborne human pathogen associated with a high mortality rate. Given the remarkable ability of this bacterium to persist in food processing facilities, ready-to-eat food products are common vehicles of *L. monocytogenes.* In the present study, we report the use of whole-genome sequencing (WGS) to investigate the diversity among *L. monocytogenes* isolates associated with a hospital outbreak of listeriosis occurred in Portugal.

Materials | Methods

To investigate the potential source of 3 cases of hospital-acquired *L. monocytogenes* infections, we performed WGS (on a MiSeq Illumina instrument) on strains isolated from: patient (n= 3); environment, collected by swabbing surfaces in the establishment that produces the foodstuffs for the hospital (n = 20); and ready-to-eat foods prepared in this establishment and served in the hospital (n = 4). Samples from the environment included surfaces of the processing area and equipment, and were collected spaced by 2 weeks, with cleaning and disinfection procedures performed during this interval. To infer the genetic relatedness among all the strains isolated, we performed reference-based mapping followed by the identification of bona fide core Single Nucleotide Polymorphisms (SNPs) (using samtools/bcftools). SNP-based phylogenetic relationships among the isolates were visually depicted in a minimum spanning tree constructed using the goeBURST algorithm implemented in the PHYLOViZ platform.

Results

L. monocytogenes infections were confirmed in the 3 patients admitted to a central hospital in Portugal, being the meals prepared in a food establishment and distributed in the hospital the presumable pathogen's vehicle. WGS-based investigation of the relatedness between clinical isolates and multiple isolates collected from that establishment and ready-to-eat foods (Figure 1) revealed:

i) a high degree of genetic homogeneity between clinical and environmental isolates, with a maximum SNP distance among all isolates of only 12 SNPs;

ii) 14 unique sequences profiles, indicating that at least 14 distinct clones coexisted outbreak' source;

iii) that 2 clinical cases genetically match to several clones collected from diverse locations in the establishment that produces the foodstuffs for the hospital, whereas the third patient isolate was closely linked with a single location;
iv) a repertoire of loci likely involved in microevolutionary events (where non-synonymous mutations are overrepresented) potentially driving *L. monocytogenes* environmental persistence;
v) that clones isolated from ready-to-eat foods perfectly matched to clones circulating in the surfaces of the processing area and equipments, corroborating that cross-contamination may still occur.
Further analyses are currently underway to investigate the potential existence of mobile genetic elements in order to increase the resolution power, and perhaps, to establish the genetic link between the two indistinguishable clinical isolates and a particular location in the outbreak source.

Conclusion

In this study, we show how the high discriminatory power of WGS may identify potential links within outbreak scenarios and unveil how *L. monocytogenes* microevolution may pose some challenge in attributing meaningful similarity thresholds for food source attribution. It can also contribute to identify genes targeted by microevolution that could contribute to the *L. monocytogenes* persistence in food processing facilities.