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IEGULDĪJUMS TAVĀ NĀKOTNĒ

Bacterial Whole Genome Sequencing in Investigation of *Salmonella* Typhimurium Infection Outbreak, Riga, Latvia

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Introduction

Salmonellosis is one of the most important food-borne diseases that can cause significant morbidity and mortality worldwide. It is estimated that 93.8 million cases of gastroenteritis due to *Salmonella* species occur globally each year, with 155,000 deaths [1]. In 2017, 91,662 confirmed human salmonellosis cases were reported in the European Union by all the member states [2]. In Latvia, 234 cases of human *Salmonella* infections were registered in 2017. *Salmonella enteric* serovar Enteritidis accounted for 70% of cases, and *Salmonella enteric* serovar Typhimurium accounted for 17, 5% [3].

Fast and accurate identification and characterization of *Salmonella spp.* isolates is one of the most important public health monitoring tasks in outbreak investigation and epidemiology [4].

Next Generation Sequencing (NGS) technologies and simplified sample preparation allow for complete genome sequencing of bacteria and virus isolates in less than 48 hours. The speed of these technologies and improved cluster resolution make it an alternative to traditional molecular typing techniques [5].

Routine typing with whole genome sequencing allows for early detection of outbreaks and helps public health professionals find the likely source of the outbreak.

Background

A large outbreak of *Salmonella enterica* serotype Typhimurium infection occurred in Riga during January and February 2018. At least 98 children from 14 different kindergartens had been infected. First symptoms of salmonellosis appeared on January 10 and later.

Objective

The aim of this study was to evaluate the usefulness of whole genome sequencing in molecular typing of *Salmonella* Typhimurium.

Sample selection

• 107 *Salmonella* isolates from human samples obtained from October 2017 to February 2018.
• 25 *Salmonella* Typhimurium isolates from food and veterinary samples obtained from a national monitoring program from 2015 to 2017.
•No *Salmonella* Typhimurium isolates from food or environmental samples were obtained in connection with the particular outbreak.

Methods

• *Salmonella* from food and veterinary sources were isolated and detected by standard protocol of ISO 6579. Salmonella from human feces were isolated and detected according to Clinical Microbiology Procedures Handbook (Fourth Edition, USA, 2016).
• Genomic DNA was prepared using a DNA Extraction Kit (NORGEN Biotek, Canada).
• *Salmonella* Typhimurium isolates were subjected to whole genome sequencing (WGS) using the Illumina MiSeq system with 2x300 base pairs reads length. DNA libraries were constructed with the Nextera XT DNA Library Preparation Kit (Illumina).
• Reads were assembled *de novo* using the Velvet algorithm program 1.1.04.
•For data processing and analysis Ridom SeqSphere + version 4.1.9 was used.
• To analyze the relationships between *Salmonella* Typhimurium isolates multi locus sequence typing (MLST) and core genome MLST were performed. cgMLST nomenclature accordingly to Ridom Nomenclature server www.cgmlst.org was used.
•The 3002 locus cgMLST scheme was used with the cluster alert threshold of ≤7 differences.

Results

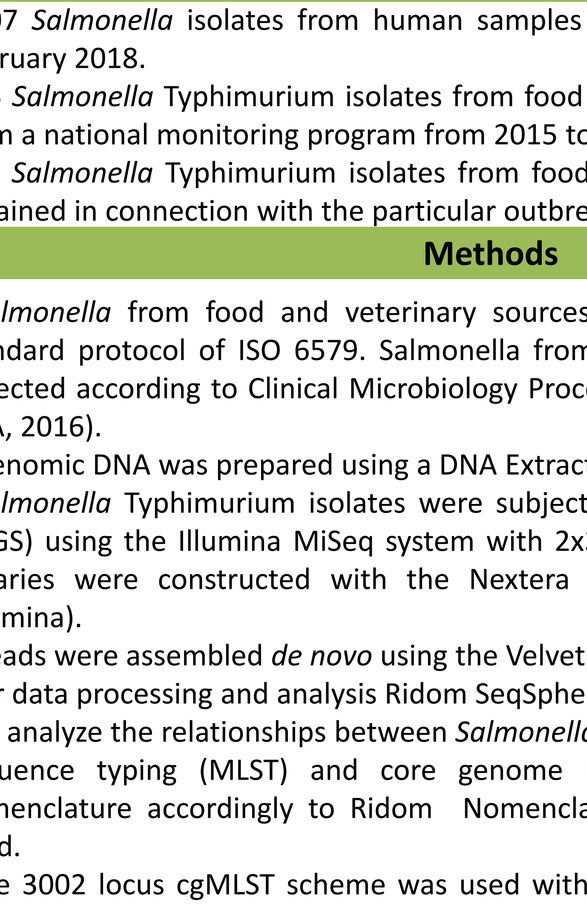

Fig.1. Epidemic curve of weekly reported human *Salmonella* cases in Riga, Latvia. In red – MLST- 19, green – MLST-34.

MLST and cgMLST classification of *Salmonella* isolates

Group of isolates	Number of isolates	MLST type (N)	cgMLST genotype (N)
Isolates from human samples	107	MLST-19 (102) MLST-34 (5)	cgMLST – 1013 (97) cgMLST - 1014 (1) cgMLST - 1291(4) cgMLST - 379 (1) cgMLST - 1017 (1) unique cgMLST(3)
Isolates from food and veterinary sources	25	MLST-19 (25)	cgMLST – 1096 (3) cgMLST – 1015 (1) cgMLST – 1016 (7) cgMLST - 1018 (5) cgMLST – 1080 (3) cgMLST – 1083 (1) cgMLST – 1085 (1) cgMLST – 1086 (2) cgMLST – 1088 (1) cgMLST – 1100 (1)

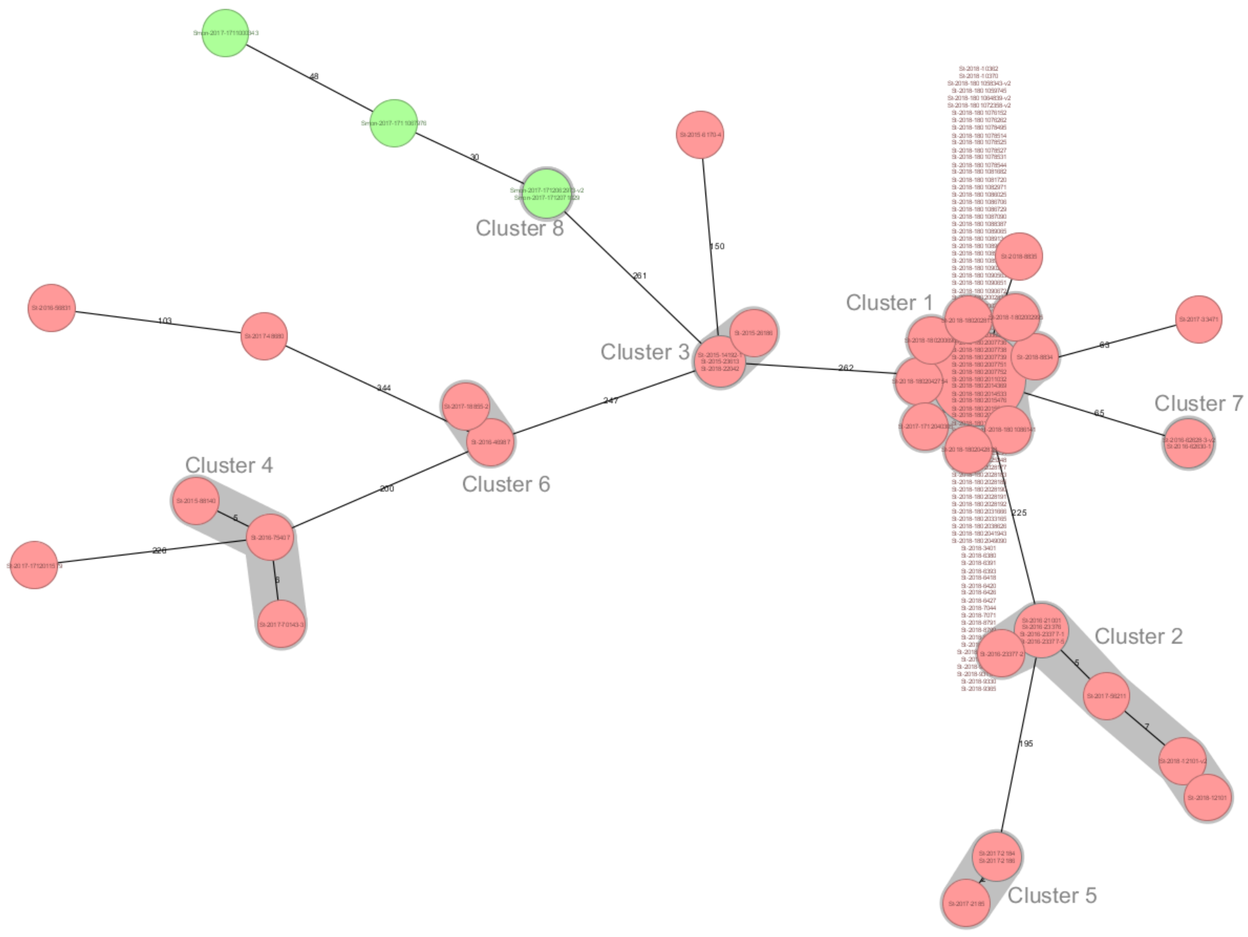

Fig.2. Minimum spanning tree of *Salmonella* Typhimuriu isolates based on core genome multilocus sequence typing analysis. In red – MLST- 19, green – MLST-34.

Task Templates: S. enterica cgMLST v2.0, S. enterica MLST v1.0
S. enterica cgMLST Complex Type / Cluster-Alert distance: 7
Ridom SeqSphere+ MST for 125 Samples based on 3002 columns, pairwise ignoring missing values, logarithmic scale
Distance based on columns from S. enterica cgMLST (3002)

Conclusion

• 97 *Salmonella* Typhimurium isolates from human sources were genetically related and belonged to MLST-19 type and cgMLST – 1013 genotype.
•The first case with cgMLST 1013 was on December 12, 2017.
• The most related isolates to the outbreak cgMLST 1013 clinical strain were veterinary isolate from May 2017 (63 different alleles) and two food isolates from September 2016 (65 different alleles).

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