User-friendly WGS analysis of *Salmonella* Enteritidis PT4 outbreaks

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Introduction

Food-borne outbreaks of *Salmonella* Enteritidis (*S*. Enteritidis) linked to eggs and egg-related products still frequently occur in Europe [1]. In April and May 2014, two geographically separated *S*. Enteritidis outbreaks were investigated by the Belgian NRL-FBO. Since isolates from food and human cases were available for both outbreaks, they were used as a case study for a retrospective outbreak investigation with whole genome sequencing (WGS).

Materials and Methods

Epidemiological information gathered from both outbreaks through standard questionnaires and food samples were sent to the NRL-FBO, which isolated and identified *Salmonella* in 3 samples. The NRCSS received *Salmonella* isolates from 3 human cases. The serotype, phage type, MLVA profile and minimal inhibitory concentration (MIC) for 14 antimicrobials were determined in the 6 isolates.

The 6 *Salmonella* isolates were sequenced on the Illumina Hiseq 2000 using 100 bp paired-end reads. Whole genome sequencing (WGS) data analysis was performed on a Windows 7 platform with CLC Genomics Workbench 8.0, tools available on the Center for Genomic Epidemiology's server and BRIG [2].

Results

The 2 food and 2 human isolates of the Flemish outbreak and 1 food and 1 human isolate of the Walloon outbreak were serotyped as *S*. Enteritidis. They were assigned MLVA profile 3-10-5-4-1 and phage type PT4, except for 1 human isolate (Flemish) that was phage typed as PT4a. Together with the epidemiological investigation, these results linked the human cases to the egg-containing food samples for both outbreaks.

The WGS analysis started with *de novo* assembly of the quality trimmed FASTQ reads with CLC Genomics Workbench, after which the contigs were uploaded to MLST server [3], PlasmidFinder [4] and ResFinder [5]. All isolates were reported as ST-11, having a single plasmid and no antimicrobial resistance genes, apart from the PT4a isolate which also was determined as ST-11, but with an additional plasmid and a bla_{TEM} gene. Antimicrobial susceptibility tests confirmed the detected resistance gene phenotypically and BRIG analysis indicated that the bla_{TEM} gene was located on the additional plasmid. SNP calling on FASTQ reads through the CSI Phylogeny server [6] specified 0 to 2 SNPs difference within all 4 isolates of the Flemish outbreak and no SNP difference within the 2 isolates of the outbreak in Wallonia, whereas there were 51 to 53 SNPs detected between the 2 outbreaks.

Discussion

WGS analysis confirmed the link between human and food isolates that was identified through the classical epidemiological and microbiological outbreak investigation, but could additionally distinguish the 2 outbreaks based on SNP analysis and fine-tune the results via exploration of the present plasmids. This illustrates the added value of the use of WGS for outbreak investigation. An interesting finding was the presence of an additional resistance plasmid in 1 human isolate that may have been acquired in the intestine during the foodborne infection. The WGS analysis with available user-friendly software and tools shows that the use of WGS for outbreak investigation is not strictly limited to specialised bioinformaticians and that it is feasible within the setting of an NRL.

References

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