GLOBALLY INTEGRATED *SALMONELLA* WGS TRACKING USING OXFORD NANOPORE TECHNOLOGIES LONG-READ SEQUENCING

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IAFP 2025 Cleveland Ohio JULY 27–30

P3-203

ABSTRACT

Introduction: The reduced cost and faster turnaround time of next-generation sequencing (NGS) have enabled its seamless integration into high-throughput microbiology service laboratories. Eurofins laboratories in Madison (Wisconsin), and Nantes (France), conducted a proof of concept (POC) to implement a harmonized *Salmonella* serotyping and tracking method using Oxford Nanopore Technologies (ONT) and internally developed bioinformatics pipelines.

Purpose: To demonstrate a POC for a reliable end-to-end NGS method to serotype and globally track *Salmonella* isolates originating from the food industry, using Oxford Nanopore Technologies long-read sequencing system.

Methods: Three independent replicates of a reference strain *Salmonella enterica* subsp. *arizonae* DSM 9386, and three independent replicates of three wild-type *Salmonella sp* (n=9) were grown overnight on XLD or TSA agar in Madison as well as Nantes. DNA from isolated colonies was extracted, purified, fragmented, and normalized according to internal/ONT protocols. The DNA was prepared for sequencing using the ONT Native Barcoding Kit 24 V14 and run on an R10.4.1 flow cell using the ONT GridION until each barcode generated at least 300 Mb of data. Both FASTQ data sets were then centralized for bioinformatic analyses using an internally developed and validated pipeline for *Salmonella* serotype identification and single nucleotide variant calling (SNV).

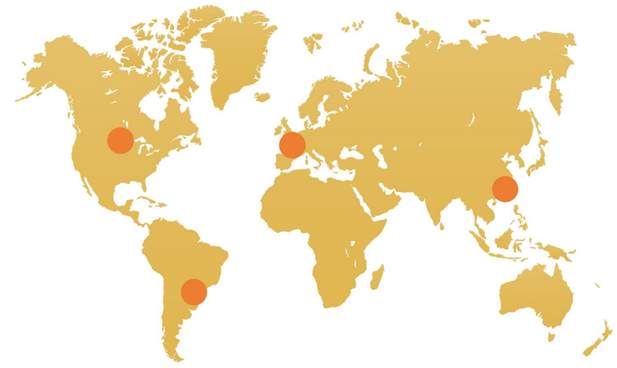
Results: The sequenced and *de novo* assembled genomes of the reference species *Salmonella enterica* subsp. *arizonae* DSM 9386 differed by less than 3 SNVs between replicates. The genomes of the wild-type *Salmonella* differed by less than 1 SNV between replicates. Less than 3 contigs were obtained per genome, and a coverage depth of more than 30X was obtained on 99.6% of the genome. These results indicate that the method is accurate and reproducible.

Significance: The harmonized NGS sequencing system allows for the identification and tracking of *Salmonella* serotypes using laboratories in Europe and North America without the need to ship pathogens internationally. This advancement has significant implications for global food safety and pathogen tracking.

GLOBAL COVERAGE

Laboratories across 4 continents have established the same WGS protocols with the bioinformatic analyses being centralized in Nantes, France. The network allows for rapid and cost-effective solutions for WGS strain tracking.

The centralized bioinformatics pipeline is based on Snakemake integrating Nanofilt tools for data filtration, Flye for de novo genome assembly, Medaka for polishing, Nanocaller assembly for SNV calling and Seqsero2 for serotype prediction.



Samples/DNA were prepared in accordance with ONT ligation sequencing protocol

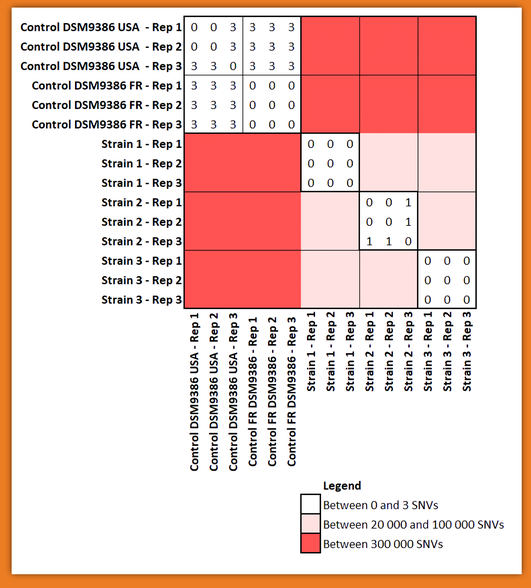
DNA was sequenced using Oxford Nanopore Technologies GridION instrument.

Sequence data analyzed by internally validated pipeline for *Salmonella* serotype identification and SNV calling

Sample type	Mb (> 300)	Number of contigs	Length of contigs (Mb)	Mean read depth after assembly	Percentage of the assembly > 30x	Minimum depth on 90% of the assembly	Length of the largest contig (Mb)	Predicted serovar
Positive control ATCC	2359	1	4.4	516	99	412	4.4	IIIa -:z4,z23:-
	9974	2	4.5	215	99	181	4.4	IIIa -:z4,z23:-
	3702	2	4.5	398	99	345	4.4	IIIa -:z4,z23:-
Customer strain 1	3538	1	4.8	354	99	325	4.8	Livingstone
	3441	1	4.8	344	99	315	4.8	Livingstone
	3346	1	4.8	335	99	303	4.8	Livingstone
Customer strain 2	1456	2	4.7	302	99	268	4.6	Infantis
	1553	2	4.7	323	99	288	4.6	Infantis
	1408	2	4.7	292	100	257	4.6	Infantis
Customer strain 3	2962	1	4.7	304	99	276	4.7	Braenderup
	1323	1	4.7	272	99	246	4.7	Braenderup
	1127	1	4.7	232	99	207	4.7	Braenderup

Table 1. Madison, WI microbiology laboratory *Salmonella* sequence data and predicted serovars. The genome of the reference strain differed by <3 SNVs per replicate. The genomes of the wild type *Salmonella* differed by <1 SNV per replicate.

RESULTS



CONCLUSIONS

- **The proof of concept demonstrated that a harmonized ONT-based NGS workflow, implemented across laboratories in the U.S. and Europe, provides accurate, reproducible, and high-resolution *Salmonella* serotyping and strain tracking.**
- **The method showed minimal genomic variation between replicates and achieved high-quality genome assemblies, confirming its robustness for routine use in microbiology service labs.**
- **This harmonized approach enables global pathogen surveillance without the need for international sample shipment, offering a scalable solution for enhancing food safety.**

INTRODUCTION

- Next Generation Sequencing is ideally suited for tracking pathogens in food manufacturing operations.
- Its resolution allows for early detection of contamination, source tracking, and detection of a small genetic evolution of a pathogen.

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