Shotgun metagenomics as a ONE Health tool for better protecting human health

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Introduction

Conventional methods for bacterial pathogen detection and characterization in various samples have several drawbacks:
- *A priori* knowledge of what to look for
- Culture dependent
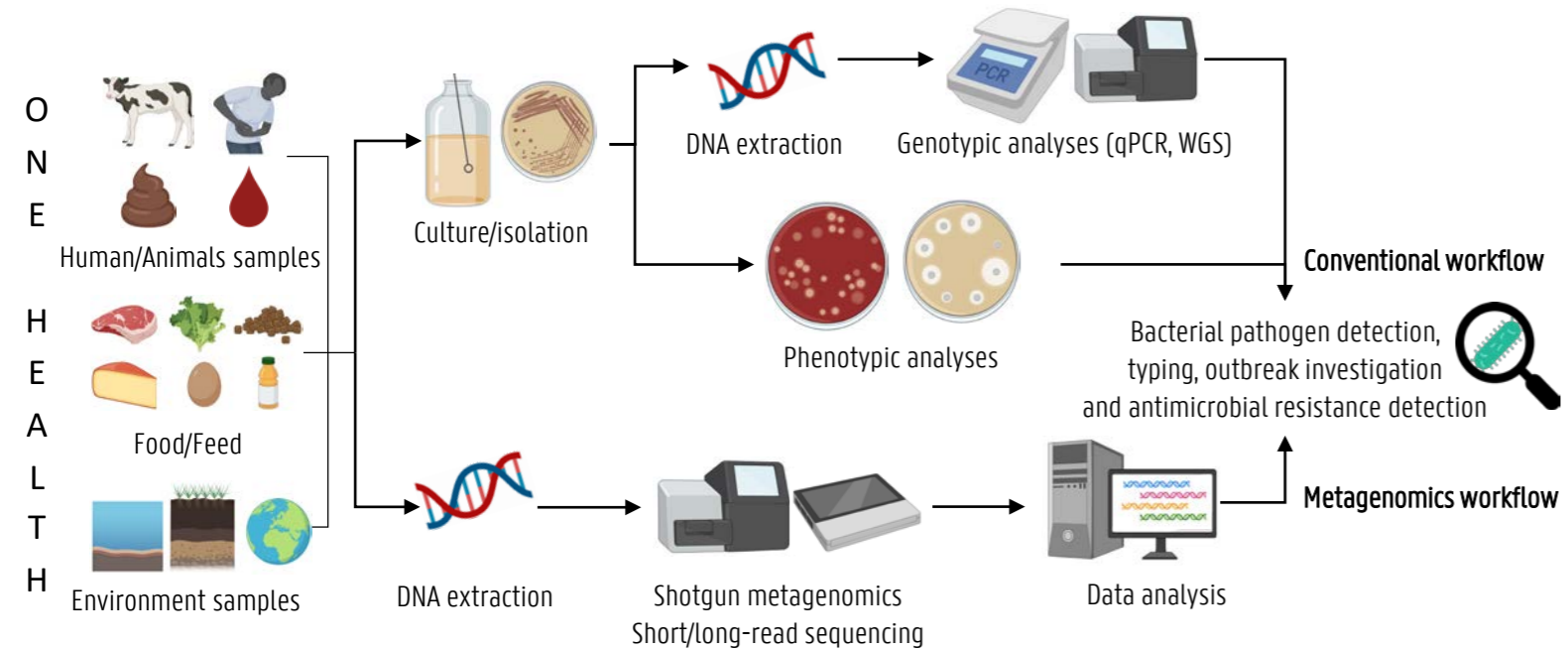- Time consuming and not complete

In comparison, shotgun metagenomics is:
- Open approach (all sample's genetic material sequenced)
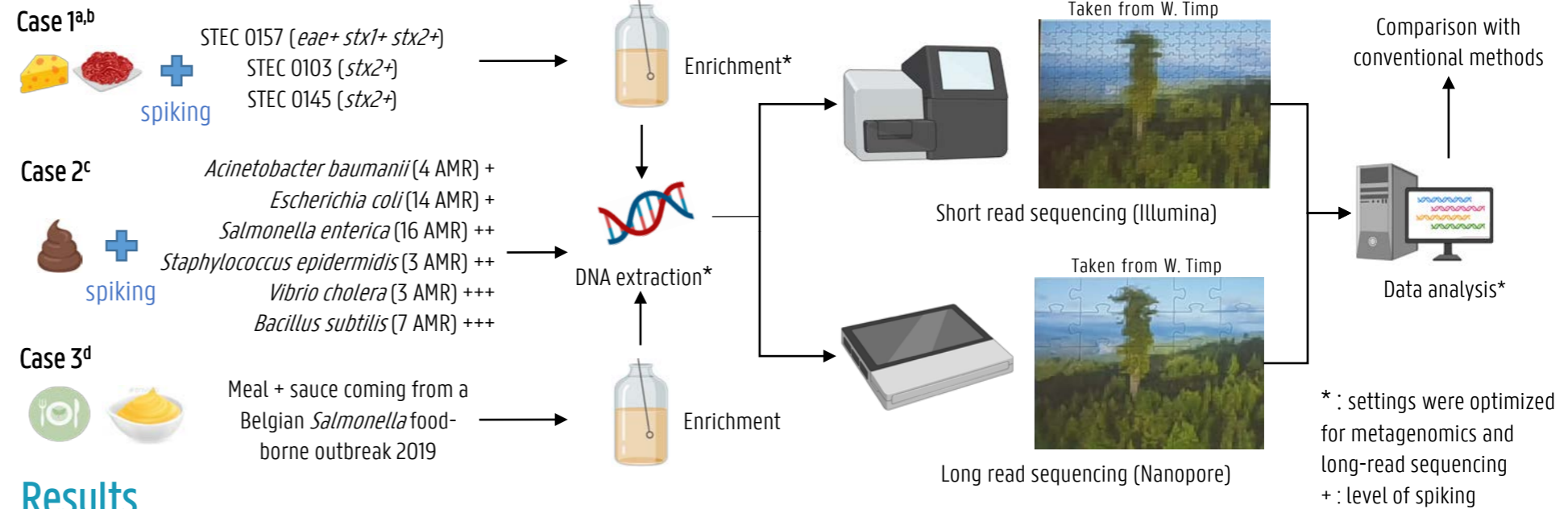- Fast, efficient and accurate

→ could transform the way specific key public health questions can be addressed, in a ONE Health context.

Adapted from C. Chiu – UCSF, edited from Fields (2001) Science

ONE HEALTH

Human/Animals samples
Food/Feed
Environment samples

Culture/isolation
DNA extraction
Genotypic analyses (qPCR, WGS)
Phenotypic analyses

Conventional workflow

Bacterial pathogen detection, typing, outbreak investigation and antimicrobial resistance detection

DNA extraction
Shotgun metagenomics Short/long-read sequencing
Data analysis

Metagenomics workflow

Further information

Aknowledgments

Literature cited
[a]Buytaers FE et al., Microorganisms, 2020
[b]Buytaers FE et al., Frontiers in Microbiology, 2021
[c]12 months FARMED yearly report, 2021
https://onehealthejp.eu/wp-content/uploads/2021/06/OHEJP-Annual-Report-2020_final-1.pdf
[d]Buytaers FE et al., Microbial genomics, 2021

Methodology

Case 1[a,b]
STEC O157 (*eae+ stx1+ stx2+*)
STEC O103 (*stx2+*)
STEC O145 (*stx2+*)
spiking
Enrichment*

Case 2[c]
Acinetobacter baumanii (4 AMR) +
Escherichia coli (14 AMR) +
Salmonella enterica (16 AMR) ++
Staphylococcus epidermidis (3 AMR) ++
Vibrio cholera (3 AMR) +++
Bacillus subtilis (7 AMR) +++
spiking
DNA extraction*

Case 3[d]
Meal + sauce coming from a Belgian *Salmonella* food-borne outbreak 2019
Enrichment

Taken from W. Timp
Short read sequencing (Illumina)

Taken from W. Timp
Long read sequencing (Nanopore)

Comparison with conventional methods
Data analysis*

* : settings were optimized for metagenomics and long-read sequencing
+ : level of spiking

Results

Case 1[a,b]: Using a 24h enrichment, strain inference, relatedness and full characterization of the 3 STEC strains was possible, both with Illumina and Nanopore sequencing, as previously obtained with conventional methods.

Case 2[c]: DNA extraction was optimized for production of long-fragments. All the species could be detected except the lowest abundant ones (+), also not detected by conventional methods. For the most abundant ones (+++), long-read sequencing allowed to link species and AMR identifications using bioinformatics. This was not possible with short-reads.

AMR gene
Long-read used for species ID

Case 3[d]: *Salmonella* was identified in 2 metagenomics samples from suspected food. Both could be linked to human cases from the Belgian outbreak and discriminated from a Polish outbreak (previously investigated by WGS)
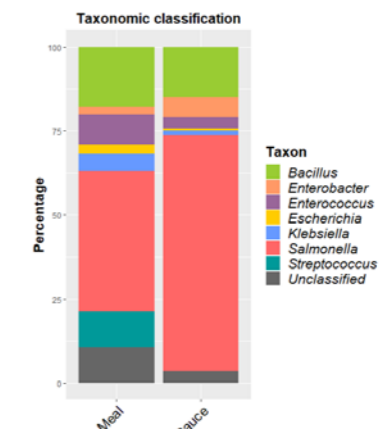
Taxonomic classification

	Reference coverage
S19FP07575	90%
S19FP07382	91%
S19FP07414	91%
S19BD06641	95%
metagenomics_sauce_salmonella	97%
S19FP07457	89%
metagenomics_meal_salmonella	85%
S19BD06627	93%
S19BD06633	95%
S19BD06604	92%
S19BD06642	92%
S19FP07576	89%
S19BD03708	89%
S19BD04434	92%
S19BD03792	87%
S19BD04892	89%
S18BD04909	92%
TIAC3667	49%
TIAC3663	39%
TIAC3651	41%
S17BD05926	93%
SRR3285443	97%
S19BD01323	91%
TIAC3662	32%
TIAC3670	40%
S17BD07382	92%
S19BD04853	91%
S19BD05008	88%
S19BD04888	88%
S19BD04889	85%
S19BD04976	89%
S19BD04986	90%
ERR2173854.1	97%
SRR4063739	97%
TIAC3660	43%
TIAC3659	71%
S19BD01263	93%
SRR4063700	97%

Belgian outbreak UI-608 09/2019

Polish outbreak UI-367 2016-2020

Conclusions

The 3 case studies showed the added value of shotgun metagenomics compared to conventional methods, in a ONE Health context. Indeed, this technique was used for accurate pathogen identification and characterization in various samples as well as for outbreak investigation. Moreover, long-reads increased the resolution of AMR detection and their location in the genome. Future work will focus on the method's portability for easy, fast and direct use on-site and on environmental samples.