New insights in identifying antimicrobial acquired genes by using short and long read sequencing technologies: a pilot study on *Salmonella* under One-Health settings.

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Introduction

To reduce the risk of spreading antimicrobial resistance, there is a need to better understand gene transfer dynamics and in particular for *Salmonella* under one health setting. Mobile elements play a crucial role in the transmission pathways of acquired antibiotic resistance genes (ARGs).

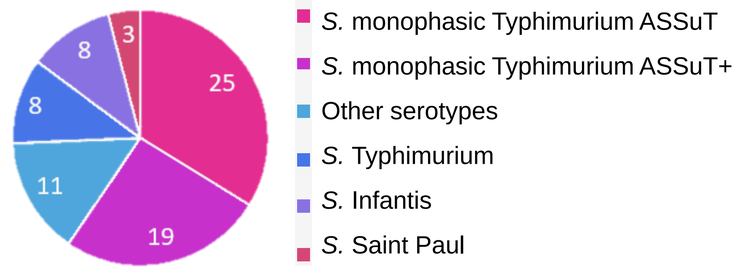
Does plasmid reconstruction of MDR *Salmonella* spp. provide additional information about AMR profile?

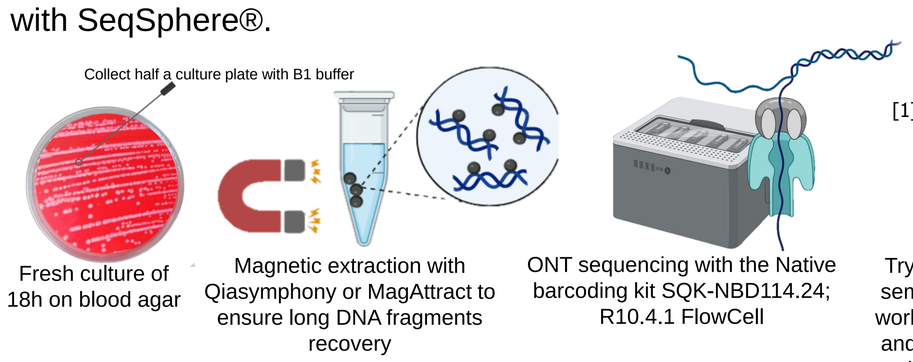
In this pilot study, a pipeline for plasmid reconstruction was developed from long reads sequencing data. A comparison of the ARGs detected by WGS with short and long reads technologies was conducted.

Material & methods

The selected panel is composed of 74 *Salmonella* strains of 47 human strains and 27 strains from diverse sources (pig, N=6; food, N=5; poultry, N=2; pets, N=2; feed, N=2; Bovine, N=1; environmental N=7, Kinder outbreak, N=2) isolated in 2023 and 2024. This panel was selected according to their predicted resistance to at least three antimicrobial classes by using Illumina sequencing data processed with SeqSphere®.

Distribution of serotype N=74





[1] Trycycler

[2]

Trycycler-centric pipeline with semi-automatized Snakemake workflow enabling the complete and accurate reconstruction of chromosome and plasmids

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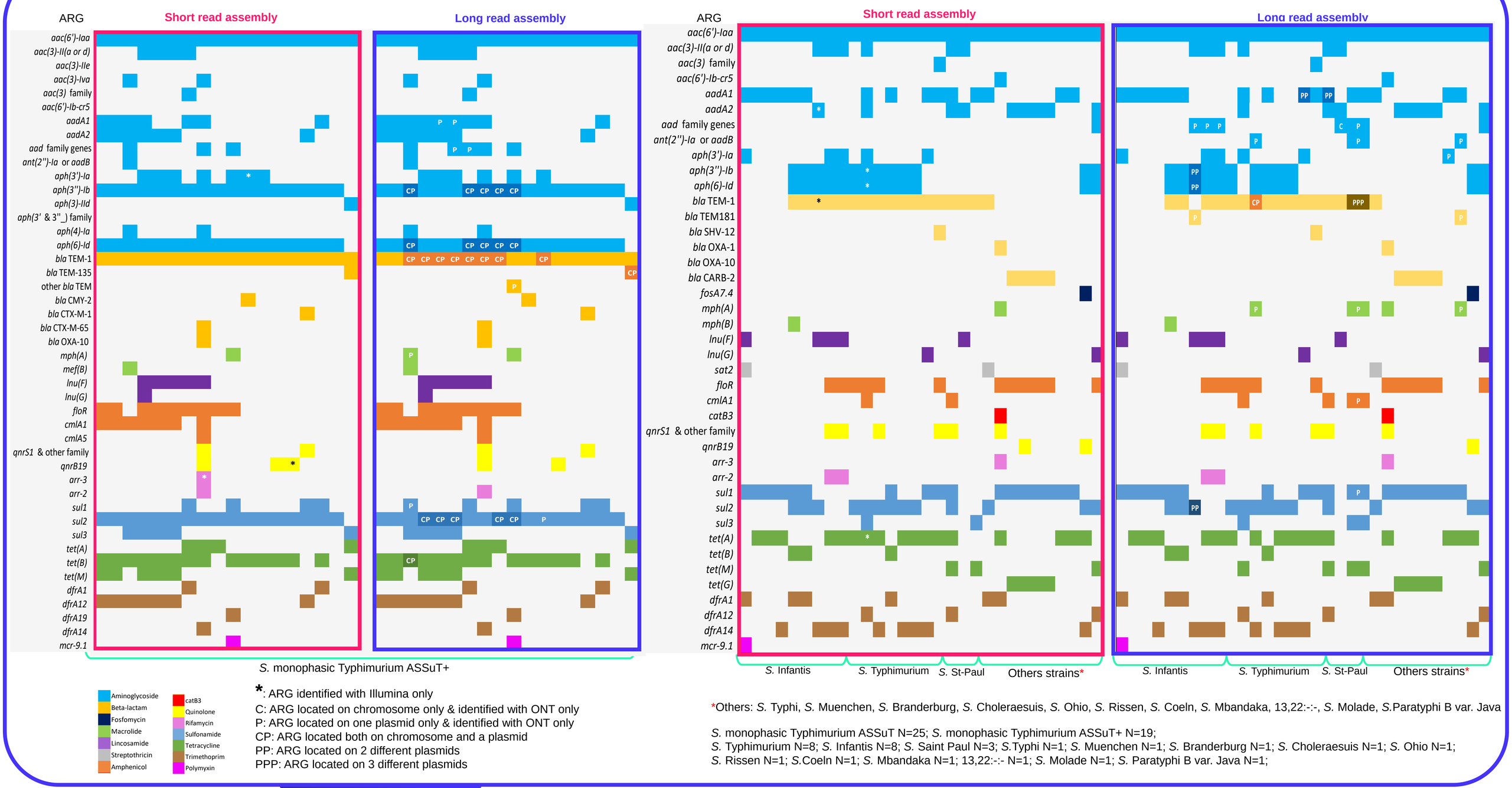
AMRfinder+ Resfinder

Assemblies were subjected to *AMRFinderPlus, Resfinder and CARD* databases to detect ARGs and results were correlated with ARGs detected with Illumina data

AMR profiles defined by both sequencing strategies and location of multiple or extra copies of ARGs



*: ARG identified with Illumina only

C: ARG located on chromosome only & identified with ONT only
P: ARG located on one plasmid only & identified with ONT only
CP: ARG located both on chromosome and a plasmid
PP: ARG located on 2 different plasmids
PPP: ARG located on 3 different plasmids

*Others: *S.* Typhi, *S.* Muenchen, *S.* Branderburg, *S.* Choleraesuis, *S.* Ohio, *S.* Rissen, *S.* Coeln, *S.* Mbandaka, 13,22:-:-, *S.* Molade, *S.*Paratyphi B var. Java

S. monophasic Typhimurium ASSuT N=25; *S.* monophasic Typhimurium ASSuT+ N=19;
S. Typhimurium N=8; *S.* Infantis N=8; *S.* Saint Paul N=3; *S.*Typhi N=1; *S.* Muenchen N=1; *S.* Branderburg N=1; *S.* Choleraesuis N=1; *S.* Ohio N=1;
S. Rissen N=1; *S.*Coeln N=1; *S.* Mbandaka N=1; 13,22:-:- N=1; *S.* Molade N=1; *S.* Paratyphi B var. Java N=1;

Discussion

- Both sequencing platforms were concordant in detecting 88.8% of ARGs.
- No plasmid hosting ARGs were detected from any *S.* monophasic Typhimurium with ASSuT profile
- By short-read sequencing, 8 additional genes were detected, mainly associated with aminoglycoside class (N=4 strains).
- By using long-read sequencing, 24 genes were uniquely identified and 33 extra copies of genes were detected either on both chromosome and plasmids (N=26 Copies) or on plasmids only (N=7 copies).
- One *S.* Typhimurium from human origin harbored 3 *bla*TEM-1 copies

Conclusion

Long read sequencing combined to the plasmid reconstruction pipeline identified duplicated genes as well as their located on different genomic entities (chromosome and/or plasmid). Strains with multiple copies of ARGs from the same antibiotic class might have an enhanced resistance phenotype that remains to be investigated.

References

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