IMPLEMENTATION OF WHOLE-GENOME SEQUENCING FOR CHARACTERIZATION OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* AT THE BELGIAN NATIONAL REFERENCE CENTRE

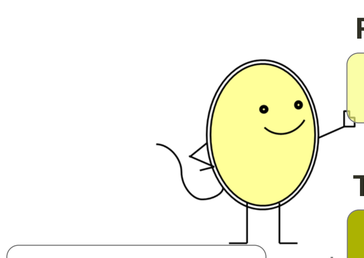
F. Crombé[1], K. De Rauw[1], O. Soetens[1], B. Caljon[2], D. Piérard[1]

1. Department of Microbiology, National Reference Centre for Shiga toxin-producing *Escherichia coli*, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium
2. Brussels Interuniversity High Throughput core (BRIGHTcore), Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium

INTRODUCTION

- Whole genome sequencing (WGS) has been implemented for routine molecular surveillance of STEC in some public health laboratories.
- The WGS technology enables to fully characterize STEC isolates, facilitating epidemiologic surveillance and enabling data comparison in case of multi-country outbreak.
- In order to validate the implementation of WGS at the Belgian National Reference Centre (NRC) for STEC, we compared the results obtained by traditional methods for O:H-typing and virulence typing (*stx* subtype, *eae*A, *ehx*A, *aai*C and *agg*R) to those obtained from Illumina-based sequencing data using the *E. coli* genotyping plugin tool v.1.2 of the BioNumerics software v.7.6 (Applied Maths, Biomérieux, Belgium).

MATERIALS & METHODS



40 STEC strains

Voluntarily provided by clinical laboratories to the NRC STEC

Routine typing[1]

O-serotyping (the ‘Big 6’) | Motility (MIU) | *Stx*1, *stx*2 & *stx*2f-multiplex PCR | *Stx*2a-PCR | *Stx*2d-PCR | *eae*A-PCR

Three-monthly typing[1]

Stx1 & 2-subtyping PCRs | *ehxA*-PCR | *aaiC aggR-PCR* | *flicH7-PCR* | *gnd*-PCR and sequencing

Illumina-based WGS

[1] Link to the NRC website
https:nrchm.wiv-isp.be/nl/ref_centra_labo/shiga_toxine_verotoxine/default.aspx

VIRULENCE GENES DETECTION

- All 24 *stx*1-positive isolates were detected by BioNumerics *in silico* PCR. There was 100% agreement with *stx*1 subtyping.
- All 31 *stx*2-positive isolates were detected by *in silico* PCR. There was a good agreement with *stx*2 subtyping except for 4 strains carrying *stx*2a and *stx*2c.
- *Eae*A, *ehx*A, *aai*C, *agg*R and *flic*H7 genes were detected correctly by *in silico* PCR.

Figure 1: Comparison of results obtained for *stx*1- and *stx*2-subtyping with traditional PCR and BioNumerics *in silico* PCR.

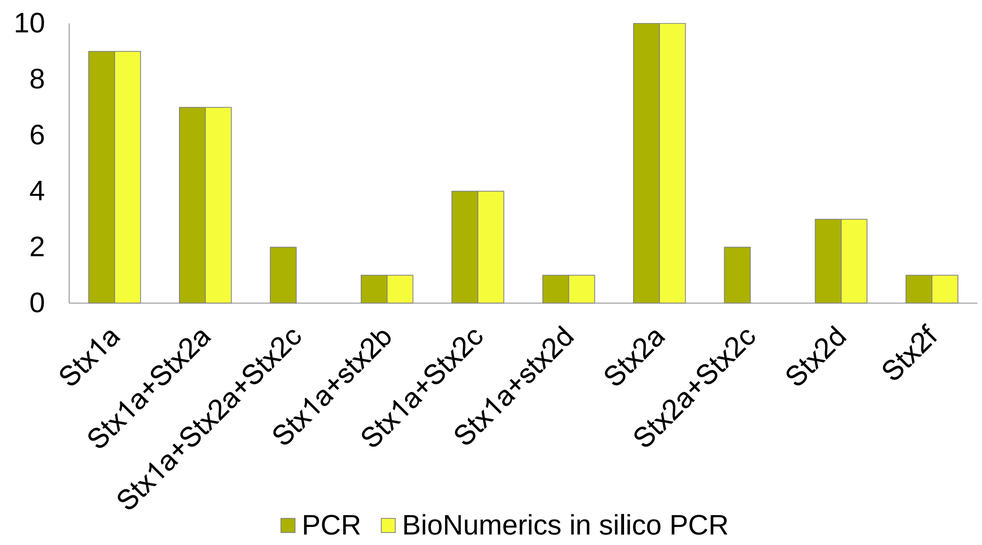
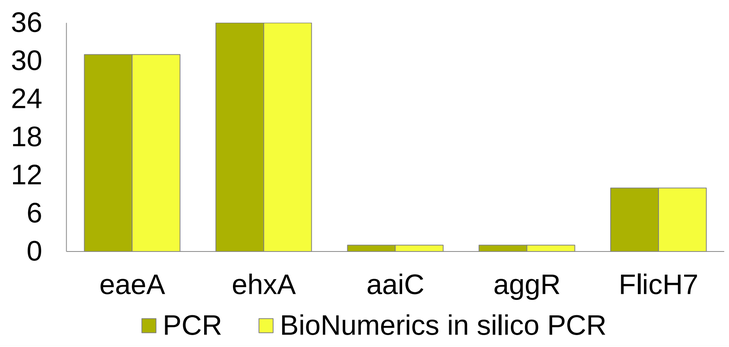


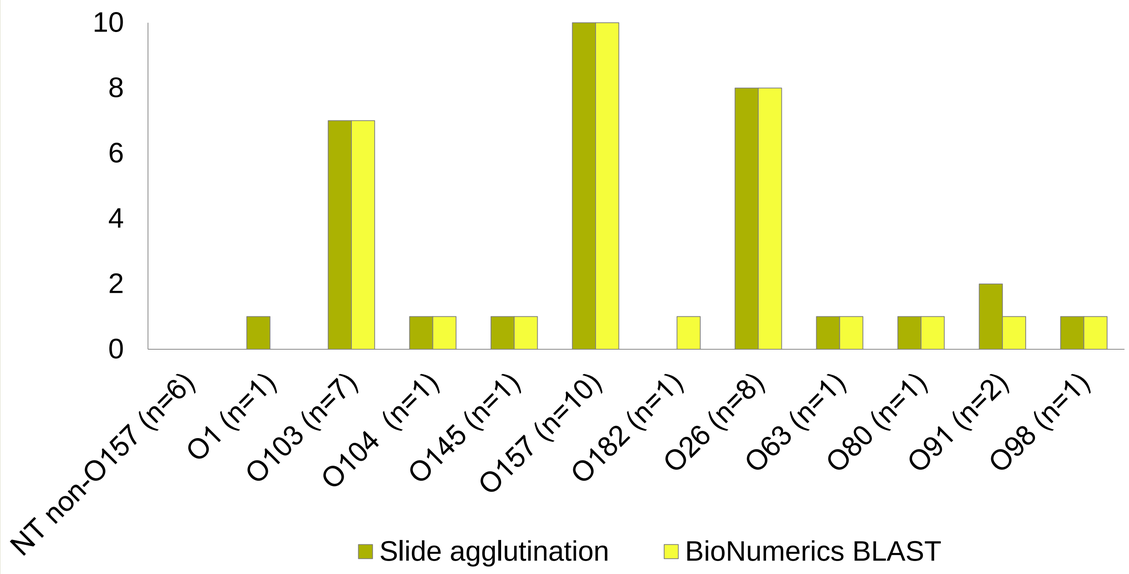
Figure 2: Comparison of results obtained for *eae*A, *ehx*A, *aai*C, *agg*R and *flic*H7genes with traditional PCR and BioNumerics *in silico* PCR.



O-TYPING

- All 26 isolates belonging to the “Big 6” serotypes (O26, O103, O111, O121, O145 and O157) were predicted correctly by BioNumerics BLAST.
- Five isolates out of the remaining 14 non-O157 isolates were determined correctly, 6 were untypable (NT) by both methods and 3 were only determined by one of these methods.

Figure 3: Comparison of results obtained for O-type with traditional slide-agglutination and BioNumerics BLAST.



CONCLUSION

- A good agreement was found between the results obtained by the traditional methods and WGS for O:H-types and virulence genes.
- WGS will be used at the NRC for molecular surveillance although some additional work is necessary to unravel the inconsistenties found in some isolates.

PART OF THIS WORK WAS PERFORMED IN THE FRAME OF THE BELGIAN NATIONAL REFERENCE CENTRE FOR SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*, SUPPORTED BY THE MINISTRY OF SOCIAL AFFAIRS THROUGH A FUND WITHIN THE NATIONAL HEALTH INSURANCE SYSTEM.