Evaluation of **Whole-Genome Sequencing** combined with a bioinformatics tool for the complete characterization of ***Streptococcus agalactiae*** infection

Sabrina Egrek [1,2], Rosalie Sacheli [1,2], Cécile Meex [1,2], Maria Isabel Taveira Da Silva Pereira[2], Marie-Pierre Hayette [2], Pierrette Melin [1,2]
(1) National Reference Centre *Streptococcus agalactiae*, Clinical microbiology, University Hospital of Liege, CIRM, Liege, Belgium
(2) Clinical microbiology, University Hospital of Liege, CIRM, Liege, Belgium

21st Lancefield International Symposium on Streptococci and Streptococcal Diseases
Stockholm, Sweden | 7 - 10 June 2022 | ESCMID

1 Objective

The aim of this work was to evaluate the **Whole-Genome Sequencing** (WGS), as a new tool, in order to implement the method within the activities of the **Belgian National Reference Centre *Streptococcus agalactiae*** (NRC). The analysis of the bacterial genome by WGS could replace some current standard methods used at the NRC, which can :

→ **unify laboratory workflow**,
→ **decrease workload**,
→ **enhance molecular surveillance**.

2 Methods

To determine the reliability of the WGS, the results were compared with those obtained by the standard methods used by the NRC (multiplex PCRs and MLST). The raw sequence data from WGS were analyzed with the bioinformatics tool "**WGS-typer**" (Hedera22, Liège, Belgium), specially designed for the NRC needs.

Validation panel
37 strains* selected in the NRC collection (2018) to include the different evaluated characteristics :
- **16 strains of vaginal colonization**
- **15 strains (5 males & 10 females) of invasive infections in adults:**
  - Bacteremia, endocarditis, skin infections
  - Male : 46 – 90 years
  - Female : 28 – 86 years
- **6 strains (3 EOD & 3 LOD) of invasive infections in infants**
  - Bacteremia, meningitis
  - Early Onset Disease (EOD) : 0 – 3 days
  - Late Onset Disease (LOD) : 63 – 112 days

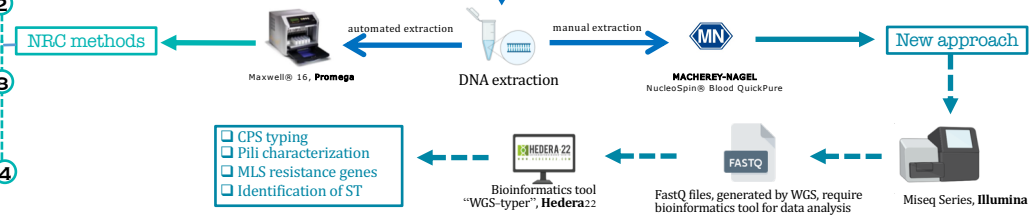
*: Only 14 strains of vaginal colonization were compared with MLST

CPS typing by genotyping, PCR : types Ia, Ib, II to IX (1)
- Multiplex PCR, types Ia, Ib, II to VIII, Poyart, C. *et al. 2007 J. Clin. Microbiol. 45, 1085-8*
- Simplex PCR, type IX, Kong, F. *et al. 2008 J. Clin. Microbiol. 45, 1085-8*

Pili characterization : PI1, PI2a & PI2b (2)
- Multiplex PCR (Springman, AC. *et al. 2014 BMC Microbiol. 19;14:159*)

Molecular characterization of MLS resistance (3)
- « Home made » Multiplex PCR for *Erm*B, *Erm*Tr, *Mef*A & *Lsa*C genes

Multilocus sequence typing (MLST) (4)
- Identification of the sequence type (ST) (Jones *et al, 2003, J. Clin. Microbiol 41(6) :2530*



3 Results : Accuracy of results generated from WGS, data analyzed with the « WGS-typer » *(Hedera22)*

Capsular genotyping

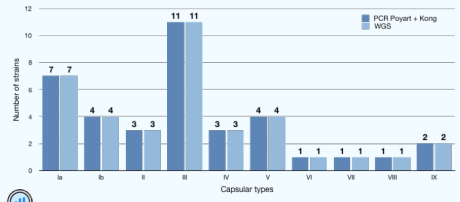


***Figure 1**: Distribution of capsular (CPS) types of 37 Streptococcus agalactiae strains with agreement of paired results between typing methods (PCR Poyart + Kong and WGS)*

The results show an **agreement of 100% (37/37)** between the CPS types generated with the PCRs used by the NRC (Poyart PCR & Kong PCR) and the new approach (WGS), see ***Figure 1***.

Pili characterization

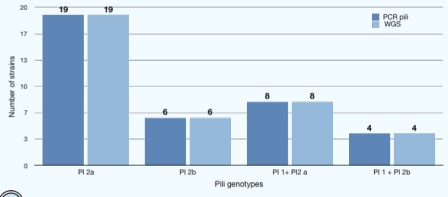


***Figure 2**: Distribution of the pili genotypes of 37 Streptococcus agalactiae strains with agreement of paired results between typing methods (multiplex PCR used by NRC and WGS)*

The results show an **agreement of 100% (37/37)** between the multiplex PCR used by the NRC (pili PCR) and the new approach (WGS), see ***Figure 2***.

Characterization of MLS resistance genes

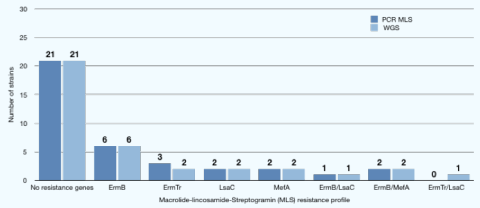


***Figure 3**: Distribution of the resistance genes to macrolide-lincosamide-streptogramin detected by the multiplex PCR MLS within the 37 Streptococcus agalactiae strains and of the paired results generated from WGS*

- Generated results with the « home made » multiplex PCR MLS used by NRC and WGS, are concordant for **36/37 strains (97%),** see ***Figure 3***.
- One strain out of 3 harbouring only the *Erm*Tr gene as detected by the multiplex MLS PCR, shows two resistance genes (*Erm*Tr and *Lsa*C) as derived from the WGS.

Identification of sequence type

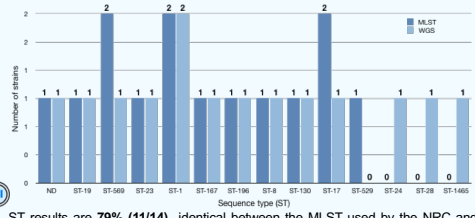


***Figure 4**: Distribution of the sequence-types of 14 Streptococcus agalactiae strains identified with MLST and of the paired results generated from WGS.*

- ST results are **79% (11/14)** identical between the MLST used by the NRC and the new approach (WGS), see ***Figure 4***.
- For one strain, the ST could not be determined (ND) by both methods MLST PCR and WGS. After characterization of the alleles by MLST and WGS, this combination was unknown to the PubMLST database. The raw sequence data was therefore submitted on the database and **ST 1655** was assigned to this combination after validation by the experts on March 04, 2021.
- Three strains characterized respectively by ST-560, ST-17 and ST-529 by MLST, are characterized by ST-28, ST-1465 and ST-24 by WGS. After the investigation of the allelic sequences extracted from the « WGS typer » tool on PubMLST database, the results obtained by WGS were confirmed. A handling error for the MLST method cannot be excluded.

Regarding capsular serotype and pili protein genes, the results reported by conventional PCR methods were perfectly confirmed by the WGS (100% concordance). However, for MLS resistance genes and sequence types, the comparison highlights one and three discrepancies respectively. A new sequence type 1655 was listed in the PUBMLST database thanks to this work.

4 Discussion & conclusion

- WGS is a promising approach that can compete with the conventional methods currently used in our laboratory such as multiplex PCRs & MLST.
- This new approach allows, at lower costs and workload, a wide characterization of the group B Streptococcus and shows a huge concordance with conventional methods. High performance of WGS is demonstrated for CPS typing, pili typing and detection of MLS resistance genes, and superiority for determination of ST. However, before implementation further validation steps are needed.
- WGS is an interesting method for epidemiological surveillance and investigation of outbreaks. It makes possible to establish phylogenetic relationships between strains. This use would be relevant to trace the source of contamination, and to follow the evolution of invasive or recurrent infections.

CHU de Liège | CIRM | LIÈGE université
Contact (1) r.sacheli@chuliege.be (2) sabrina.egrek@chuliege.be