Group B streptococcus (GBS) neonatal invasive infections in Belgium 2010-2017, and characterization of isolated strains.

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RATIONALE & OBJECTIVES

Where intrapartum antibiotic-prophylaxis (IAP) is given to pregnant women colonized with Group B Streptococcus (GBS), the incidence of neonatal early-onset disease (EOD) has been successfully reduced. Nevertheless, GBS is still the leading cause of severe disease among newborns, notably because the incidence of GBS late-onset disease (LOD) is not affected by IAP. Another strategy such as maternal immunization for prevention of both EOD/LOD is highly desirable worldwide.

The Belgian National Reference Centre (NRC) routinely performs surveillances of GBS invasive strains.

This study refers to GBS neonatal invasive diseases, EOD and LOD cases, reported to the Belgian NRC from 2010 up to 2017.

To describe epidemiology of GBS isolated from neonatal EOD or LOD cases by characterization of relevant epidemiological markers for vaccine development and their distribution:

- **Capsular polysaccharide (CPS) type**
- **Pilus island (PI) pattern**
- **Multi-Locus Sequence-Type (MLST)**

STUDY POPULATION AND METHODS

In Belgium, GBS invasive infection is not a reportable disease; but a regular surveillance of diseases and characterization of GBS isolates is performed by the Belgian National Reference Centre (NRC) for Streptococcus agalactiae (GBS).

Population:

- Overall through years 2010 to 2017, on a voluntary base, laboratories belonging to the national sentinel network sent to the NRC a total of **292 strains of GBS** isolated from bloodculture or cerebro-spinal fluid of newborns with invasive disease. They were issued from 149 EOD (0-6days) and 143 LOD (1 week-3 months).

Identification and CPS Typing (types Ia, Ib, II to IX)

- ***Identification,*** upon reception of isolates, GBS identification was confirmed (MALDI-TOF mass spectrometry, Bruker) and strains were stored at -80°C.
- ***Serotyping***, Latex agglutination (Strep B Latex, Statens Serum Institut, Denmark)
- ***Genotyping***, PCR.
  - Multiplex PCR, Types Ia, Ib, II to VIII, Poyart, C. *et al. 2007 J. Clin. Microbiol. 45, 1985–8*
  - PCR type IX, Kong, F. *et al. 2008 J. Clin. Microbiol. 46, 2745–50.*

Pilus island characterization (PI-1, PI-2a & PI-2b)

- Multiplex PCR (Springman, AC. *et al.* *2014 BMC Microbiol. 19;14:159*)

Multiple-Locus Sequence-Typing (MLST)

- Seven housekeeping genes (*adhP, pheS, atr, glnA, sdhA, glcK,* and *tkt*) were amplified and sequenced as described by Jones et al, 2003, *J. Clin. Microbiol. 41, 2530-6*
- The STs were determined by the *S.agalactiae* MLST website (http://pubmlst.org/sagalactiae/).

RESULTS

Distribution of CPS types among GBS isolated from neonatal EOD and LOD

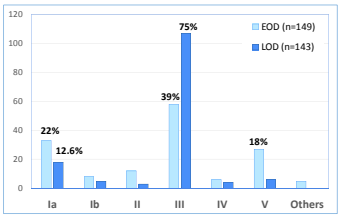

Figure 1: *Distribution of CPS types of 292 GBS strains isolated from newborns with invasive disease.* *(Y axis: number of cases)*

- The most prevalent CPS type was III either among EOD cases (39%) or LOD cases (75%), followed mainly by types Ia, V and II for EOD and type Ia for LOD. *(Fig. 1 & 2)*
- These distributions did not vary significantly during the study period. *(Fig.2)*

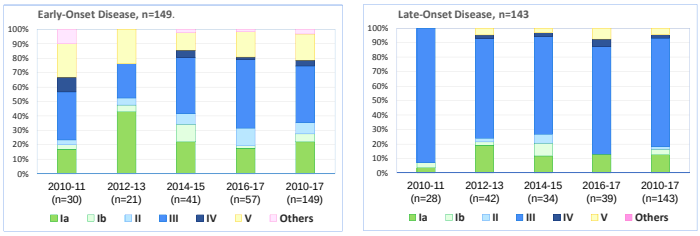

Figure 2: *Biennial distribution of CPS types of 292 GBS strains isolated from newborns with invasive disease.*

Distribution of pilus island patterns among GBS isolated from neonatal EOD and LOD (2014-2017)

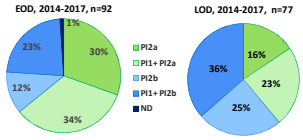

Figure 3: *Distribution of pilus island patterns of 169 GBS strains isolated from newborns with invasive disease. (ND= No gene detected)*

- All GBS strains harbored at least one PI gene, singly or in combination, except one isolate from EOD.
  - With 64% harboring PI-2a and 35% PI-2b among GBS from EOD, and 61%, harboring PI-2b and 39% PI-2a among strains isolated from LOD. *(Fig.3)*
  - The PI-2a singly is mainly found in CPS type Ia. *(Fig.4)*
  - The PI-2b singly or in combination is mainly found in type III. *(Fig.4)*

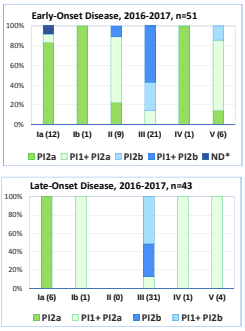

Figure 4: *Distribution of pilus island patterns among the different CPS types of 94 GBS strains isolated through 2016-2017 from newborns with EOD and LOD.*

Distribution of STs among GBS isolated from neonatal EOD and LOD (2016 – 2017)

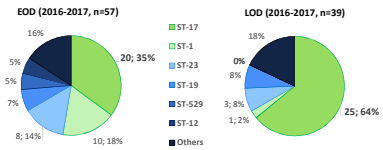

Figure 5: *Distribution of ST of 96 GBS strains isolated from newborns with invasive disease, EOD or LOD.*

- The strains were classified into 18 individual sequence types.
  - A high prevalence of ST-17 was detected : 35% of isolates from EOD and 64% from LOD. *(Fig.5)*
  - A strong correlation was shown between CPS type III and ST-17, between CPS Ia and ST-23. *(Fig.6 & 7)*
  - More diversity of STs was observed for CPS type V. *(Fig.6 & 7)*

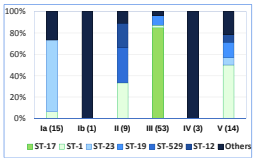

Figure 6: *Distribution of the 6 most represented ST within the different CPS types.*

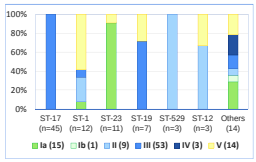

Figure 7: *Serotype distribution within each of the 6 most represented ST.*

DISCUSSION & CONCLUSION

- **CPS type III predominated in neonatal infections, EOD and LOD ;** CPS type Ia, II and V were 2nd and 3rd rank for EOD.
- **The reported distribution of CPS types of GBS isolated from neonatal EOD/LOD did not vary significantly overtime** when compared to the Belgian reported data concerning GBS isolated from neonatal invasive diseases during the previous decade 1998-2007. *(Fig.8)*
- **This distribution of CPS types was also consistent with reported European data.** *(Fig.8)*
- **One or two pilus island genes were detected in all strains of GBS** (except one isolate).
- PI genes were not evenly distributed within CPS types: **PI-2b singly or in combination demonstrated a correlation with type III, and PI-2a singly was mainly found in CPS type Ia.**
- **Strains of CPS type III were mainly represented by the hypervirulent GBS ST-17** (85%) ; and, GBS ST-17 were only found in CPS type III.

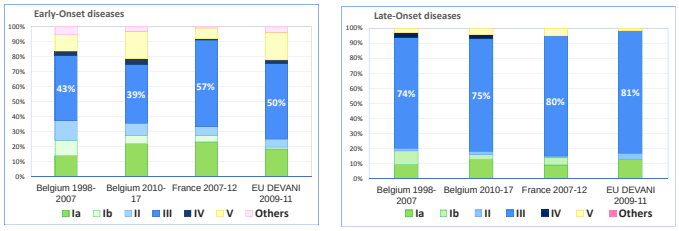

Figure 8: *Distribution of CPS types of 292 GBS strains isolated from newborns with invasive disease in Belgium (2010-2017) compared to 301 GBS isolated previously in Belgium (1998-2007), 438 GBS in France through 2007-2012 (39.7% EOD and 60.3% of LOD)[1] and 159 GBS from 8 European countries participating in the DEVANI Project (EU FP7) 2009-2011 (52.9% EOD and 47.1% LOD)[2].*

1- Joubrel et al, 2015, Clin Microbiol Infect 21:510-6
2- Melin, DEVANI Consortium team et al. EU-Framework Program Seven, XVIII LISSSD September 2011, Sicily

I have no conflicts of interest to declare.