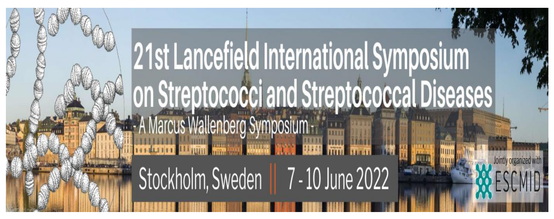

Characterization of Group B Streptococcus strains isolated from neonatal invasive diseases in Belgium, 2018

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OBJECTIVES

Despite advances in preventive strategies, Group B Streptococcal (GBS) disease is still a leading cause of severe neonatal infections. The Belgian National Reference Centre (NRC) routinely performs surveillances of GBS invasive strains.

To provide an overview of bacteriological characteristics of GBS causing early and late onset invasive diseases in infants during the year 2018, in Belgium.

- **To characterize relevant epidemiological markers of the isolated GBS strains.**
  - Determination of their **capsular-polysaccharide (CPS) types** and distribution.
  - Determination of their **pili types** and distribution.
  - Determination and description of their **antimicrobial susceptibility profile**.
  - Determination and distribution of macrolide /lincosamide **(MLS) resistance genotypes.**

STUDY POPULATION AND METHODS

In Belgium, GBS invasive infection is not a notifiable 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 during the year 2018, on a voluntary base, laboratories belonging to the national surveillance network sent to the NRC a total of **56 GBS strains** isolated from blood culture, cerebro-spinal fluid, or synovial liquid of newborns with invasive disease. Laboratories were asked to fill a short Case Report Form for each reported case.

CPS Typing: types Ia, Ib, II to IX

- ***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.*

Pili characterization: PI1, PI2a & PI2b

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

Antimicrobial susceptibility testing (EUCAST 2017)

- ***Disk diffusion, categorization S.I.R.***
  - **Dtest screening** for inducible resistance to lincosamide.
  - **Screening for reduced susceptibility to beta-lactams**, using oxacilline, ceftizoxime and ceftibuten disks according to Kimura et al (2009, *J. Clin. Microbiol. 47, 4154–7)*
- ***Determination of MICs***
  - **Etest diffusion method**
  - **Microdilution method, using Sensititre®** system with customized microplates

Molecular characterization of MLS resistance

- Multiplex PCR for ErmB, ErmTr, MefA and LSaC genes

RESULTS

Early-onset and Late-onset Diseases (EOD, LOD)

- A total of **56 GBS strains** isolated from neonatal invasive diseases received by the NRC in 2018: **33 from EOD and 23 from LOD**, see ***Figure 1, year 2018.***
- Considering the estimated incidence of GBS neonatal infections in Belgium, this collection of isolates sent to the NRC represented for 2018 about 40% of GBS neonatal invasive diseases.
- Not shown, through the past decade, the number of laboratories participating has more than doubled, and therefore the number of reported cases.
- The ratio between EOD and LOD has evolved these recent years, EOD cases were higher than LOD in the beginning of the 21th century. Later LOD were higher than EOD until 2013 (p=0,0008). Thanks to preventive strategies focused on EOD decrease. However, more recently, a stabilization of the EOD/LOD ratio has been noticed, see ***Figure 1***.

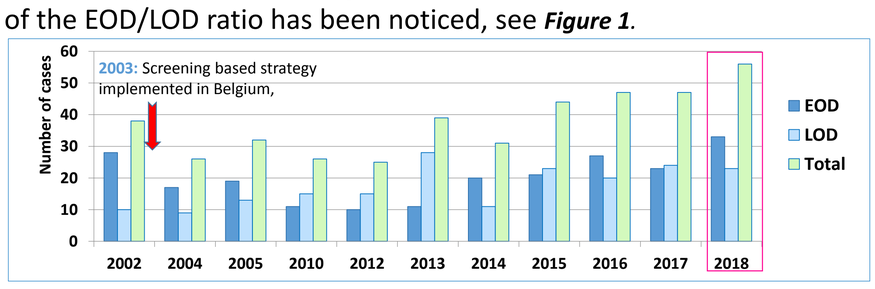

Figure 1: *Overview of the yearly distribution of GBS EOD and LOD cases (2002 – 2018).*

Distribution of CPS types and pili types

- A CPS type was assigned to all isolates:
  - **The most prevalent CPS type was type III either among EOD cases (45,5%) or LOD cases (82,6%). *See Figure 2.***
- Every isolate of GBS harboured at least one pili gene or a combination of genes except four strains (1 EOD case and 3 LOD cases, investigation still ongoing).
- Pili genes were not evenly distributed among strains isolated from EOD or LOD. ***See Figure 3.***
  - Among the EOD cases, the combination PI1,PI2a (39,5%) and the pili genes PI2a (30.3%) were predominant, followed by PI2b (15,1%) and PI1, PI2b (12,1%). Among the LOD cases, strains harboured mainly a combination of pili genes with the majority of PI1,PI2b (47.8%).

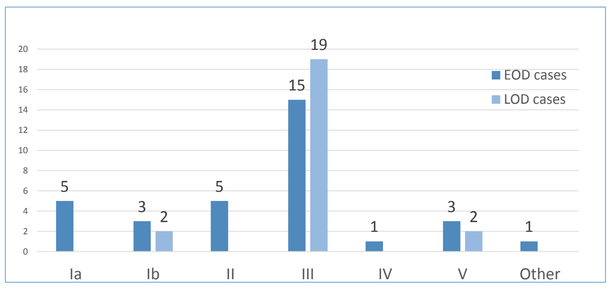

Figure 2: *Distribution of CPS types of 56 GBS strains isolated in 2018 from newborns with invasive disease: 33 EOD and 23 LOD*

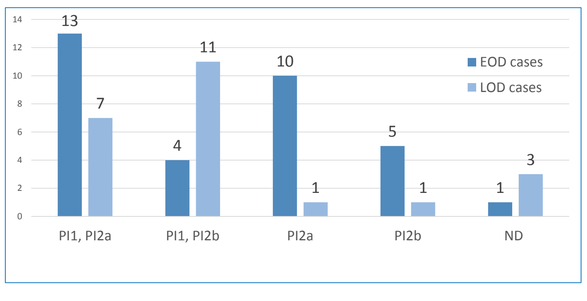

Figure 3: *Distribution of the pili genotypes of 56 GBS strains isolated in 2018 from newborns with invasive disease: 33 EOD and 23 LOD*

Antimicrobial susceptibility profile

- ***Susceptibility to beta-lactams***
  - **All isolates were fully S to penicillin** with MICs ranging from 0.03 to 0.125 mg/L.
  - According to Kimura's procedure and interpretative criteria, no decreased susceptibility to beta-lactams was detected.
- ***Susceptibility to macrolide-lincosamide***
  - Out of 56 isolates, **15 (26,8%) were resistant to erythromycin** and **clindamycin,** one strain has **an isolated resistance to erythromycin.** Regarding EOD, 12/33 (39,5%) were resistant to macrolides and lincosamides, while in LOD only 3/23 (13,04%) strains were resistant to these antibiotics. This is important to consider for intrapartum prophylaxis.
  - As shown in ***Figure 4*** the **major phenotype of resistance was cMLS** (87,7% of resistant isolates), the constitutive resistant phenotype. The inducible, iMLS, phenotype was demonstrated in one resistant GBS; one isolate showed isolated resistance to erythromycin (M phenotype). No strain harboured the L phenotype (isolated resistance to clindamycin).

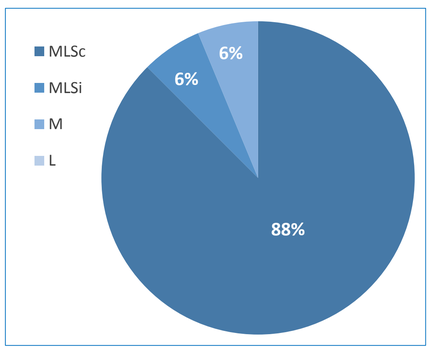

Figure 4: *Distribution of resistance phenotypes to macrolide-lincosamide among the 16 GBS isolates expressing resistance.*

cMLS: constitutive resistance to macrolides and lincosamides
i**MLS**: inducible resistance to lincosamides
M: isolated resistance to macrolides
L: isolated resistance to lincosamides

- The distribution of the genes coding for resistance, *ErmB, ErmTr, MefA* and *LsaC* harboured by these 16 isolates is shown in the following ***Figure 5.***

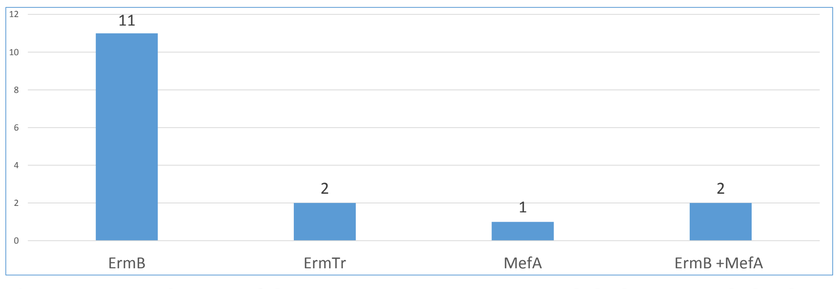

Figure 5: *Distribution of the resistance genes to macrolide-lincosamide harboured by the 16 GBS isolates expressing resistance*

DISCUSSION & CONCLUSION

- **In Belgium, GBS invasive disease is not a notifiable disease. Surveillance is organized by the NRC and strains are provided by Belgian laboratories belonging to a surveillance network. During the last decade, number of participating laboratories has increased impacting the number of reported cases.**
- **Bacteriological characteristics of GBS isolated from newborns with invasive disease (early and late-onset), CPS type distribution, pili type distribution, rate and type of resistance to macrolide/lincosamide, were consistent with previous Belgian reported data as well as from European countries and North America.**