

## OBJECTIVES

Even if GBS disease risk is highest during the first trimester of life, GBS is also an important cause of invasive infection in adults, especially the elderly, the immunocompromised or patients with chronic illnesses.

To provide an overview of bacteriological and clinical characteristics of GBS causing invasive diseases in non-pregnant adult in Belgium.

- To describe the main reported clinical manifestations and suspected focus.
- 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

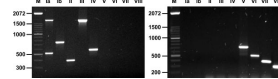
In Belgium, GBS invasive diseases are not notifiable but a regular surveillance of diseases and characterization of GBS isolates is organized by the Belgian National Reference Centre (NRC) for *Streptococcus agalactiae* (GBS).

### Population:

- Overall during the year 2015, on a voluntary base, laboratories belonging to the national surveillance network sent to the NRC a total of **143 GBS strains** isolated from invasive disease among non-pregnant adults.
- 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 oxacillin, ceftiozime 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

### Clinical manifestations

Syndrome	Number (%) N= 143
Bacteremia without focus (or not reported)	61 (+26) (42,7% + 18,2%)
Focused infection predominantly associated to bacteremia	56 (39,2%)
Skin & soft tissue infection (mainly erysipelas)	27 (19%)
Bone & joint infection	6 (4,2%)
Endocarditis	4 (2,8%)
Meningitis	2 (1,4%)
Respiratory tract infection	3 (2,1%)
Urinary tract infection	7 (4,9%)
Intra-abdominal infections	7 (4,9%)

Table: Clinical diagnoses for non-pregnant adults with invasive GBS infection.

### 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 143 isolates, **51 (35.7%)** were resistant to erythromycin and/or clindamycin. Rate of resistance was not evenly distributed among GBS of different CPS types. See **Figure 3**.
- As shown in **Figure 4** the major phenotype of resistance was cMLS (60.6% of resistant isolates), the constitutive resistant phenotype. The inducible, iMLS, phenotype was demonstrated among 17.8% of the resistant GBS; 19.6% of GBS showed isolated resistance to erythromycin (M phenotype) and the L phenotype, isolated resistance to clindamycin was detected for one isolate.
- The distribution of the genes coding for resistance, *ermB*, *ermTr*, *MefA* and *LsaC* harboured by these 51 isolates is shown in **Figure 5**.

### Distribution of CPS types and pili types

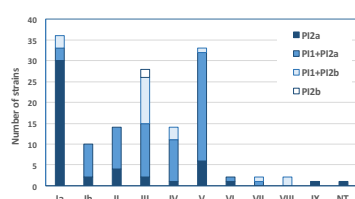


Figure 1: Distribution of CPS types of 143 GBS strains isolated from adults with invasive disease, and distribution of pili types within each serotype.

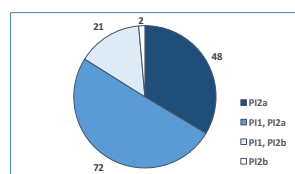


Figure 2: Distribution of the pili genotypes of 143 GBS strains isolated from adults with invasive disease.

- The most prevalent CPS types were types Ia (25.1%), V (23%) and III (19.6%), accounting together for 67.7%. CPS types II (9.8%), IV (9.8%), Ib (7%), VI (1.4%), VII (1.4%), VIII (1.4%) and IX (0.7%) represented the remaining cases. One strain remained non typeable. See **Figure 1**.
- Every isolate of GBS harboured at least one pili gene or a combination of genes. The pili PI1+ PI2a association was far predominant as detected in 50.5% of isolates. See **Figure 2**.
- Pili gene were not evenly distributed among strains with different CPS types. Among GBS with CPS type Ia, the pili type PI2a was highly the most prevalent and represented a characteristic of CPS type Ia as rarely detected alone among other CPS types. The combination PI1+PI2a was the most prevalent among CPS types V, Ib, II, and IV. Both combinations PI1+PI2a and PI1+PI2b were the major combinations of pili genes detected among CPS type III. PI2b gene alone was only detected in one CPS Type III strain. See **Figure 1**.

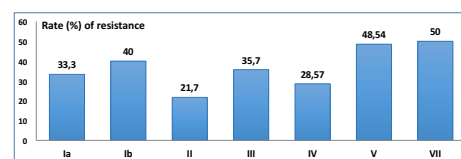


Figure 3: Rate (%) of resistance to macrolide/lincosamide among the different CPS types of GBS isolated from non-pregnant adults with GBS disease

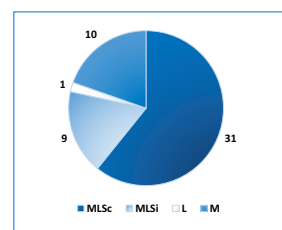


Figure 4: Distribution of resistance phenotypes to macrolide-lincosamide among the 51 GBS isolates expressing resistance.

MLS: constitutive resistance to macrolides and lincosamides; MLSi: inducible resistance to lincosamides; L: isolated resistance to lincosamides

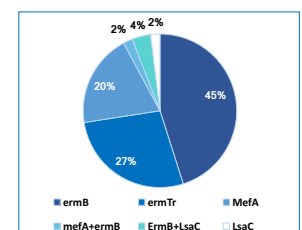


Figure 5: Distribution of the resistance genes to macrolide-lincosamide harboured by the 51 GBS isolates expressing resistance

## CONCLUSION

- In Belgium, bacteremia without identified focus and skin-soft tissue infections were the main clinical manifestations of GBS causing invasive infection among non-pregnant adults.
- Bacteriological characteristics of GBS isolated from adults with invasive disease were consistent with reported data among European countries; CPS types Ia, II and V accounting 67.7%.
- Macrolides/lincosamides resistance rate (35.7%) has slightly increased during the last decade and is mainly associated to CPS type V and to *ermB* determinants.