

Prevalence and capsular-polysaccharide type distribution of colonizing group B streptococci (GBS) isolated from recto-vaginal samples in pregnant women in Hanoi, Vietnam.

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OBJECTIVES

1. To provide data concerning GBS colonization among pregnant women in Vietnam.

- Determination of the prevalence of GBS colonization among pregnant women seeing doctors at the Bach Mai Hospital in Hanoi.

2. To characterize relevant clinical and 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

The study was organized by the Belgian National Reference Center (NRC) for *Streptococcus agalactiae* (GBS) and was carried out both in Hanoi, Vietnam and within the Belgian NRC.

Population: During November and December 2015, 888 recto-vaginal swabs were collected from pregnant women at 35-37 weeks' gestation, at the Bach-Mai Hospital in Hanoi.

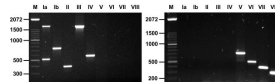
Culture method: All specimens were directly cultured on ChromID StreptoB agar (bioMérieux®) for GBS detection (pale pink to red colonies). All isolated GBS were stored deep-frozen in brain-heart-broth with glycerol (20%) and afterwards sent to the Belgian NRC for further confirmation and characterization.

CPS Typing: types Ia, Ib, II to IX

- Serotyping**, Latex agglutination (Strep B Latex, Statens Serum Institut, Denmark)

- Genotyping**, PCR.

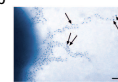
- Multiplex PCR, Types Ia, Ib, II to VIII, Poyart, C. et al. 2007 *J. Clin. Microbiol.* 45, 1985-8



- 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, 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

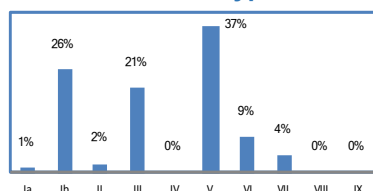
- Multiplex PCR for ErmB, ErmTr, MefA and LSaC genes

RESULTS

Prevalence of GBS colonization

- 111/888 swabs were positive for GBS: the colonization rate among pregnant women from Hanoi was 12.5%.
- 90 strains of GBS were stored and further sent to Belgium for further characterization.

Distribution of CPS types



Distribution of the CPS genotypes of 90 GBS strains isolated from pregnant women's vagino-rectal swabs, in Hanoi.

Among Vietnamese pregnant women from Hanoi, the most prevalent CPS types were V, Ib and III.

Latex agglutination allowed determination for 83% of the strains, 9% were non typable (no agglutination) and 8% presented multiple agglutinations. Genotyping with the multiplex PCR provided a genotype for all isolates. No discordance was observed between the 2 methods when the serotype was available.

Antimicrobial susceptibility profile (1)

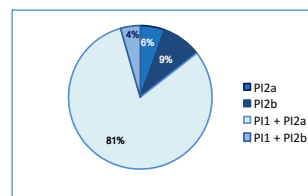
Antimicrobial agent	Susceptible %	Intermediate %	Resistant %	Type of Resistance %
Penicillin	100	0	0	
Erythromycin	26.6	1.1	72.2	
Clindamycin	22.2	0	77.8	3.3 Inducible R 74.4 constitutive R
Moxifloxacin	81.1	0	18.9	
Tetracycline	6.7	0	93.3	
Vancomycin	100	0	0	

Antimicrobial susceptibility profile of 90 GBS strains isolated from pregnant women's vagino-rectal swabs, Hanoi 2015 (EUCAST 2017 breakpoints)

Susceptibility to beta-lactams

All isolates were fully S to penicillin and according to Kimura's procedure and interpretative criteria, no decreased susceptibility to beta-lactams was detected.

Pili characterization



Distribution of the pili genotypes of 90 GBS strains isolated from pregnant women's vagino-rectal swabs, in Hanoi.

The pili PI1 + PI2a association is far predominant. Every isolate harboured at least one pili gene.

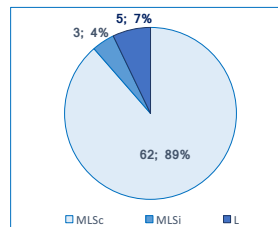
Antimicrobial susceptibility profile (2)

Susceptibility to macrolide-lincosamide

High resistance rates were determined with 72% for erythromycin and 78% to clindamycin.

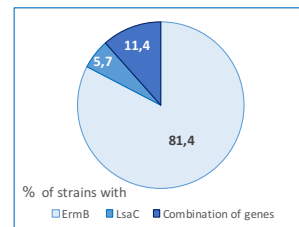
As shown in the following figure the major phenotype of resistance is MLS_C. No isolated resistance to macrolide was detected, while 5% demonstrated an isolated resistance to clindamycin.

The *ErmB* gene is present alone in the majority of the resistance strains (83%). The gene *LsaC* was detected among 6% of the resistant isolates; 11% harboured a combination of genes. None of the 4 research genes were detected among the susceptible isolates.



Distribution of the resistance phenotypes to macrolide-lincosamide among the 70 GBS isolates expressing resistance to macrolide/lincosamide.

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



Distribution of the resistance genes to macrolide-lincosamide among the 70 GBS isolates expressing resistance to macrolide/lincosamide

DISCUSSION & CONCLUSION

- The observed prevalence rate of GBS carriage among pregnant women in Hanoi, Vietnam was 12.5%. This colonization rate is quite low when compared with European or North American rates. Even if the culture methods used selective differential agar there was no selective enrichment step.
- The serotype distribution, with the most prevalent serotypes V, Ib and III, differs from what is described in Europe or North America. On the other side, the pili distribution is similar to the one observed among European countries as for Belgian strains.
- Concerning the susceptibility to antibiotics, fortunately, penicillin remains effective on all the tested strains. Nevertheless, we observe a high rate of resistance to erythromycin (73%) and clindamycin (78%) among these vietnamese strains. These results are consistent with Asian ones but higher than European ones. Resistance rate to moxifloxacin is 18.9%, much higher than in Europe (<5%) but more similar to resistance rate found in China. The high resistance rate to tetracycline is typically associated to human strains of GBS since the emergence of GBS as the most important bacterial pathogen among neonates.