

**Evolution of rate and genotypes of resistance to macrolide/lincosamide among invasive Group B Streptococcus (GBS): Development of a multiplex PCR tool for simultaneous detection of ErmB, ErmTr, MefA and LsaC resistance genes.**

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**Methods:** A multiplex-PCR, using a set of specifically designed or already described (Kataja, 1999; Malbruny, 2011) primers was developed and used to detect, in GBS, three genes for erythromycin resistance, *ermB*, *ermTR*, *mefA* and one gene for clindamycin-resistance *lsaC*. *AdhP* gene amplification was used as control for GBS identification. All(219) GBS isolates from invasive infections in newborns and adults received by the Belgian National Reference Center for GBS in 2015, and control strains were tested for erythromycin/clindamycin susceptibility (disk-diffusion/broth- microdilution) and for detection of resistance genes.

**Results:** PCR products demonstrated the expected respective sizes. The method has been validated successfully according to ISO15189 analytical requirements. Of the 219 isolates, 67(30,67%) were resistant to erythromycin and/or clindamycin: 44/67(65,78%) showed a constitutive-MLS phenotype and 10/67(14,9%) the inducible-MLS phenotype. Among the constitutive-MLS strains, 73% harboured *ErmB* gene, 13% *ErmTR*, 7% *ErmB+mefA* and 7% *ermB* together with *LsaC gene*. The inducible-MLS strains harboured mostly *ErmTr* gene (89%) and the others the *ErmB* gene. Among the 10/67(14,9%) GBS strains with an M-phenotype (isolated resistance to erythromycin), the *MefA* gene was exclusively detected. Among the 3(4,48%) strains showing an isolated resistance to clindamycin (L-phenotype), the *LsaC* gene was detected.

**Conclusion:** The developed multiplex PCR is able to detect simultaneously four genes involved in MLS resistance in GBS. In 2015, 30,6% of the invasive GBS strains isolated in Belgium were resist to macrolides and/or lincosamides. The emergence of the L-phenotype in GBS described since 2010, justifies the relevance to also detect *LsaC* gene together with *ErmB, ErmTr* and *MefA*.