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Phenotypical and Genotypical Surveillance of Macrolide and Lincosamide Resistance in Group B Streptococcus in Belgium

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ABSTRACT

Background: Constant increase of erythromycin and clindamycin resistance has been observed worldwide among group B streptococcal (GBS) isolates. In Belgium, through the 2000s, erythromycin resistance has increased from 20% to up to 30%. Therefore phenotypical and molecular surveillance of erythromycin and clindamycin resistance patterns has been conducted.

Materials and Methods: A total of 328 GBS isolates, previously serotyped by PCR, were collected: 275 clinical isolates (N1) were obtained from the Belgian surveillance for invasive GBS disease in newborns (59 isolates from 32 early- and 27 late-onset diseases) and adults (216 isolates) during 2008 to 2011 and 53 isolates (N2) from recto-vaginal colonization among pregnant women in 2010. Erythromycin and clindamycin MICs were determined by using Etest® (EUCAST interpretative criteria). Furthermore, for the erythromycin-resistant isolates, the inducible (iMLS), constitutive (cMLS) and M phenotypes were assessed by a double-disk diffusion test; the distribution of genes encoding RNA methylases (*erm*) and efflux pumps (*mef*) was investigated by PCR.

Results: Of the N1 and N2 isolates, 94 (34,2%) and 15 (28,3%) were respectively resistant to erythromycin. Among these 109 isolates, 102 (93,6%) exhibited the MLS phenotype (resistant to erythromycin and clindamycin): 74 were cMLS with erythromycin MIC_{50} ≥256 mg/L and 28 iMLS with erythromycin $MIC_{50/90}$ 16/≥256 mg/L. The M phenotype (resistant to erythromycin and susceptible to clindamycin) was expressed by 7 (6,4%) of the isolates with erythromycin $MIC_{50/90}$ 4/12 mg/L. One isolate presented a newly described resistance mechanism in GBS: the L phenotype (susceptible to erythromycin and resistant to clindamycin) with a clindamycin MIC of 8 mg/L.
For cMLS, the most common genotype was *ermB* (65%) (*P* <0,05) followed by *ermTR* (30%) and *ermB+ermTR* (5%). All iMLS isolates harbored an *ermTR* gene except 3 (2 with *ermB*, 1 with both *ermB* and *ermTR*); and all M phenotypes were positive for *mefA/B* gene.

Conclusion: 1) In Belgium, by year 2010, prevalence of macrolide resistance in GBS exceed 30%, with a significant higher rate among serotype V (*P* <0,001) and serotype IV (*P* < 0,01). 2) Resistance mechanisms were mainly MLS phenotypes (target-site modification); M phenotype (efflux resistance mechanism) was also prevalent. 3) Resistance surveillance is mandatory to guide prophylaxis and treatment of serious GBS infections but also to identify newly acquired resistance mechanisms such as the L phenotype.

INTRODUCTION

Resistance to macrolides and lincosamides has increased worldwide among group B streptococcal (GBS) isolates over the last two decades: from less than 5% to common resistance of 20% to 30%. Different known mechanisms account for acquired resistance to macrolides in streptococci. The most prevalent of these is target site modification by 23S rRNA methylases, commonly encoded by the *erm*B and *erm*A subclass *erm*TR genes. The Erm enzymes confer resistance to macrolides and inducible or constitutive resistance to lincosamides and streptogramin B, so-called MLS_B phenotype. Another mechanism, involving active drug efflux, is encoded by the *mef*A and *mef*E genes; the Mef pump affects 14- and 15- membered ring macrolides but not 16-membered macrolides, neither lincosamides nor streptogramin B (M phenotype). Apart from these worrying resistances, a other phenotype involving low-level of clindamycin resistance (with erythromycin remaining susceptible) in GBS isolates has recently been reported in many countries: the L phenotype.

AIM

To determine the phenotypical and molecular resistance patterns for erythromycin- and clindamycin-resistant group B streptococcus and to identify the resistance genes (*ermB*, *ermTR* and *mefA/B*) among erythromycin-resistant *S. agalactiae* isolated in Belgium from various clinical and colonizing origins.

MATERIALS AND METHODS

Strains

	Collection 1	Collection 2
Period	2008-2011	2010
Number	275	53
Laboratory	Belgian GBS reference laboratory	
Origin	Early and late onset neonatal disease and adult invasive disease	Recto-vaginal colonization among pregnant women
Screening method for E-CC resistance	Etest® determination of MICs/ EUCAST interpretative criteria	

Description of 328 non-redundant GBS strains from which erythromycin and/or clindamycin resistant isolates were selected

Determination of erythromycin and clindamycin MICs

- Etest® method (AB Biodisk®, Sweden)
- Inoculum 0,5 McFarland on Mueller-Hinton agar +5% sheep blood
- Incubation 18h at 35°C
- MIC resistance breakpoints: > 0,5 mg/L, EUCAST 2011

Determination of erythromycin resistance phenotypes

- Double-disk diffusion assay
- Erythromycin 15 µg paper-disks and clindamycin 2 µg paper disks (Becton Dickinson and Company®, USA)
- Disks placed 15-20 mm apart on agar plate; 18-24h incubation at 35°C
- **MLSB phenotype:**
  - **Inducible resistance (iMLS):** blunting of the clindamycin zone of inhibition proximal to the erythromycin disk, or D-Shape
  - **Constitutive resistance (cMLS):** resistance to clindamycin with no blunting of the clindamycin zones of inhibition
- **M phenotype:** susceptibility to clindamycin with no blunting of the clindamycin zones of inhibition

Identification of resistance genes

Nucleotide primers sequences used in this study

Target	Sequence 5' to 3'	Product size (bp)	Reference
ermB	GAAAAGGTACTCAACCAAATA (forward) AGTAACGGTACTTAAATTGTTTA (reverse)	639	1
ermTR	ATAGAAATTGGGTCAGGAAAAG (forward) TTGATTTTTAGTAAAAAG (reverse)	530	1
mefA/B	AGTATCATTAATCACTAGTGC (forward) TTCTTCTGGTACTAAAAGTGG (reverse)	348	1
dltS (internal control)	AGGAATACCAGGCGATGAACCGAT (forward) TGCTCTAATTCTCCCCTTATGGC (reverse)	952	2

Summary of PCR protocols using specific primers for known macrolide-resistance markers and for dltS gene

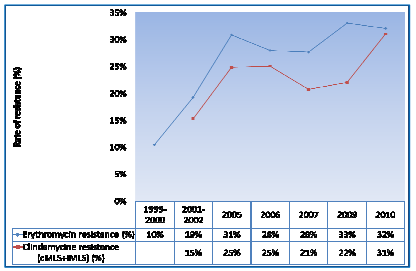
Target	Initial denaturation	Number of cycles	Denaturation	Annealing	Extension	Final extension
ermB	At 94°C for 10 min	30	At 94°C for 60 s	At 54°C for 60 s	At 72°C for 60 s	At 72°C for 5 min
ermTR		35	At 94°C for 30 s	At 45°C for 90 s		
mefA/B		30	At 94°C for 60 s	At 52°C for 60 s		
dltS (internal control)	At 95°C for 10 min	30	At 95°C for 60 s	At 55°C for 90 s	At 72°C for 90 s	

•Thermocycler: Thermo Hybaid Hbpxe 02® thermocycler (Thermo Scientific®, USA)
•Following amplification, the products were visualized by electrophotesis on 2% agarose gel by ethidium bromide staining

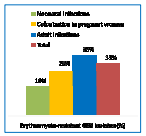
RESULTS

Erythromycin and clindamycin resistance rates

Among the 328 clinical and colonizing GBS collected between 2008 and 2011, 109/328 isolates (33,2%) and 75/328 isolates (22,9%) were resistant to erythromycin and clindamycin respectively. Rate of resistance to clindamycin was higher when inducible resistance (iMLS) was added to cMLS and L phenotypes: 103/328 (31,4%).

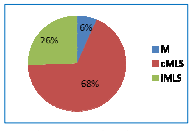


Evolution macrolide and lincosamide resistance in GBS in Belgium between 2000 and 2010 (3)

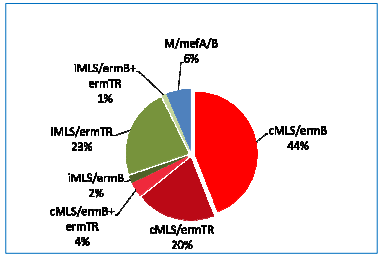


Erythromycin resistance rate among GBS isolated from various origins

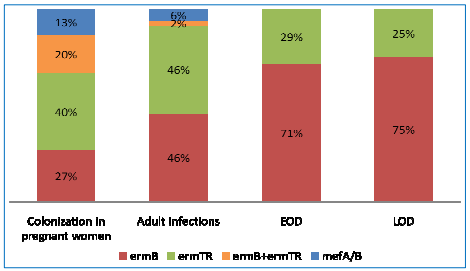
Distribution of resistance phenotypes and genotypes



Phenotypes distribution among 109 erythromycin-resistant GBS isolates



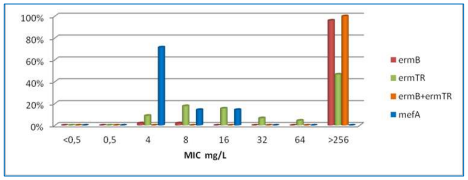
Distribution of the ermTR, ermB and mefA/B genes within the different erythromycin-resistance phenotypes



Genotypical distribution of 109 erythromycin-resistant GBS isolated from adult infections, neonatal infections (EOD and LOD) and colonization in pregnant women

RESULTS

Correlation of erythromycin MICs with genotypes



Correlation of erythromycin MICs with genotypes within 109 erythromycin-resistant GBS isolates

	ermB	*ermTR*	*ermB +ermTR*	*mefA/B*
MIC50	≥256 mg/L	64 mg/L	≥256 mg/L	4 mg/L
MIC90	≥256 mg/L	≥256 mg/L	≥256 mg/L	12 mg/L

CONCLUSIONS

- **In Belgium, by the year 2010, prevalence of macrolide and lincosamide resistance among GBS exceeded 30%.**
- **Erythromycin resistance was higher among GBS isolated from adult invasive infections than from neonatal population.**
- **MLS phenotypes, either constitutive or inducible, were predominant leading to cross-resistance to macrolides and lincosamides. These isolates harbored a target-site modification gene: *ermB* or *ermTR*, or both.**
- **Efflux resistance mechanism (M phenotype), also prevalent among GBS isolates, was always encoded by *mefA/B* gene.**
- **Erythromycin MICs were well correlated with genotypes. High level of erythromycin-resistance was associated with *ermB* isolates while low level of resistance was present in *mefA/B* isolates.**
- **The highest proportion of MLS phenotypes (cMLS and iMLS) and the emergence of L phenotype could explain the recent increase in clindamycin resistance rate.**
- **Constant increase of macrolide and lincosamide resistance rates stresses the importance of performing susceptibility testing for GBS isolates issued from prenatal screening in women who are at high risk for penicillin anaphylaxis, where clindamycin is the recommended antibiotic alternative.**
- **Resistance surveillance is mandatory to guide prophylaxis and treatment of serious GBS infections but also to identify newly acquired resistance mechanisms such as the L phenotype.**

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