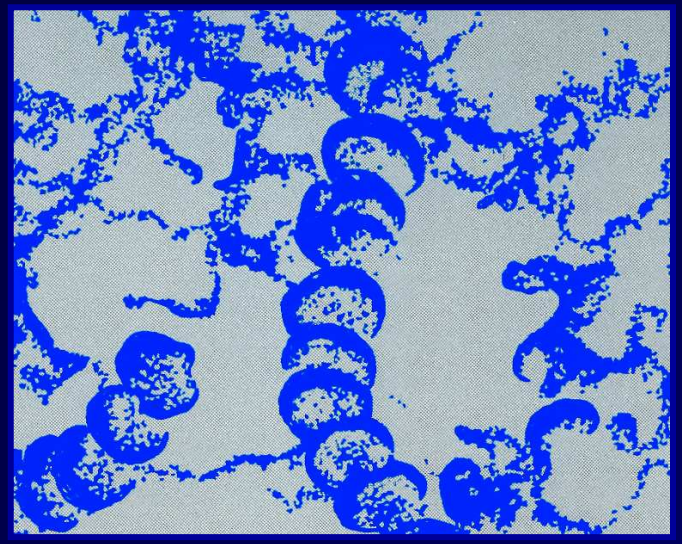

Abstract C2-168
47th ICAAC, Chicago, September 2007

Susceptibility profile to penicillin, erythromycin and clindamycin of clinical isolates of group B streptococci recently isolated in Belgium and detection of erythromycin resistance genes

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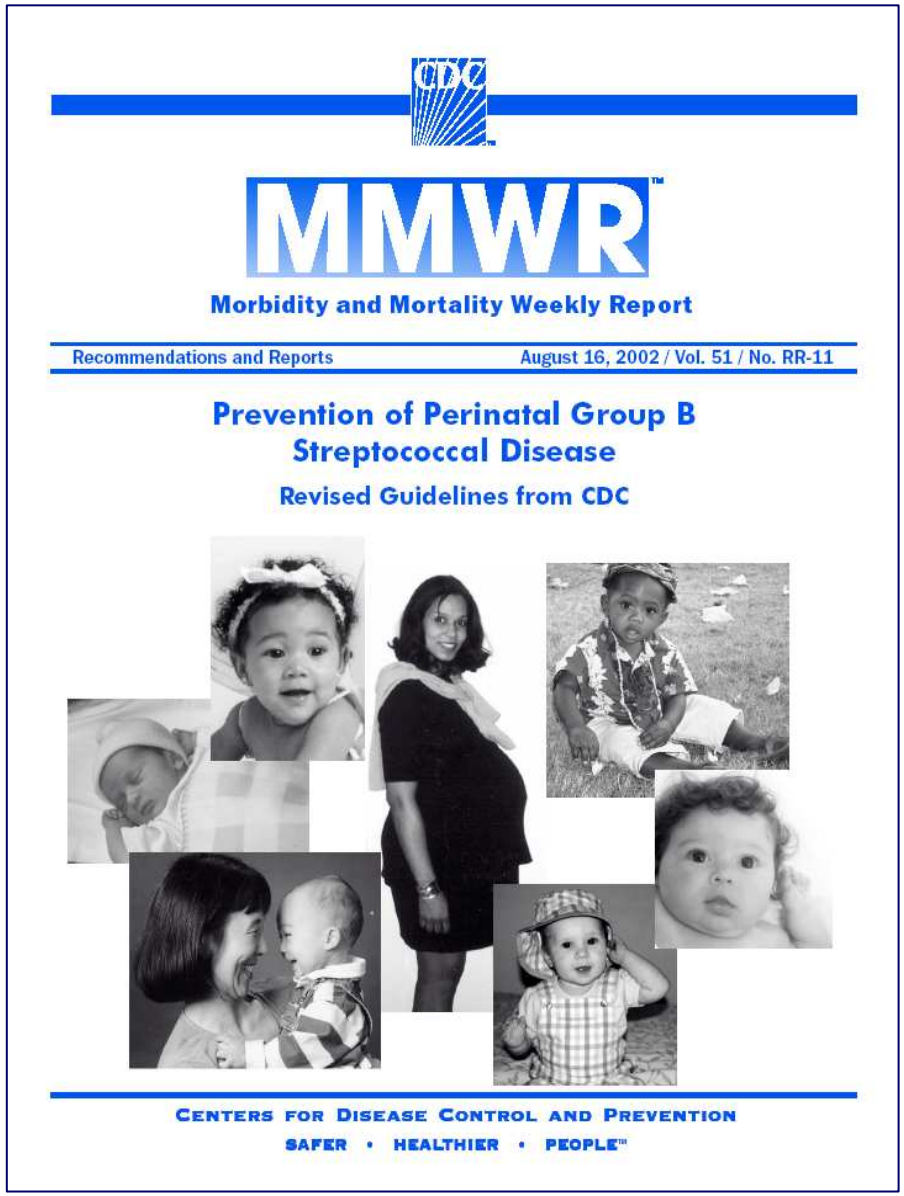
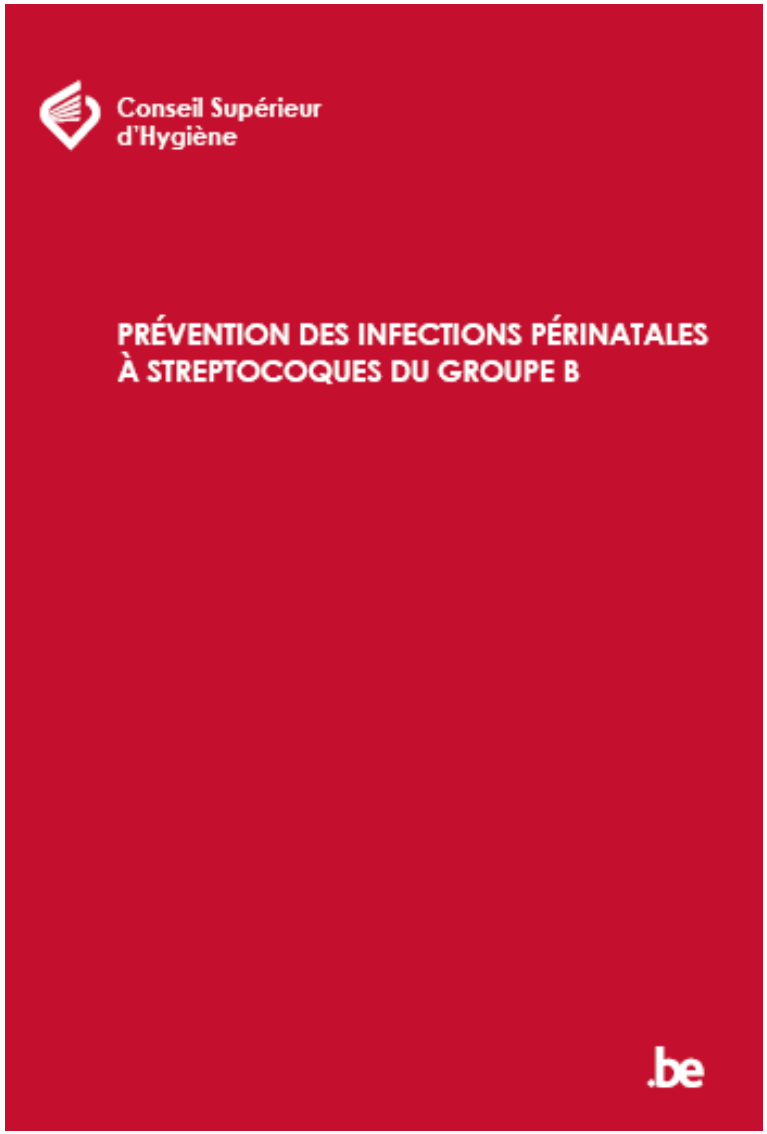
Background
Group B streptococci or *S.agalactiae*

- **Since the 1970s, leading cause of life-theatening infections in newborns**
  - **Neonatal illness/death**
  - **Long-term disabilities**

Of major concern

- **Maternal morbidity**
  - **Along pregnancy**
  - **Peripartum**
- **Serious diseases among elderly and adults with underlying diseases**
  - **Significant mortality**


MMWR
Morbidity and Mortality Weekly Report
Recommendations and Reports
August 16, 2002 / Vol. 51 / No. RR-11
Prevention of Perinatal Group B Streptococcal Disease
Revised Guidelines from CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION
SAFER • HEALTHIER • PEOPLE™


Conseil Supérieur d'Hygiène
PRÉVENTION DES INFECTIONS PÉRINATALES À STREPTOCOQUES DU GROUPE B
.be

Prevention of perinatal GBS EOD

- Intrapartum antibiotics
  - Highly effective at preventing EOD in women at risk of transmitting GBS to their newborns (≥ 4 h)

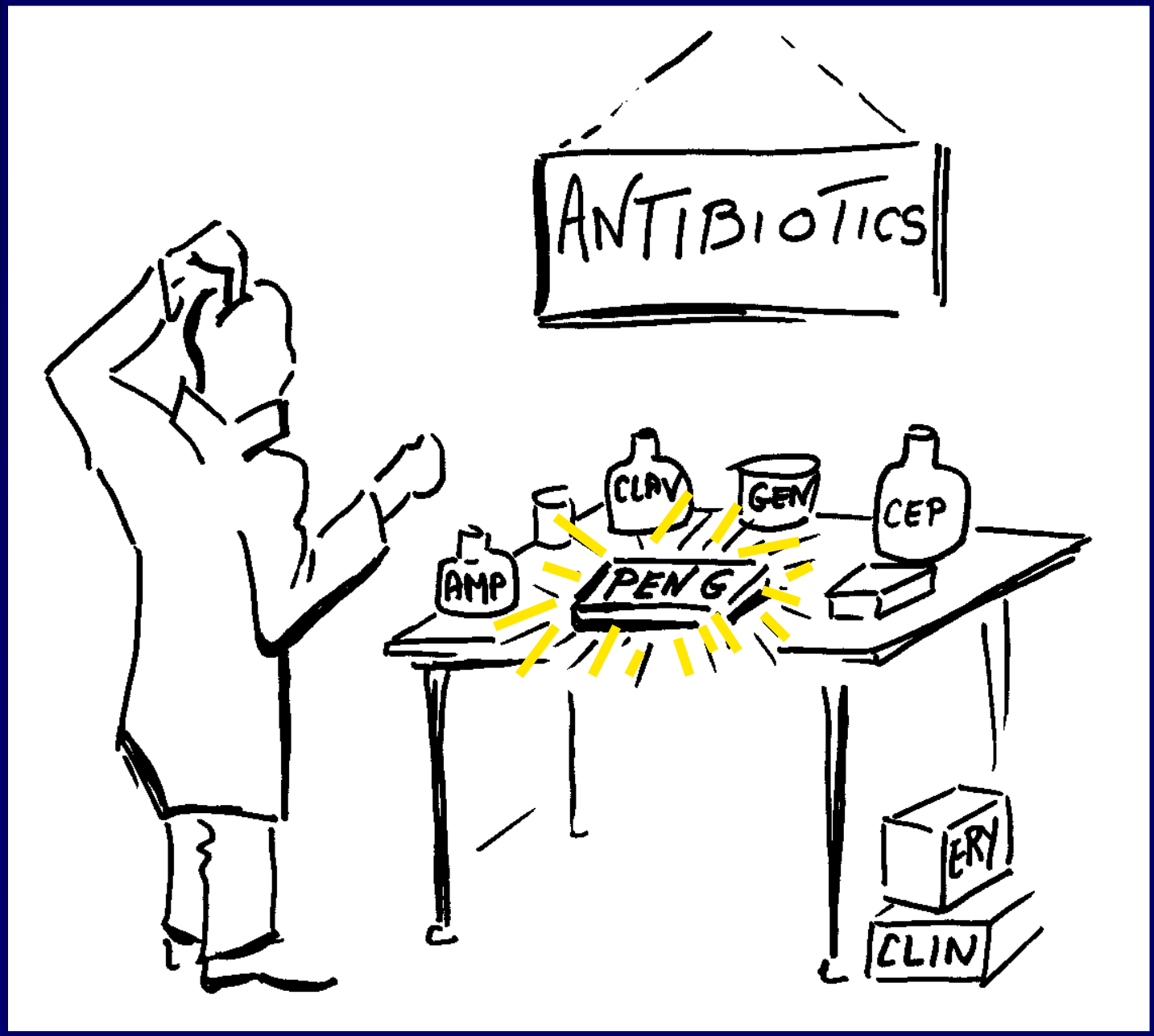
INTRAPARTUM ANTIMICROBIAL PROPHYLAXIS

Main goal :

- To prevent 70 to 80 % of GBS EO cases

Secondary :

- To reduce peripartum maternal morbidity



From P. De Mol

Concerns

- Increase of resistance to erythromycin and clindamycin
- Susceptibility to penicillin
  - Very few R isolates recently characterized in Japan

Objectives

Among GBS recently isolated in Belgium

- From adults with severe infections
- From early or late onset neonatal diseases

- To monitor penicillin susceptibility
- To determine rates of erythomycin and clindamycin resistance
- To assess the distribution of macrolide resistance phenotypes
- To identify genes coding for resistance to erythromycin

Methods : Isolates

- Clinical isolates
  - Sent to the Belgian reference laboratory for GBS from January 2005 and June 2006
  - From blood, CSF or any deep normally sterile site
  - 178 isolates
    - 22 from neonatal EOD
    - 10 from neonatal LOD
    - 146 from adult invasive disease
    - Serotypes
      - Ia (17%); Ib (6%); II (10%); III (33%); IV (6%); V (21%); others (7%)
- Reference strains
  - Positive either for *erm*B, *erm*TR or *mef*A genes
  - Negative for these 3 genes

Methods : Susceptibility testing

- Disk diffusion
  - For all isolates
  - Erythromycin (15 µg) and clindamycin (2 µg) disks
  - 18 mm apart
- Etest
  - Benzylpenicilline strips
    - For all isolates
  - Erythromycin and clindamycin strips
    - For all erythromycin resistant isolates
- Macrolide resistant phenotypes - Dtest
  - MLS_B phenotypes
    - Inducible Resistance
    - Constitutive Resistance
  - M Phenotype

Interpretation criteria *(MH with blood)*
(CLSI 2006)

	Zone Diameter (mm)			MIC (mg/L)		
	S	I	R	S	I	R
Penicillin	≥ 24	-	-	≤ 0.12	-	-
Erythromycin	≥ 21	16-20	≤ 15	≤ 0.25	0.5	≥ 1
Clindamycin	≥ 19	16-18	≤ 15	≤ 0.25	0.5	≥ 1

Methods : Detection of R genes

- DNA extraction
  - QIAmp DNA Mini Kit (Qiagen)
- PCR amplification with specific primers and protocols
  - Detection of *erm*B, *erm*TR and *mef*A genes
- Caracterisation of PCR products
  - Separation by electrophoresis
    - 2% Agarose gel + ethidium bromide staining
    - Visualization under UV light

Targets	PCR Product sizes (bp)
*erm*B	640
*erm*TR	530
*mef*A	348

Results
Antimicrobial susceptibility profile of 178 GBS clinical isolates

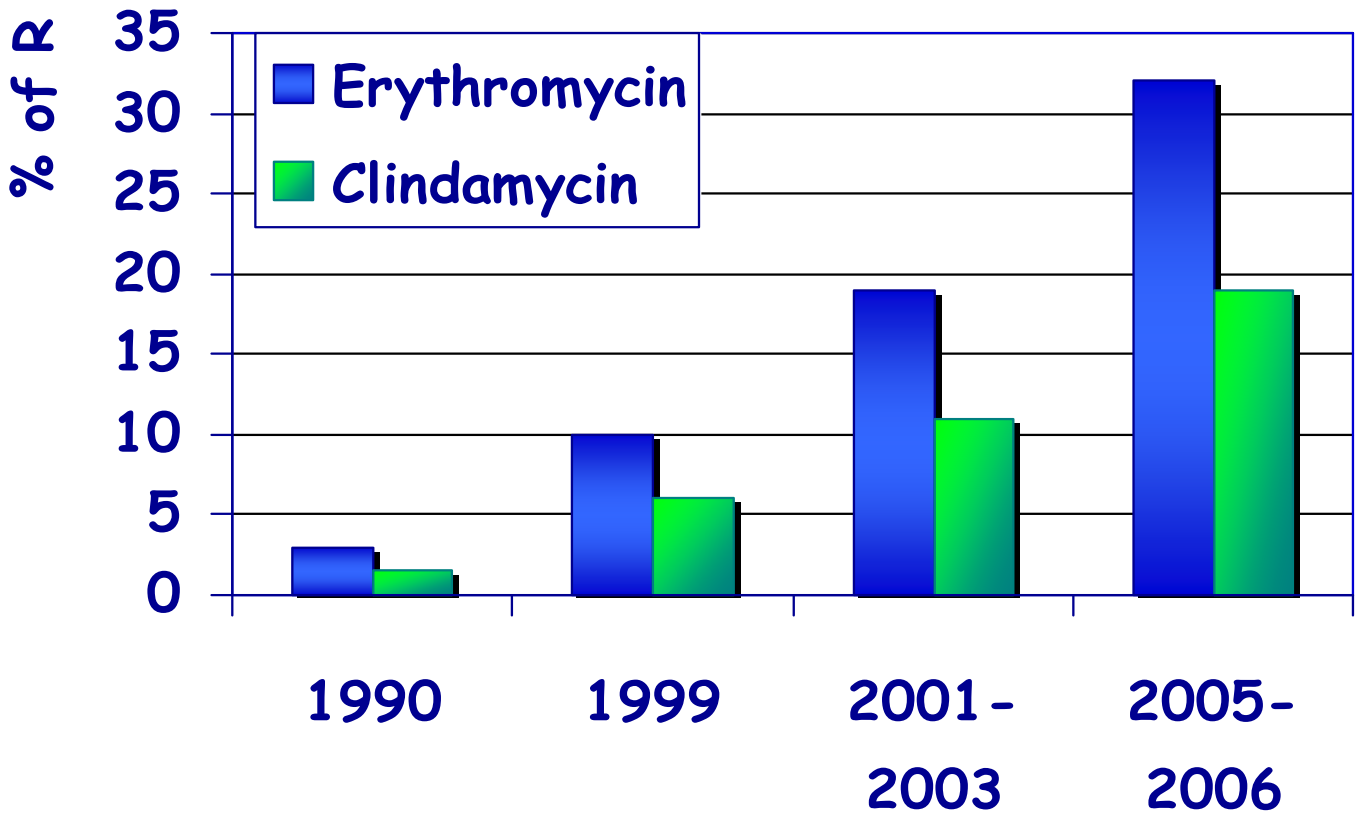
	% of Resistance	MIC_{90} (mg/L)	MIC Range (mg/L)
Penicillin*	0	0.094	0.016-0.094
Erythromycin	32		
Clindamycin	19 (25**)		

** : same interpretation for ampicillin and cefazolin*

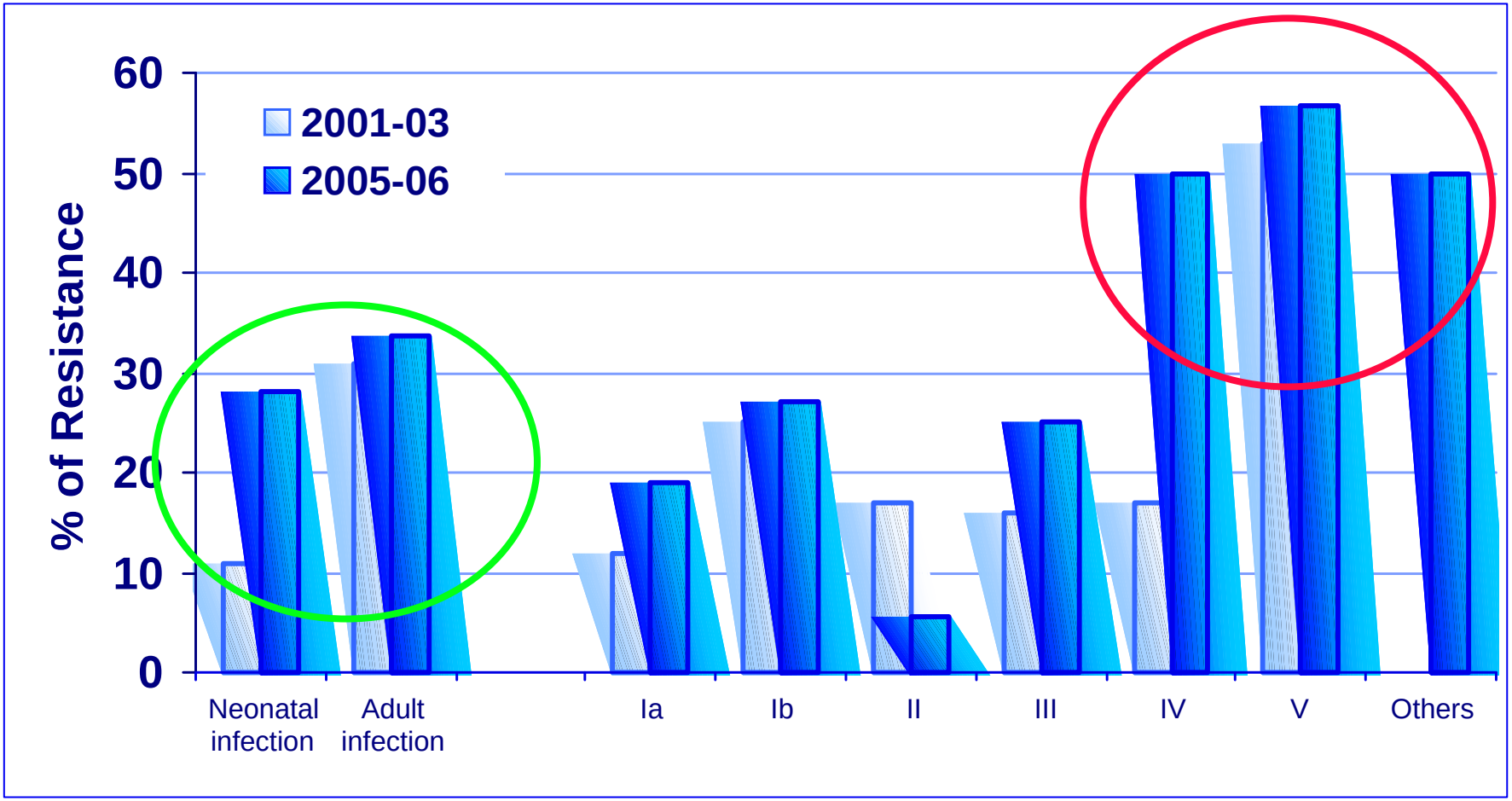
***: iMLS resistant phenotype included*

Results

Erythromycin and clindamycin resistance Evolution among Belgian GBS isolates



Results
Erythromycin resistance among Belgian clinical GBS isolates



Results
MLS Resistance phenotypes

Phenotype		%	Ery MIC_{50} / MIC_{90} (mg/L)
MLS	*Constitutive*	45	>256 / >256
	Inducible	34	4 / >256
M		21	4 / 12

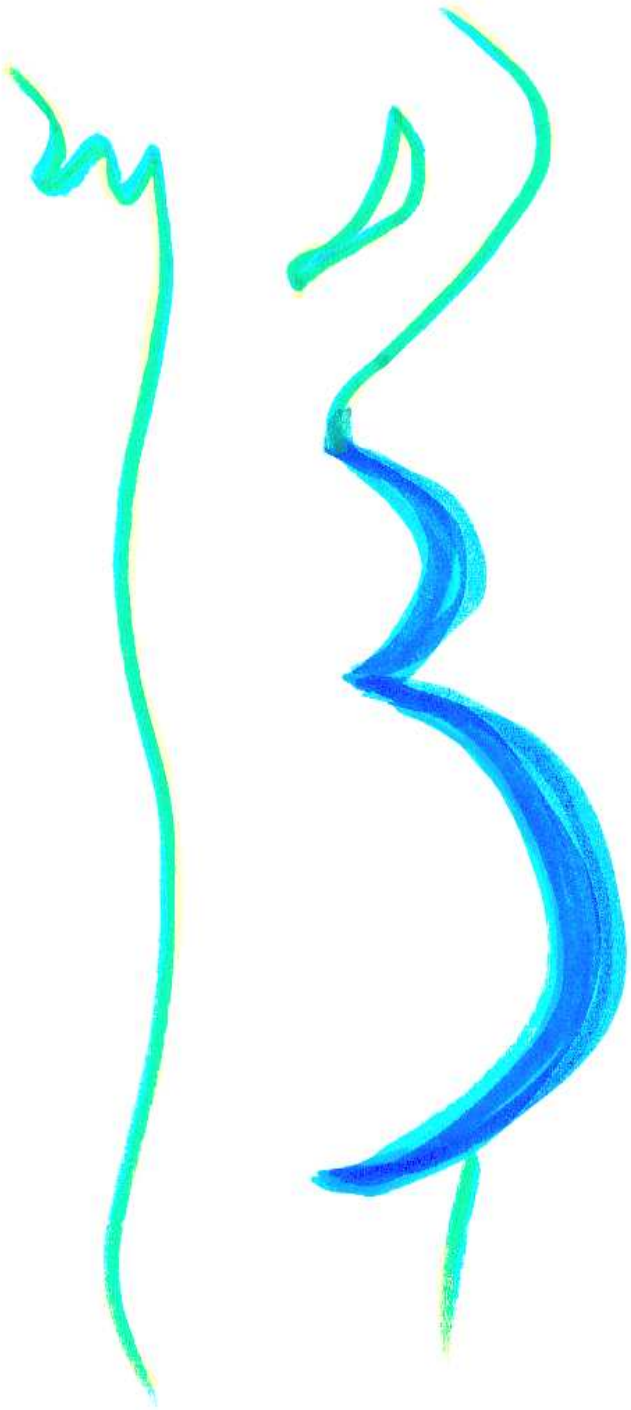
- **Dtest**
  - cMLS Erythro R & Clinda R
  - iMLS Erythro R & Clinda S/I/R with Dtest +
  - M Erythro R & Clinda S with Dtest -

Results
Distribution of macrolide R genes

Resistance phenotype	Resistance genotype	Number of isolates (% per phenotype)
MLS constitutive (26 isolates)	***erm*B**	**19 (73)**
	*erm*TR	2 (8)
	*erm*B & *erm*TR	5 (19)
MLS inducible (20 isolates)	*erm*B	1 (5)
	***erm*TR**	**16 (80)**
	*erm*B & *erm*TR	1 (5)
	*erm*TR & *mef*A	1 (5)
	unknown	1 (5)
M (12 isolates)	*erm*TR	3 (25)
	***mef*A**	**8 (67)**
	*erm*B & *erm*TR	1 (8)

Conclusion

- All GBS isolates fully susceptible to penicillin
- Increase of resistance to macrolides : a relevant problem.
  - Level: similar to rates observed in France, a neighbour country.
  - No more difference among isolates from either adults or neonates.
  - Most of macrolide R isolates had a MLS phenotype.
  - Detection of MLS-IR is important
    - simple and reliable double-disk diffusion test strongly recommended.
- Neither macrolides no lincosamides should no longer be used without susceptibility testing.



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Laboratories of the Belgian surveillance network