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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Belgian reference laboratory for GBS
Background
Group B streptococci or *S. agalactiae*

- Since the 1970s, leading cause of life-threatening 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
Prevention of Perinatal Group B Streptococcal Disease
Revised Guidelines from CDC
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 erythromycin 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**
  - $\text{MLS}_B$ phenotypes
    - Inducible Resistance
    - Constitutive Resistance
  - M Phenotype
### Interpretation criteria *(MH with blood)*
*(CLSI 2006)*

<table>
<thead>
<tr>
<th>Zone Diameter (mm)</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Penicillin</td>
<td>≥ 24</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≥ 21</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≥ 19</td>
</tr>
</tbody>
</table>
Methods: Detection of R genes

- DNA extraction
  - QIAmp DNA Mini Kit (Qiagen)
- PCR amplification with specific primers and protocols
  - Detection of *ermB*, *ermTR* and *mefA* genes
- Characterisation of PCR products
  - Separation by electrophoresis
    - 2% Agarose gel + ethidium bromide staining
    - Visualization under UV light

<table>
<thead>
<tr>
<th>Targets</th>
<th>PCR Product sizes (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ermB</em></td>
<td>640</td>
</tr>
<tr>
<td><em>ermTR</em></td>
<td>530</td>
</tr>
<tr>
<td><em>mefA</em></td>
<td>348</td>
</tr>
</tbody>
</table>
# Results

Antimicrobial susceptibility profile of 178 GBS clinical isolates

<table>
<thead>
<tr>
<th>% of Resistance</th>
<th>MIC$_{90}$ (mg/L)</th>
<th>MIC Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Penicillin</strong>*</td>
<td>0</td>
<td>0.094</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>19 (25**)</td>
<td></td>
</tr>
</tbody>
</table>

* : same interpretation for ampicillin and cefazolin  
**: iMLS resistant phenotype included
Results
Erythromycin and clindamycin resistance
Evolution among Belgian GBS isolates

% of R

Erythromycin
Clindamycin

0 5 10 15 20 25 30 35

Results

Erythromycin resistance among Belgian clinical GBS isolates

% of Resistance

Neonatal infection  Adult infection  Ia  Ib  II  III  IV  V  Others

2001-03  2005-06

pm-chulg - 16.11.07
### Results

**MLS Resistance phenotypes**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>%</th>
<th>Ery MIC&lt;sub&gt;50&lt;/sub&gt; / MIC&lt;sub&gt;90&lt;/sub&gt; (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLS <strong>Constitutive</strong></td>
<td>45</td>
<td>&gt;256 / &gt;256</td>
</tr>
<tr>
<td><strong>Inducible</strong></td>
<td>34</td>
<td>4 / &gt;256</td>
</tr>
<tr>
<td>M</td>
<td>21</td>
<td>4 / 12</td>
</tr>
</tbody>
</table>

#### 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**

<table>
<thead>
<tr>
<th>Resistance phenotype</th>
<th>Resistance genotype</th>
<th>Number of isolates (% per phenotype)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLS constitutive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(26 isolates)</td>
<td><strong>ermB</strong></td>
<td>19 (73)</td>
</tr>
<tr>
<td></td>
<td><strong>ermTR</strong></td>
<td>2 (8)</td>
</tr>
<tr>
<td></td>
<td><strong>ermB &amp; ermTR</strong></td>
<td>5 (19)</td>
</tr>
<tr>
<td><strong>MLS inducible</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(20 isolates)</td>
<td><strong>ermB</strong></td>
<td>1 (5)</td>
</tr>
<tr>
<td></td>
<td><strong>ermTR</strong></td>
<td>16 (80)</td>
</tr>
<tr>
<td></td>
<td><strong>ermB &amp; ermTR</strong></td>
<td>1 (5)</td>
</tr>
<tr>
<td></td>
<td><strong>ermTR &amp; mefA</strong></td>
<td>1 (5)</td>
</tr>
<tr>
<td></td>
<td><strong>unknown</strong></td>
<td>1 (5)</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12 isolates)</td>
<td><strong>ermTR</strong></td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td><strong>mefA</strong></td>
<td>8 (67)</td>
</tr>
<tr>
<td></td>
<td><strong>ermB &amp; ermTR</strong></td>
<td>1 (8)</td>
</tr>
</tbody>
</table>
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 nor lincosamides should no longer be used without susceptibility testing.
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Technical staff of the medical microbiology laboratory of the University hospital of Liège

Laboratories of the Belgian surveillance network