GBS Screening, diagnosis and clinically relevant resistance

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"Background"

- Evolution of culture methods
- Rapid non-cultural GBS screening
- Antimicrobial resistance
Vaginal (rectal) GBS colonization at delivery

- GBS carriers
  - GI tract = natural reservoir
  - 10 - 35 % of women (vagina/rectum)
  - Clinical signs not predictive
  - Dynamic condition (transient – chronic – intermittent)
  - Prenatal cultures late in pregnancy can predict delivery status
- Prevention for neonatal early onset disease
  - Antibioprophylaxis
  - Universal GBS screening-based strategy
  - Successful but cases continue occurring

Goal of GBS screening

*To predict GBS vaginal (rectal) colonization at the time of delivery*
How could you know if my mom is GBS-colonized?
Background

Critical factors influencing accuracy

- Screening methods
  - Culture
  - Non-culture
- Timing of sampling
- Swabbed anatomic sites
- Culture media
Background

Culture

Non-culture

Resistance

Optimal time for screening

35-37 weeks gestation

Culture-based screening done 1 to 5 or ≥ 6 weeks before delivery (Yancey, 860 cases; Melin, 531 cases)


Melin et al. ICAAC 2000
Optimal time for screening
35-37 weeks gestation

Culture-based screening done 1 to 5 or ≥6 weeks before delivery (Yancey, 860 cases; Melin, 531 cases)

Melin, 13-16% GBS Pos
PPV = 56%
NPV = 95%
or 5% False negative
or 30% of GBS pos in labor not detected with prenatal screening!

Melin et al. ICAAC 2000
Choice of the anatomic sites

Vagina + rectum

Vagina & rectum > vagina or rectum > cervix

Badri et al., J Infect Dis 1977;135:308-12

- Rectum
  - = reservoir, source of vaginal colonization
- Rectum GBS positive and vagina negative
  - 15 to 20% of GBS positive pregnant women
- Lower vaginal area
  - To exclude use of speculum for collection
Evolution of culture methods
Use of selective enrichment broth

- To maximize the isolation of GBS
- To avoid overgrowth of other organisms

<table>
<thead>
<tr>
<th>Nb women, medium</th>
<th>Direct culture 48hrs GBS+</th>
<th>Sub-culture from SEB % GBS+</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>200, Granada</td>
<td>88 %</td>
<td>100 %</td>
<td>Tazi A et al, 2008</td>
</tr>
<tr>
<td>500, Granada</td>
<td>72 %</td>
<td>99 %</td>
<td>Melin P et al, 2008</td>
</tr>
<tr>
<td>StrepB select</td>
<td>74 %</td>
<td>96 %</td>
<td></td>
</tr>
<tr>
<td>288, Blood /Lim</td>
<td>52 %</td>
<td>82 %</td>
<td>Shibuya R, 2009</td>
</tr>
<tr>
<td>New Granada</td>
<td>52 %</td>
<td>100 %</td>
<td></td>
</tr>
</tbody>
</table>
Evolution of culture methods
Use of selective enrichment broth

- **Todd Hewitt broth**
  + colistin + nalidixic acid
  
  = LIM broth

- **Todd Hewitt broth**
  + gentamicin + nalidixic acid (+ 5% sheep blood)
  
  (C.Baker, 1973 Applied Microbiology)

  = « Trans-Vag™ broth »

- **Granada biphasic broth**

(CDC 2002 - Belgian SHC 2003)
Revised guidelines from CDC (2002)

- **Sub-culture < selective enrichment broth**
  - **Blood agar**
    - **Advantage**
      - Growth of all GBS Isolates beta-hemolytic or not
    - **Disadvantage**
      - Difficulty in seeing rare GBS colonies within mixed flora
      - Difficulty in recognizing non-hemolytic GBS in mixed flora
Evolution of culture methods

Use of differential agar media

Recommended by some European guidelines

GRANADA
(M.de la Rosa, JCM)

1983, 1992

Strepto B Select

2005 2007

Pigment-based

Chromogenic media
Granada medium agar

M de la Rosa Fraile, JCM 1983 & 1992

- Orange color: GBS pigment, Granadaene

- 100% specific for GBS // β-hemolysis

- Group B Streptococcus Differential Modified Granada Medium™ (BD)
- Carrot Medium (Hardy)

Does not show non-hemolytic strain !
(<5 % of invasive isolates)
Strepto B ID agar (BioMérieux)

High sensitivity for growth of GBS
GBS = pink to red colonies

Chromogenic media

Not 100 % specific for GBS: Id to confirm (latex)
Strep B Select agar (BioRad)

GBS = pale to dark blue-turquoise colonies

Chromogenic media

Not 100 % specific for GBS: ld to confirm (latex)
### Granada (BD) - StreptoB ID - StrepB Select versus Blood agar +/- CNA

500 genital swabs (29.4 % GBS Positive)

<table>
<thead>
<tr>
<th></th>
<th>Number of GBS Positive culture (%)</th>
<th>Direct culture</th>
<th>Lim sub-culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strep B Select</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BioRad)</td>
<td></td>
<td>103 (70.1)</td>
<td>134 (91.1)</td>
<td>139 (94.6)*</td>
</tr>
<tr>
<td><strong>Granada</strong></td>
<td></td>
<td>90 (61.2)</td>
<td>123 (83.7)</td>
<td>124 (84.4)</td>
</tr>
<tr>
<td>(BD)</td>
<td></td>
<td>93 (63.2)</td>
<td>124 (84.3)</td>
<td>128 (87.1)</td>
</tr>
<tr>
<td><strong>Strep B ID</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(bioMérieux)</td>
<td></td>
<td>93 (63.2)</td>
<td>124 (84.3)</td>
<td>128 (87.1)</td>
</tr>
<tr>
<td><strong>BA + CNA</strong></td>
<td></td>
<td>76 (51.7)</td>
<td>113 (76.9)</td>
<td>120 (80.6)</td>
</tr>
<tr>
<td><strong>&gt;=1 Medium</strong></td>
<td></td>
<td></td>
<td></td>
<td>147 (100)</td>
</tr>
</tbody>
</table>

* StrepB Select > BA (p<0.5)
 Granada (BD) - StreptoB ID - StrepB Select versus Blood agar +/- CNA

« False-Positive »
= Characteristic colonies not confirmed as GBS

<table>
<thead>
<tr>
<th>Identified as</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep B Select</td>
<td>GAS, GCS, GDS-enterococci, Staphylococci, <em>S.bovis</em>, $\alpha$-hemolytic colonies, (yeasts, Gram negative bacilli)</td>
</tr>
<tr>
<td>Granada</td>
<td>/</td>
</tr>
<tr>
<td>Strep B ID</td>
<td>GCS, Staphylococci, $\alpha$-hemolytic colonies, (Gram negative bacilli)</td>
</tr>
<tr>
<td>BA +/- CNA</td>
<td>GAS, GCS, GFS, Staphylococci, GDS-enterococci, (Gram negative bacilli)</td>
</tr>
</tbody>
</table>

P. Melin, 2008 ECCMID P1388
Positive predictive value
Granada (BD) - StreptoB ID - StrepB Select versus Blood agar +/- CNA

<table>
<thead>
<tr>
<th></th>
<th>PPV Primoculture</th>
<th>PPV Lim sub-culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep B Select</td>
<td>71,5 %</td>
<td>77,9 %</td>
</tr>
<tr>
<td>Granada</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Strep B ID</td>
<td>80,9 %</td>
<td>87,9 %</td>
</tr>
<tr>
<td>BA +/- CNA</td>
<td>62,8 %</td>
<td>65,7 %</td>
</tr>
</tbody>
</table>

**Sensitivity**
Strep B Select > Granada - Strep B ID > CNA

**Specificity**
Granada > Strep B ID > Strep B Select > CNA

P. Melin, 2008 ECCMID P1388
Which agar or which combination?
+/- Blood agar

Workload – costs – extra-testing to be considered
Vagino-rectal swab or Vaginal & rectal swabs

Inoculate swab(s) in 1 LIM broth

LIM broth

Overnight
And subculture following
at 35-37°C
to one of the media

Granada agar
Anaero
48 h at 35-37°C

StrepB Select
Ambient air

ID StreptoB
Ambient air

POSITIVE GBS Screening if
Orange colonies
- GBS

Blue-turquoise colonies
- suggestive GBS
Id. to confirm

Pink colonies
- suggestive GBS
Id. to confirm

Negative GBS Screening if
No orange colonies
No blue-turquoise colonies
No pink colonies
**Crucial conditions to optimize SCREENING**

- **WHEN**: 35-37 weeks
- **WHO**: ALL the pregnant women
- **Specimen**: Vaginal + rectal swab(s)
- **Collection**: WITHOUT speculum
- **Transport**: Transport/collection device (non nutritive medium: Amies/Stuart)
- **Request form**: To specify prenatal « GBS » screening + expected address for delivery
- **Laboratory procedure**

*(CDC 2002 - Belgian SCH 2003)*
Adhesion to a common protocol is a key for success
Multidisciplinary collaboration is mandatory
Prenatal culture-based screening

- Limiting factors
  - Positive and negative predictive values
    - False-negative results
      - Failure of GBS culture (oral ATB, feminine hygiene) or new acquisition
      - Up to 1/3 of GBS women at time of delivery
      - Continuing occurrence of EO GBS cases
    - False-positive
      - Unnecessary IAP
  - Need for more accurate predictor of intrapartum GBS vaginal colonization
Alternative to prenatal GBS screening: intrapartum screening

Turnaround time
Collect specimen at admission

Optimal management of patient

Specimen analysis

Results
30-45 minutes, 24/24 hrs and 7/7 d, robust

Benitz et al. 1999, Pediatrics, Vol 183 (6)
Optimal time for IAP efficiency >= 4 hour

Cumulative histogram (% of patients) of time elapsed between admission to labor room and delivery for 532 women (sites CHR & CHBA)

- GBS Positive: 28.7%
- GBS negative: 26.9%

pm-chulg GBS workshop 28.05.2009
Rapid non-cultural GBS screening

- Available antigenic tests
  - Variety of Immuno-assays
  - Lack of sensitivity
    - Announced $5.10^5$ CFU, but not confirmed
- Hybridization tests
  - Not enough rapid
  - Lack of sensitivity if no enrichment step
Real Time PCR for intrapartum screening

- Advance in PCR techniques & development of platforms
  - BD GeneOhm™ Strep B Assay (+/- 1 hr) (in laboratory)
  - Xpert GBS, Cepheid (+/- 75 min) (can be performed as a POC)
Rapid non-cultural GBS screening
Real-time PCR

- **IDI Strep B** (BD GeneOhm)
  - Sensitivity: 94%
  - Specificity: 96%
  - PPV: 84% and NPV: 98.6%

- **Xpert™ GBS**
  - Sensitivity: 92%
  - Specificity: 95.6%
  - PPV: 86.7% and NPV: 97.4%

*HD Davies et al., CID 2004*

Surpass sensitivity of antenatal cultures
Real-time PCR, very promising, but …

- Still an expensive technology
- Logistic
  - 24/24 hours and 7/7 days
  - In the lab?
  - In the obstetrical department?
- In combination with prenatal screening strategy?
- No antimicrobial result
  - In the future detection of R genes, but mixed flora!
Antimicrobial resistance
### Clinically relevant Antimicrobial resistance?

<table>
<thead>
<tr>
<th>AB agent</th>
<th>IAP</th>
<th>Therapy</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>X</td>
<td>X</td>
<td>“No”, but ↑ MIC</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>X</td>
<td>X</td>
<td>10 - 30 %</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>X</td>
<td>X</td>
<td>Up to 20 %</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>(X)</td>
<td>(X)</td>
<td>No</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>(X)</td>
<td></td>
<td>Few cases</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>(X)</td>
<td></td>
<td>No HLR</td>
</tr>
</tbody>
</table>
Susceptibility to penicillin

- Very few « not S » isolates recently characterized in Japan
  - Mutation in *pbp* genes, especially in *pbp2x*
  - MIC = 0.25 - 1 mg/L

  *Noriyuki Nagano et al, AAC 2008*

- To recommend to all laboratories
  - To send « non-S » isolate to reference lab.
## AST interpretation criteria

**CLSI 2009 (Diffusion MH + blood) & EUCAST 2009**

<table>
<thead>
<tr>
<th></th>
<th>CLSI Zone Diameter (mm)</th>
<th>CLSI MIC (mg/L)</th>
<th>EUCAST MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Penicillin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S I R</td>
<td>S I R</td>
<td>S I R</td>
</tr>
<tr>
<td></td>
<td>≥ 24 - -</td>
<td>&lt; 0.12 - -</td>
<td>&lt; 0.25 - -</td>
</tr>
<tr>
<td><strong>Erythromycin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S I R</td>
<td>S I R</td>
<td>S I R</td>
</tr>
<tr>
<td></td>
<td>≥ 21 16-20 &lt; 15</td>
<td>&lt; 0.25 0.5 &gt; 1</td>
<td>&lt; 0.25 0.5 &gt; 1</td>
</tr>
<tr>
<td><strong>Clindamycin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S I R</td>
<td>S I R</td>
<td>S I R</td>
</tr>
<tr>
<td></td>
<td>≥ 19 16-18 &lt; 15</td>
<td>&lt; 0.25 0.5 &gt; 1</td>
<td>&lt; 0.5 - &gt; 1</td>
</tr>
</tbody>
</table>

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pm-chulg – GBS workshop 28.05.2009
Erythromycin and clindamycin resistance Evolution among Belgian GBS isolates
Erythromycin Resistance of Belgian clinical GBS isolates

2001-2003 187 invasive isolates, Melin et al, ICAAC 2003, #C2-81
2005-2006 178 invasive isolates, Melin et al, ICAAC 2007 #C2-168
MLS Resistance phenotypes

D-test recommended

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>%</th>
<th>Ery MIC_{50} / MIC_{90} (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constitutive</td>
<td>45</td>
<td>&gt;256 / &gt;256</td>
</tr>
<tr>
<td>Inducible</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 -

- **Vitek2**: not always reliable, to be improved

Neither macrolides no lincosamides should no longer be used without susceptibility testing
SUMMARY

- **Culture-based GBS prenatal screening**
  - To optimize critical factors
  - Improved by selective differential agars
  - False +/False - !

- **Rapid intrapartum screening**
  - Real time PCR
    - Yes but costs, logistic, …

- **Antimicrobial R**
  - Surveillance of Penicillin by NRC
  - To perform AST for macrolides/lincosamides