GBS Screening, diagnosis and clinically relevant resistance

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- **Background**
- **Prenatal GBS culture-based screening**
  - Evolution of culture methods
- **Intrapartum rapid non cultural GBS screening**
- **Antimicrobial resistance**
- **Summary**
**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
Background

Prevention for neonatal GBS EOD

- Prevention for neonatal early onset disease
  - Intrapartum 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
  - Swabbed anatomic sites
  - Timing of sampling
  - Screening methods
    - Culture
      - Procedure
      - Media
    - Non-culture
Choice of the anatomic sites

Lower vagina + rectum

Vagina & rectum > vagina or rectum > cervix

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

- **Rectum** (*through anal sphincter !*)
  - = reservoir, source of vaginal colonization
  - Rectum GBS positive and vagina negative
    - 15 to 20% of GBS positive pregnant women
- **Lower vaginal area**
  - For collection: use of speculum out of question
- A single combined specimen
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)

Not 100 % as colonization is dynamic

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
From direct plating on blood agar: 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 and « Granada-like » broths**

  - Also as transport media

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

- Sub-culture < selective enrichment broth
  - Blood agar +/- colistin and nalidixic acid
    - Advantage
      - Growth of all GBS Isolates beta-hemolytic or not
    - Disadvantage
      - Difficulty in seeing rare GBS colonies within mixed vaginal-rectal flora
      - Difficulty in recognizing non-hemolytic GBS in mixed flora

Sensitivity and specificity to be improved
Evolution of culture methods
Use of differential agar media

Recommended by some European guidelines (+ CDC 2010)

GRANADA
(M.de la Rosa, JCM)

1983, 1992

Pigment-based

2005, 2007

Chromogenic media

Strepto B Select

Strepto B ID

pm-chulg – UK GBS Symposium 17.06.2010
Granada medium agar
(Anaerobic incubation)

M de la Rosa Fraile, JCM 1983 & 1992

- Orange color: GBS pigment, Granadaene
- 100% specific for GBS // β-hemolysis

- Granada original, bioMérieux
- 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)
Strep B Select agar (BioRad)

High sensitivity for growth of GBS
- pink to red colonies
- or pale to dark blue-turquoise colonies

Chromogenic media
Not 100% specific for GBS: Id to confirm (latex)
(GAS, GCS, Staphylococci, alpha-hemolytic colonies, etc.)
### Number of GBS Positive culture (%)

<table>
<thead>
<tr>
<th></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>
</tr>
<tr>
<td>(BioRad)</td>
<td>103 (70.1)</td>
<td>134 (91.1)</td>
<td>139 (94.6)*</td>
</tr>
<tr>
<td>« Granada »</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BD)</td>
<td>90 (61.2)</td>
<td>123 (83.7)</td>
<td>124 (84.4)</td>
</tr>
<tr>
<td><strong>Strep B ID</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(bioMérieux)</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>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>147 (100)</td>
</tr>
</tbody>
</table>

* StrepB Select > BA (p<0.5)

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 > BA+ CNA

\[ Specificity before Id confirmation \]

Granada > Strep B ID > Strep B Select > BA+ CNA

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

Workload - costs - extra-testing - non β-hemolytic
GBS detection to be considered
### Crucial conditions to optimize SCREENING

<table>
<thead>
<tr>
<th>WHEN</th>
<th>35-37 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>ALL the pregnant women</td>
</tr>
<tr>
<td>Specimen</td>
<td>Vaginal + rectal swab(s)</td>
</tr>
<tr>
<td>Collection</td>
<td>WITHOUT speculum</td>
</tr>
<tr>
<td>Transport</td>
<td>Transport/collection device /condition</td>
</tr>
<tr>
<td></td>
<td>(non nutritive medium: Amies/Stuart or Granada like tube)(Length and T°)</td>
</tr>
<tr>
<td>Request form</td>
<td>To specify prenatal « GBS » screening + expected address for delivery</td>
</tr>
</tbody>
</table>

**Laboratory procedure**

*(CDC 2002 - Belgian SCH 2003)*
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 admision

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

Optimal management of patient

Specimen analysis

Benitz et al. 1999, Pediatrics, Vol 183 (6)
**Background Culture**

**Non-culture**

**Resistance Summary**

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

**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**
- **GBS negative**

- **28.7%**
- **26.9%**

P. Melin, 2004 ICAAC #G499

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Rapid non-cultural GBS screening

- Available antigenic tests
  - Variety of Immuno-assays
  - Lack of sensitivity
    - Announced $5 \times 10^5$ 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)

(Images of BD GeneOhm™ and Xpert GBS devices)
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%

Surpass sensitivity of antenatal cultures

Sensitivity // inoculum density = real time risk

*HD Davies et al., CID 2004*
Real-time PCR, very promising, but …

- Still an expensive technology
- Logistic
  - 24 hours 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

- Very few in the U.S.
- All laboratories should send to ref.lab.
  - Any « non-S » isolate for confirmation
  - All invasive isolates for resistance surveillance.
Erythromycin and clindamycin resistance
Evolution among Belgian GBS isolates

% of R

Erythromycin
Clindamycin

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

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MLS Resistance phenotypes

**D-test recommended**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>%</th>
<th>Ery MIC(<em>{50} / ) MIC(</em>{90}) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLS 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.

P.Melin, LISSSD 2008 P215
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
Adhesion to a common protocol is a key for success
Multidisciplinary collaboration is mandatory