Prevention for GBS perinatal disease: Which improvements for GBS screening?

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Medical Microbiology, University Hospital of Liege
INTRODUCTION & BURDEN
Streptococcus agalactiae or GBS

Gram positive cocci
- Catalase -
- β-hemolytic
- CAMP test +
- Hippurate +
- Esculine-
- Orange pigment

10 capsular serotypes (Ia, Ib, II-IX)

1887, Noccard-Mollereau, bovine mastitis
1933, Group B Antigen
1964, severe neonatal sepsis

1970, N° 1 in neonatal infections
Group B streptococcal diseases in neonates

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

- Maternal morbidity
  - Along pregnancy
  - Peripartum

- Serious diseases among elderly and adults with underlying diseases
  - Significant mortality

GLOBAL public health major concern!
Also in developing countries
GBS Neonatal Infections

A. Schuchat, Clin Microb Rev 1998;11:497-513
GBS Neonatal Infections

A. Schuchat, Clin Microb Rev 1998;11:497-513

80 % EOD

LOD

EOD: 80-90% occur
# Burden of neonatal GBS early onset diseases in European countries

<table>
<thead>
<tr>
<th>Location</th>
<th>Incidence per 1,000 live-births</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>2</td>
<td>Melin, Indian J Med Res 2004</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>0.2 - 4</td>
<td>Trijbel-Smeulders, Pediatr Infect Dis J 2004</td>
</tr>
<tr>
<td>Western Europe</td>
<td>0.3 - 2</td>
<td></td>
</tr>
<tr>
<td>The Netherlands</td>
<td>1.9</td>
<td></td>
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<tr>
<td>Scandinavia</td>
<td>0.76 - 2</td>
<td></td>
</tr>
<tr>
<td>Southern Europe</td>
<td>0.57 - 2</td>
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Data assessing more accurately the true burden are needed

- Carriage rate?
- Ethnicity?
- Sub-reporting?
- Systematic diagnostic approach?
- Virulence?
GBS EOD vertical transmission

GBS colonized mothers

Non-colonized newborns

Colonized newborns

60 - 40 %

40 - 60 %
GBS EOD vertical transmission

GBS colonized mothers

60 - 40 %
Non-colonized newborns

40 - 60 %
Colonized newborns

96 - 98 %
Asymptomatic
GBS EOD vertical transmission

GBS colonized mothers

Non-colonized newborns: 60 - 40 %
Colonized newborns: 40 - 60 %

Risk factors

2 - 4 % GBS EOD

Risk factors

96 - 98 % Asymptomatic

Sepsis, pneumonia, meningitis, long term sequelae

CDC
GBS maternal colonization

Risk factor for early-onset disease (EOD): *vaginal GBS colonization at delivery*

- **GBS carriers**
  - 10 - 35% of women
  - Clinical signs not predictive
  - Dynamic condition
  - Intestinal reservoir
  - Prenatal cultures late in pregnancy can predict delivery status
Additional Risk Factors for Early-Onset GBS Disease

- Obstetric factors:
  - Prolonged rupture of membranes,
  - Preterm delivery,
  - Intrapartum fever
- GBS bacteriuria
- Previous infant with GBS disease
- Immunologic:
  - Low specific IgG to GBS capsular polysaccharide

No difference in occurrence either in GBS Positive or Negative women, except intrapartum fever

Lorquet S., Melin P. & al. J Gynecol Obstet Biol Reprod 2005
GBS EOD - Belgian data

- **Incidence**
  - 1985 -1990: 3/1000 live births
  - 1999, estimation: 2/1000 live births
  - 2010, estimation: < 1/1000 live births

- **Meningitis**: 10%

- **Mortality**: 5 - 10%

- **60% EOD (130 cases)**: WITHOUT any maternal/obstetric risk factor except colonization

- **Prenatal screening**
  - Recto-vaginal cultures: 13-35% GBS Positive

*P. Melin - 2001, 2007 - Reference laboratory for GBS.*
Stages in the pathogenesis of GBS

neonatal EOD: Bacterial & individual factors

Colonization: adhesion to epithelial cells
different virulence factors (pili, scpB, ...)

MBG colonization

Meningitis

Brain barrier
Pili,
β-hemolysin, ...

GBS pathogenesis

Sepsis

β-hemolysin, invasins (pneumonia)

β-hemolysin, invasins (amnionitis)

Phagocytes cells, Antibodies, Complement

Resistance to phagocytose
- Capsule
- C5a peptidase
- .....
- Universal prenatal screening-based strategy
- Risk-based strategy
- No guideline

GUIDELINES FOR PREVENTION OF GBS PERINATAL DISEASE
Which prevention strategy for GBS perinatal diseases?

- Intrapartum antibiotic prophylaxis
- Immunoprophylaxis
Stages in the pathogenesis of GBS neonatal EOD: *Bacterial & individual factors*

**Colonization:** adhesion to epithelial cells different virulence factors (pili, scpB, …)

**Intrapartum antibioprophylaxis**

> 4 (2) hours before delivery
Stages in the pathogenesis of GBS neonatal EOD: **Bacterial & individual factors**

**GBS vaccine** « still expected »

Colonization: adhesion to epithelial cells, different virulence factors (pili, scpB, ...)

GBS pathogenesis

- Resistance to phagocytose
  - Capsule
  - C5a peptidase
- Ascendant transmission (amnionitis)
- β-hemolysin, invasins (pneumonia)

Phagocytes cells, **Antibodies**, Complement
Prevention of perinatal GBS EOD

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

*(clinical trials in late 80s)*

Who is the women at risk?

---

Risk-based strategy or Screening-based strategy
Prevention of perinatal GBS EOD

- Screening-based strategy

**INTRAPARTUM ANTIMICROBIAL PROPHYLAXIS**

Main goal:

- To prevent 70 to 80% of GBS EO cases

Secondary:

- To reduce peripartum maternal morbidity
Impact of prevention practices Early- and Late-onset GBS Diseases in the 1990s, U.S.

**Group B Strep Association formed**

**1st ACOG & AAP statements**

**CDC draft guidelines published**

**Consensus guidelines:**
- Screening
- Risk-based

**Screening >50% more effective than RF**

*S. Schrag, New Engl J Med 2000
Why is Screening more protective than the risk-based approach?  

Broader coverage of « at-risk » population

- Captures colonized women without obstetric RF
- High level of compliance with recommendations
- Enhanced compliance with risk-based approach cannot prevent as many cases as universal screening
Impact of prevention practices
Early- and Late-onset GBS Diseases, U.S.

Incidence of early- and late-onset invasive group B streptococcal disease in selective Active Bacterial Core surveillance areas, 1989-2008 (CDC 2010)

Before national prevention policy
Transition
Universal screening
INTRODUCTION & BURDEN

GUIDELINES

SCREENING

VACCINE

CONCLUSION

Prevention of Perinatal Group B Streptococcal Disease
Revised Guidelines from CDC, 2010

Continuing Education Examination available at http://www.cdc.gov/mmwr/cmw/cmw6888.html

SHC, Belgium July 2003
Revision ongoing

PRÉVENTION DES INFECTIONS PÉRINATALES À STREPTOCOQUES DU GROUPE B
European strategies for prevention of GBS EOD

- **Intrapartum anti-opiophylaxis recommended**
  - **Screening-based strategy**
    - France, 2001
    - Belgium, 2003, revision ongoing 2012
    - Germany, 1996, revised 2008
    - Switzerland, 2007
  - **Risk-based strategy**
    - UK, the Netherlands, Denmark
- **No guidelines**
  - Bulgaria, ...
Universal screening-based strategy for prevention of GBS perinatal disease

Vagino-rectal GBS screening culture at 35-37 weeks of gestation

For ALL pregnant women

Unless patient had a previous infant with GBS invasive disease or GBS bacteriuria during current pregnancy or delivery occurs < 37 weeks’ gestation *

GBS Neg
Not done, incomplete or unknown GBS result

GBS POS

> 1 Risk factor:
- Intrapartum fever ≥ 38°C**
- ROM ≥ 18 hrs

if NO
Intrapartum prophylaxis NOT indicated

if YES
!
Facultative!
Intrapartum rapid GBS test**

if YES
INTRAPARTUM ANTIBIOPROPHYLAXIS INDICATED

INTRODUCTION & BURDEN GUIDELINES SCREENING VACCINE CONCLUSION
Remaining burden of GBS EOD
Missed opportunities

In spite of universal screening prevention strategy
In spite the great progress
Cases still occur

- Among remaining cases of EOD
  - Some may be preventable cases
    - Missed opportunities for (appropriate) IAP
    - False negative screening

CDC revised guidelines 2010
DEVANI project, unpublished data 2011
SCREENING FOR GBS COLONIZATION
Antenatal GBS culture-based screening

Goal of GBS screening

To predict *GBS vaginal* (rectal) colonization at the time of delivery

- Critical factors influencing accuracy
  - Swabbed anatomic sites
  - Timing of sampling
  - Screening methods
    - Culture
      - *Procedure*
      - *Media*
    - Non-culture
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

Use of differential agar media
Recommended by some European guidelines (+ CDC 2010)

- GRANADA
  (M.de la Rosa, JCM)
- 1983, 1992
  Pigment-based
- 2005
  Chromogenic media
- 2007
  Strepto B Select
  StreptoB ID
Which agar or which combination?
+- Blood agar

**Workload** - costs - extra-testing - non β-hemolytic GBS detection to be considered
## 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/condition**
  - (non nutritive **medium**: Amies/Stuart or Granada like tube)
  - (type of **swab**)(Length and **T°**)
- **Request form**: To specify prenatal « GBS » screening
- **Laboratory procedure**

*(CDC 2010 - Belgian SCH 2003)*
## Crucial conditions to optimize SCREENING

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*(CDC 2010 - Belgian SCH 2003)*
Transport-collection system & transport-storage condition

- Specimen storage in transport medium and detection of group B streptococci by culture.


Recovery of group B streptococci (GBS) was assessed in 1,204 vaginorectal swabs stored in Amies transport medium at 4 or 21°C for 1 to 4 days either by direct inoculation onto Granada agar (GA) or by culture in blood. These data indicate that viability of GBS is not fully preserved by storage of vaginorectal swabs in Amies transport medium, mainly if they are not stored under refrigeration.

- Belgian Guidelines (2003, SHC)

"Specimens should be placed in a non-nutritive transport medium (e.g., Amies or Stuart's without charcoal). In these conditions, viability of GBS is warranted for at least 48 h at room temperature or in a fridge (2 - 8 °C).

Specimen labels should clearly identify that specimens are for group B streptococcal culture. Swabs should reach the lab within 48 h of collection."
Crucial conditions to optimize SCREENING

### Transport-collection system & transport-storage condition

*Preliminary results (2012, NRC GBS)*

| Use of a selective enrichment Lim broth  
*BD, Copan, bioMérieux* | Use of a selective enrichment Granada medium  
*bioMérieux* |
<table>
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**Transport-collection system & transport-storage condition**

*Preliminary results* (2012, NRC GBS)

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<td>- Sustained viability &gt; 4 days</td>
<td>- Sustained viability at RT°</td>
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<td>Between 4-8° C</td>
<td>- ≥ 24 hours, continuous decrease of life GBS</td>
<td>- Abrupt lost of viability at 35° C ≥ 48-72h</td>
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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 positive women at time of delivery
    - Continuing occurrence of EO GBS cases
  - False-positive
    - Positive prenatal screening /negative at time of delivery
    - Unnecessary IAP

Need for more accurate predictor of intrapartum GBS vaginal colonization
Prenatal culture-based screening: Limiting factors

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Need for more accurate predictor of intrapartum GBS vaginal colonization
Prenatal culture-based screening combined with *illumigene*® Group B Streptococcus assay

A loop mediated isothermal amplification (LAMP) assay by Meridian Bioscience, Inc

- Broth enrichment followed by *illumigene*® GBS
  - Speed and accuracy
Evaluation of the illumigene® GBS

242 vagino-rectal swabs

CHU Lg
& AZ St-Lucas Gent

Granada agar

18 – 48h at 35°C/ana

Overnight subculture

LIM broth

at 35°C C and

illumigene

GBS

Primary culture GBS
Pos (1-4+)/Neg

Enrichment GBS
Pos (1-4+)/Neg

illumigene GBS
Pos/Neg/Invalid
Evaluation of the *illumigene® GBS*

<table>
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<tr>
<th>GBS culture</th>
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<td>illumigene GBS</td>
<td>45</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>188</td>
</tr>
<tr>
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<td>50</td>
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**GBS Positive cultures:** 20.7%

**illumigene GBS vs GBS reference culture** (all discrepancies were retested)

- **Sensitivity**: 90.0 %
- **Specificity**: 98.9 %
- **PPV**: 95.7 %
- **NPV**: 97.4 %
- **Efficiency**: 97.1 %
## Evaluation of the *illumigene® GBS*

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### GBS Positive cultures: 20.7%

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<td>193</td>
</tr>
<tr>
<td></td>
<td>3 very rare GBS</td>
<td></td>
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Evaluation of the *illumigene*® GBS

- **Easy to perform**
  - But training very important
    - Molecular amplification method
    - Need for different skill
    - Workflow

- **Invalid results**
  - CHU Liege
    - 1 negative retested “invalid”
  - AZ Sint Lucas, Gent, decreased rate with experience
    - 5 resolved as negative
    - 3 resolved as positive

- **Short hands-on-time**
- **Short turn-around-time**
Evaluation of the *illumigene*® GBS

- **Speed and accuracy**
- **Good comparison to reference culture method**
  - 100% specificity and positive predictive value
  - High sensitivity and negative predictive value
  - Identification of an 0.8% additional GBS positive specimen
  - Overall cost and logistic to be considered
Prenatal culture-based screening: Limiting factors

- **Unknown GBS status at presentation for delivery**
  - Screening performed but result not available
  - Women with no prenatal care

**Risk based strategy**
- 60% at GBS risk not identified
- > 10% of unnecessary IAP

**Need for rapid accurate predictor of intrapartum GBS vaginal colonization**
Alternative to GBS prenatal screening: intrapartum screening
Theranostastic approach

**Turnaround time**
collect specimen at admission

Optimal management of patient

Specimen Analysis “POCT”?

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

*Benitz et al. 1999, Pediatrics, Vol 183 (6)*
Intrapartum screening theranostic approach: expected advantages

- Inclusion of women without prenatal screening/care
- Identification of women with change of GBS status after 35-37 wks gestation
- Increased accuracy of vaginal GBS colonization status at time of labor & delivery

IAP addressed to right target

- Reduction of inappropriate/unnecessary IAP
- Broader coverage of « at GBS risk women »

Improvement of prevention
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 (35-45 min) (can be performed as a POCT)
Real-time PCR, very promising, BUT ...

- Rapid, robust & accurate technology
- Still an expensive technology (specific equipment)
  - Cost effective ?
    - Need for more cost-effective clinical study
- Logistic
  - 24 hours 7 days
  - In the lab?
  - In the obstetrical department as a POCT ?
- In combination with prenatal screening strategy ?
  - CDC 2010 : for women with premature delivery or no prenatal care
- No antimicrobial result
  - In the future detection of R genes, but mixed microbiota !
CONCLUSION

Take home messages
In Europe, as globally

- Neonatal GBS diseases
  - EOD and LOD, a public health concern
  - IAP efficient for prevention of EOD
    - Best strategy still a matter of debate
    - Not 100% efficient
    - No effect on LOD
  - IAP not widely recommended
  - Need better data assessing more accurately the true burden
- GBS vaccine eagerly expected
Summary

“Screening” Prevention strategies

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

- **Culture-LAMP combined GBS prenatal screening**
  - High sensitivity and negative predictive value
  - 100% specificity and positive predictive value
  - Identification of an 0.8% additional GBS positive specimen

- **Rapid intrapartum screening**
  - Real time PCR
    - Yes but costs, logistic, ...
    - Need for more clinical and cost effectiveness trial
Evaluation of *illumigen®* GBS

**CHU Liège**
Magali Dodémont  
Gilles Sarlet  
Julie Descy  
Cécile Meex  
Raphaël Boreux  
& Bacteriology Staff

**AZ Sint-Lucas, Gent**  
Karlien Vanhouteghem  
Anne Marie Van den Abeele  
& Bacteriology Staff

**CHR Citadelle Liege** 
Jean Marc Senterre

*Meridian for supplying the test kits*