

***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**



Required RF for GBS EOD

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**
 - **Intrapartum antibioprohylaxis**
 - **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 GBS Screening

- **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

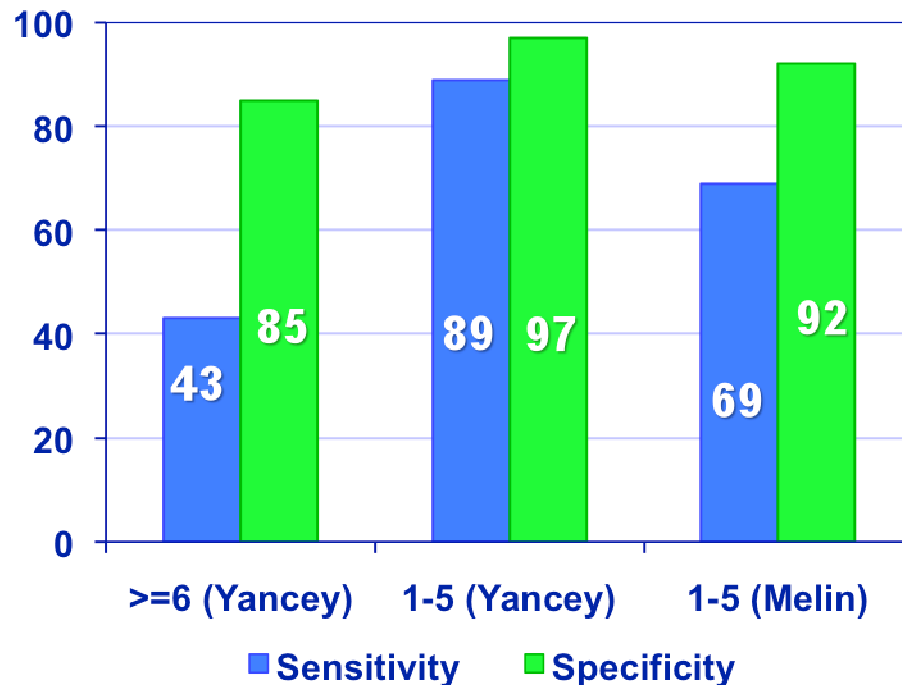
Vagina & rectum > vagina or rectum > cervix

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)



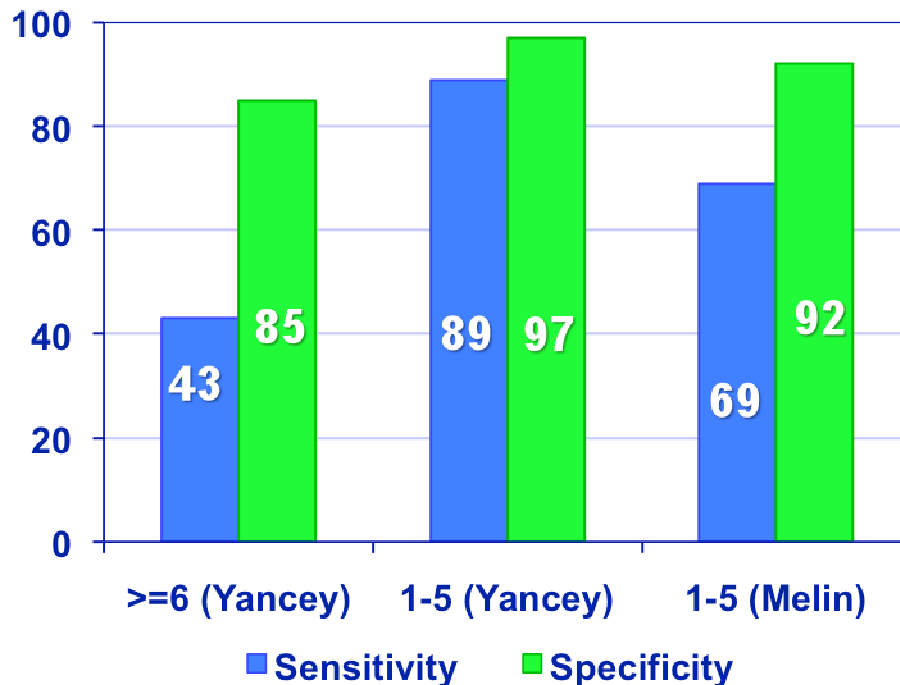
**Not 100 % as
colonization is dynamic**

Yancey MK et al. Obstet Gynecol 1996;88:811-5

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 !

Yancey MK et al. Obstet Gynecol 1996;88:811-5

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**

Nb women, medium	Direct culture 48hrs GBS+	Sub- culture from SEB % GBS+	Authors
200, Granada	88 %	100 %	Tazi A et al, 2008
500, Granada	72 %	99 %	Melin P et al, 2008
StrepB select	74 %	96 %	
288, Blood /Lim	52 %	82 %	Shibuya R, 2009
New Granada	52 %	100 %	

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**

Evolution of culture methods

Blood agar +/- CNA

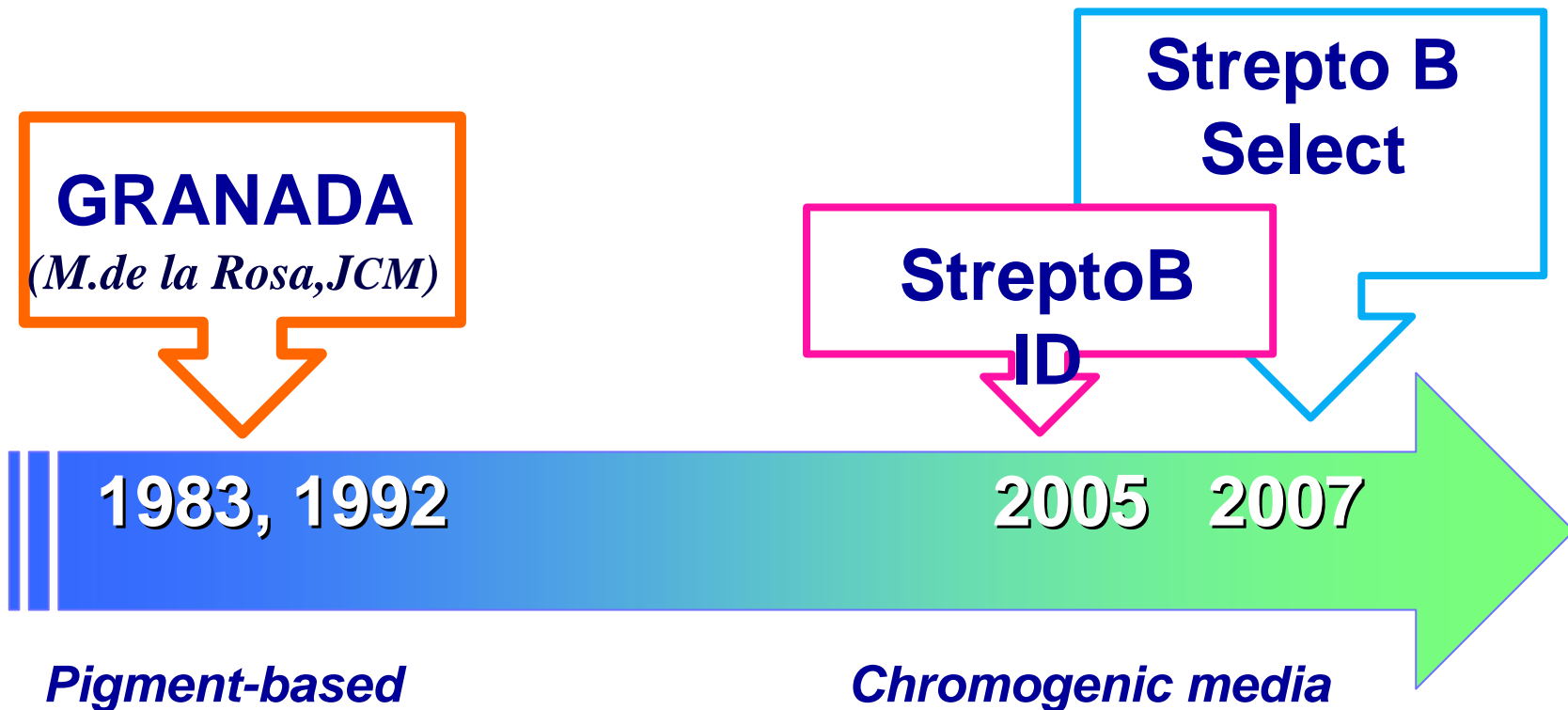
Revised guidelines from CDC (2002)

- **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 medium agar

(Anaerobic incubation)

M de la Rosa Fraile, JCM 1983 & 1992



- **Orange color: GBS pigment, Granadaene**

- **100% specific for GBS // β -hemolysis**

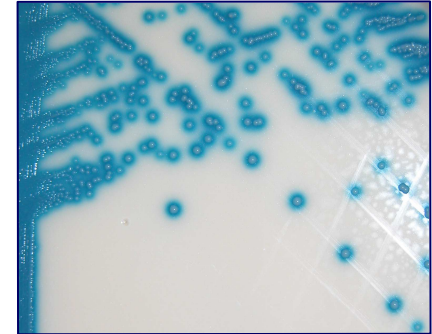
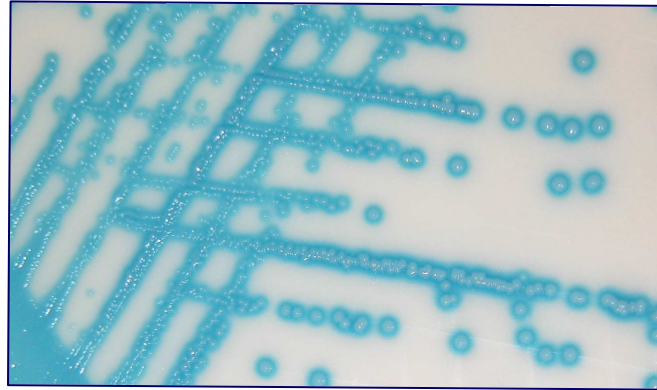
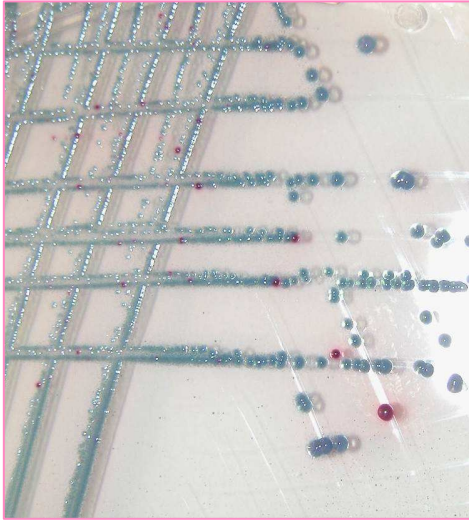
- **Granada original, bioMérieux**
- **Group B Streptococcus Differential Modified Granada Medium™ (BD)**
- **Carrot Medium (Hardy)**



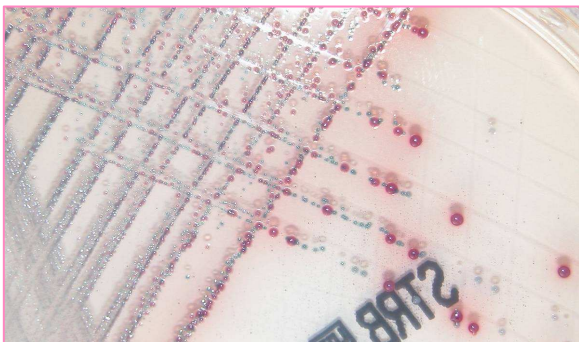
Does not show non-hemolytic strain !
(**<5 % of invasive isolates**)

Background
Culture
Non-culture
Resistance
Summary

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.)

Granada (BD) - StreptoB ID - StrepB Select versus Blood agar +/- CNA

500 genital swabs (29.4 % GBS Positive)

	Number of GBS Positive culture (%)		
	Direct culture	Lim sub-culture	Total
Strep B Select (BioRad)	103 (70.1)	134 (91.1)	139 (94,6)*
« Granada » (BD)	90 (61.2)	123 (83.7)	124 (84.4)
Strep B ID (bioMérieux)	93 (63.2)	124 (84.3)	128 (87.1)
BA ± CNA	76 (51.7)	113 (76.9)	120 (80.6)
>=1 Medium			147 (100)

* StrepB Select > BA (p<0,5)

Positive predictive value Granada (BD) - StreptoB ID - StrepB Select versus Blood agar +/- CNA

	PPV Primoculture	PPV Lim sub-culture
Strep B Select	71,5 %	77,9 %
Granada	100 %	100 %
Strep B ID	80,9 %	87,9 %
BA +/- CNA	62,8 %	65,7 %

Sensitivity

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

Specificity before Id confirmation

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

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)(Length and T°)
- ❖ **Request form** To specify prenatal « GBS »
screening + *expected address for delivery*
- ❖ **Laboratory procedure**

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

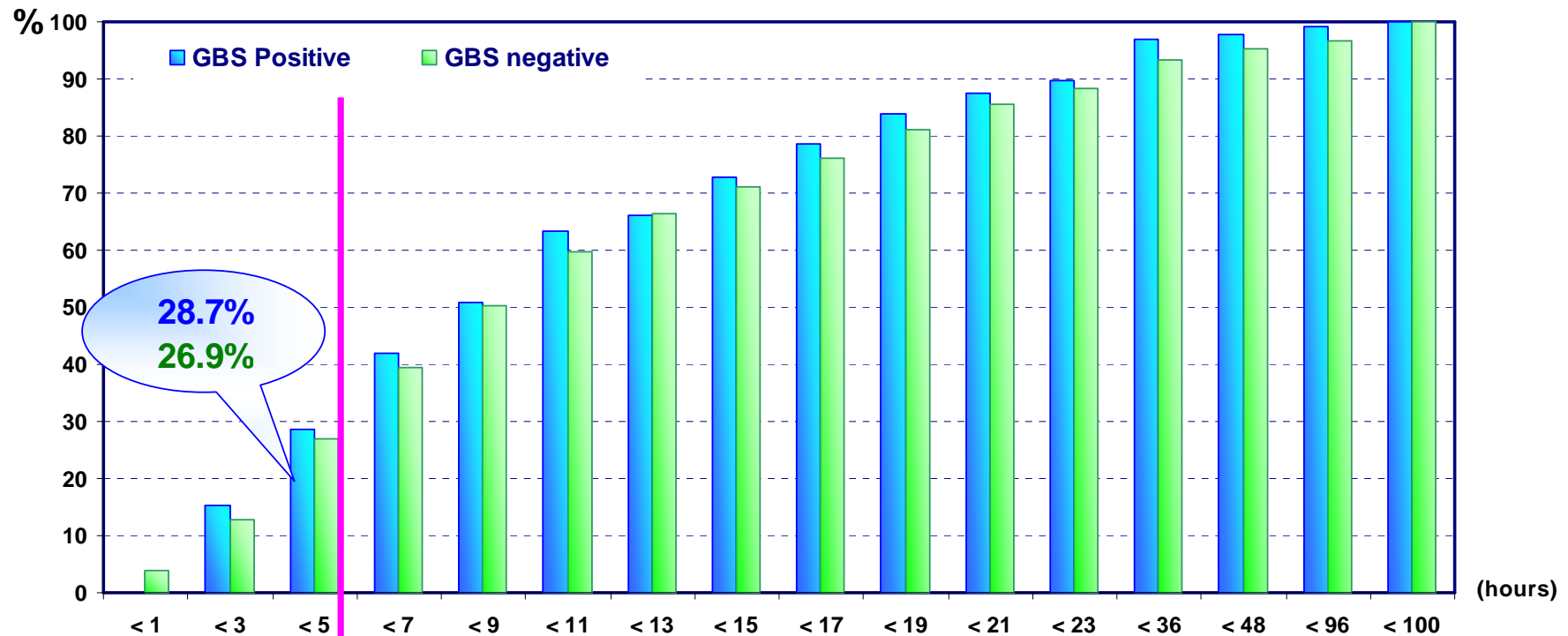
30-45 minutes, 24 hrs/7 d, robust

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

Time between admission and delivery

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)



Rapid non-cultural GBS screening

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



(Gen Expert)

Rapid non-cultural GBS screening Real-time PCR

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

HD Davies et al., CID 2004

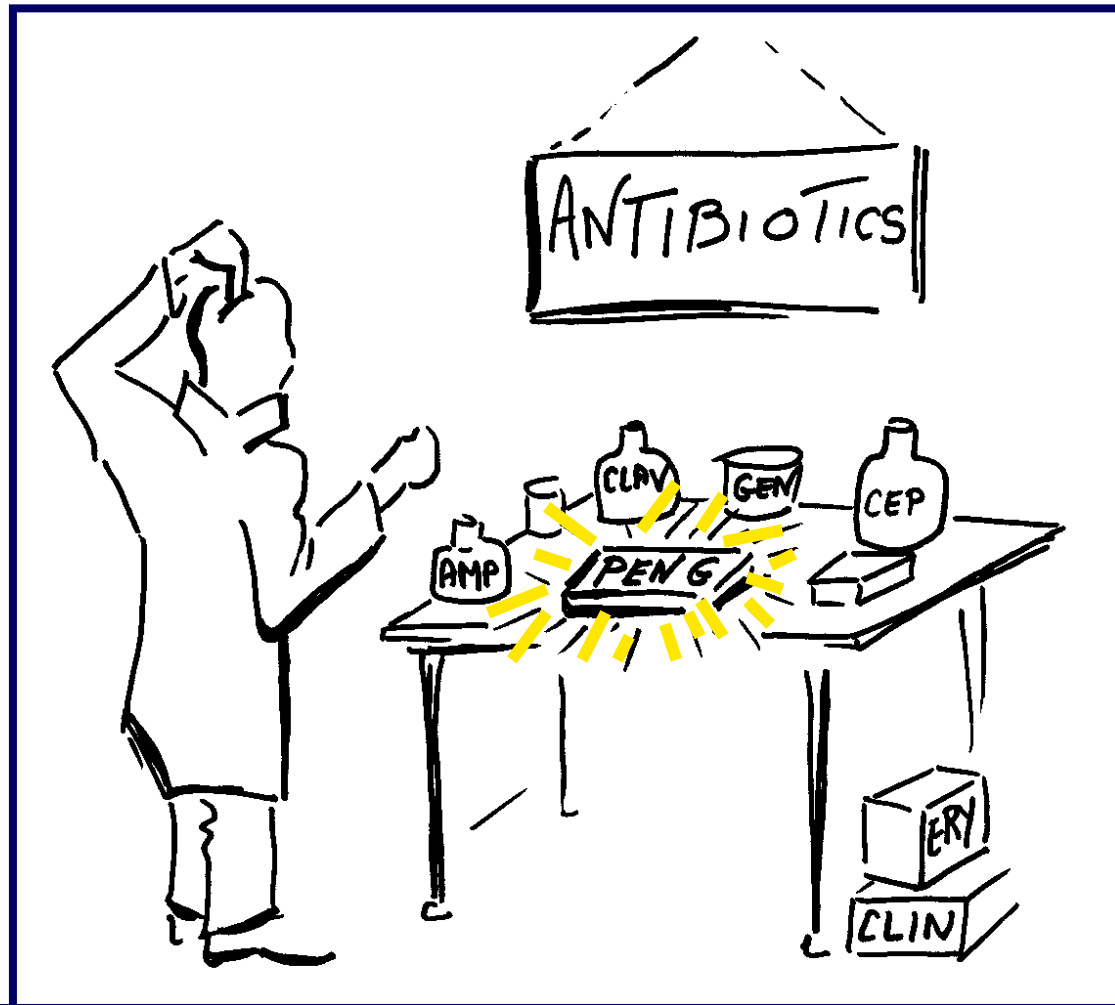
- **Xpert™ GBS**
 - Sensitivity : 92 %
 - Specificity : 95.6 %
 - PPV : 86.7 % and NPV : 97.4 %

Surpass sensitivity of antenatal cultures
Sensitivity // inoculum density = real time risk

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 ?

AB agent	IAP	Therapy	Resistance
Penicillin	X	X	“No”, but ↗ MIC
Erythromycine	X	X	10 - 30 %
Clindamycin	X	X	Up to 20 %
Vancomycin	(X)	(X)	No
Fluoroquinolone		(X)	Few cases
Aminoglycoside		(X)	No HLR

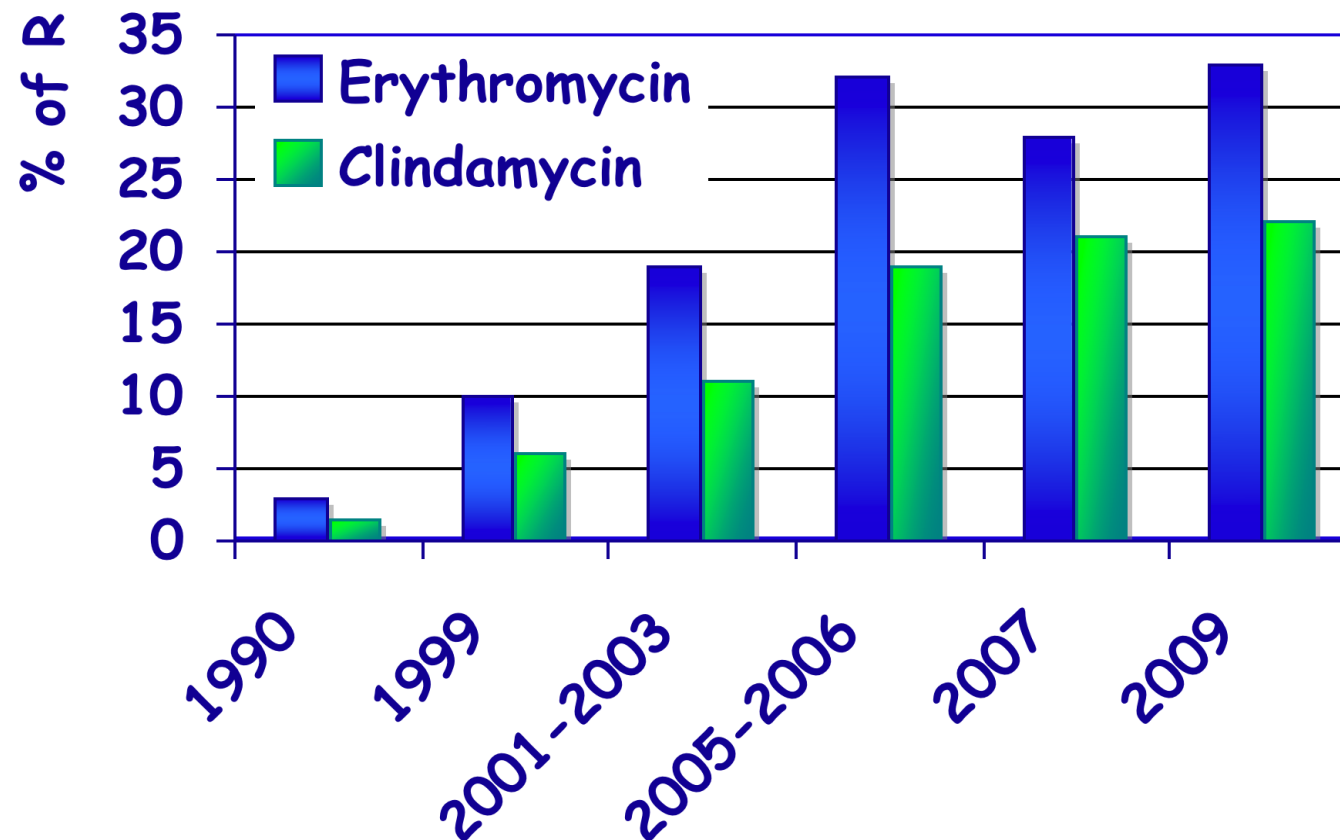
Susceptibility to penicillin

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

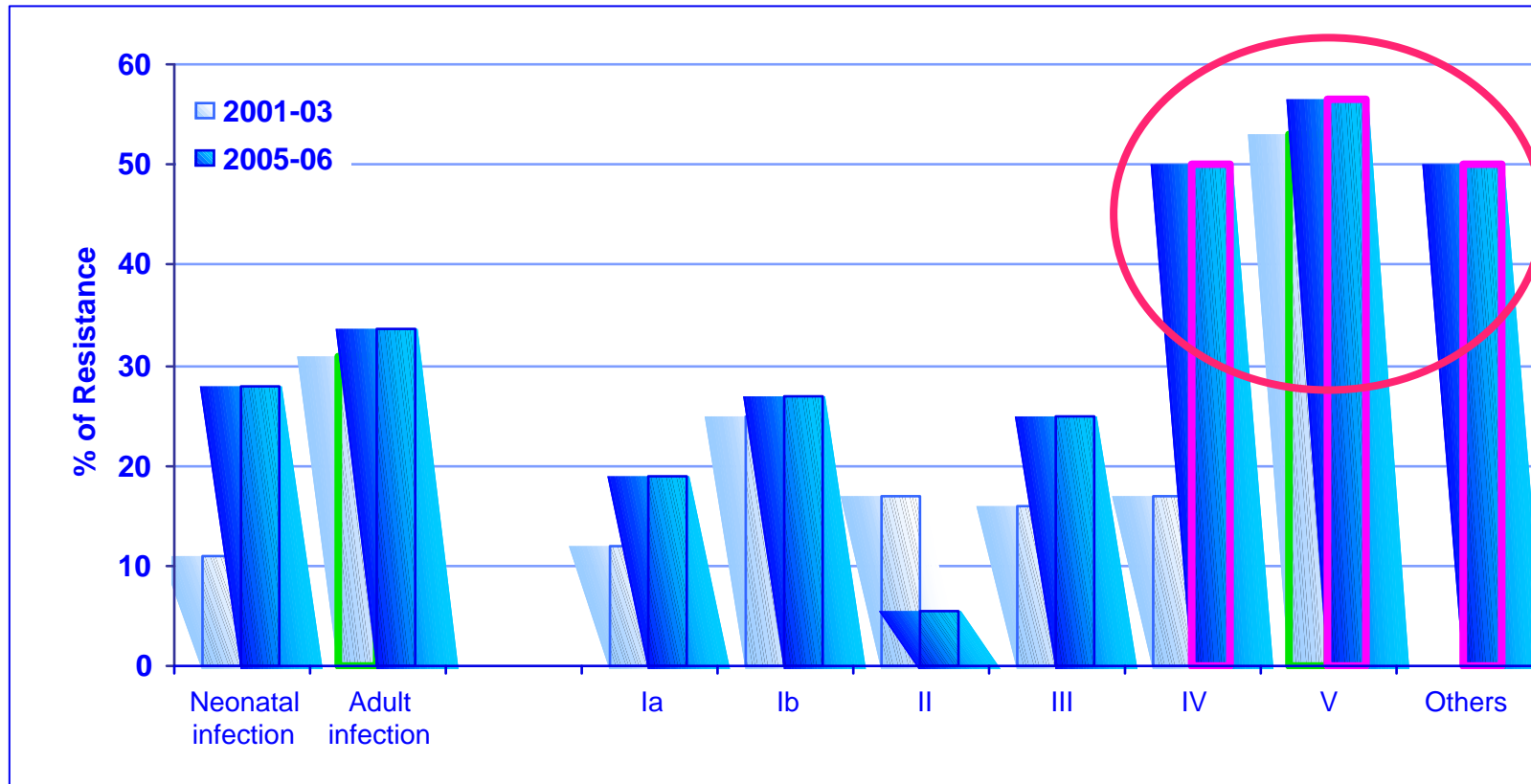
Noriyuki Nagano et al, AAC 2008

- **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



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*

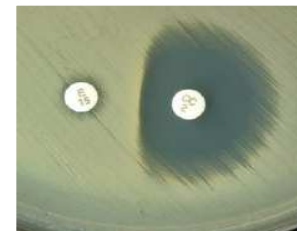
MLS Resistance phenotypes

D-test recommended

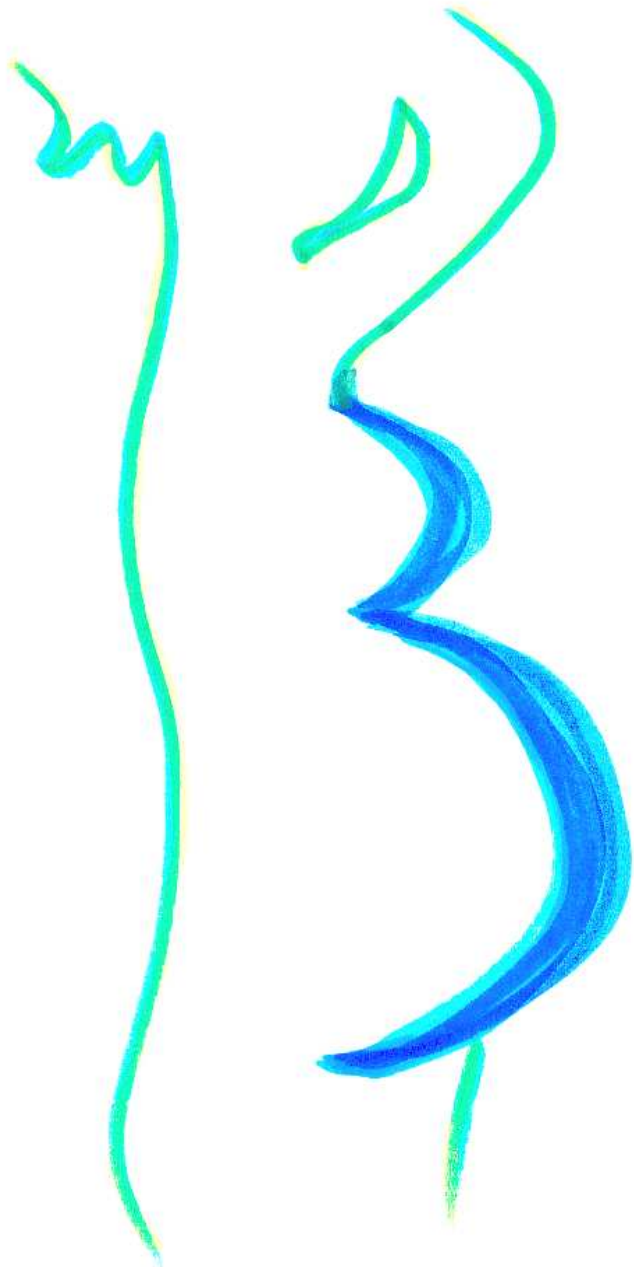
Phenotype	%	Ery MIC ₅₀ / MIC ₉₀ (mg/L)
MLS <i>Constitutive</i>	45	>256 / >256
<i>Inducible</i>	34	4 / >256
M	21	4 / 12

P.Melin, LISSSD 2008 P215

- **Dtest**
 - cMLS Erythro R & Clinda R
 - iMLS Erythro R & Clinda S//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



**Prevention
strategy for
prevention of
GBS EOD**

**Adhesion to a common protocol is a key for success
Multidisciplinary collaboration is mandatory**