ANTIMICROBIAL SUSCEPTIBILITIES OF RECENT CLINICAL ISOLATES OF GROUP B STREPTOCOCCI (GBS) FROM BELGIUM

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ABSTRACT

Background: : GBS cause severe infections in neonates, pregnant women and other adults. Empiric therapy is usually started before susceptibility results are available. Early neonatal diseases can be prevented with intrapartum antibiotic prophylaxis based on accurate susceptibility surveillance data. A previous Belgian study showed an increase of 3 to 10 % R to erythromycin (EM) through the 1990s.

Methods: 187 GBS isolates consecutively received at the reference laboratory between 2001 to March 2003 were from 73 neonates (52 early-onset and 21 late-onset diseases), 52 adults and 62 from pregnant women's vagina. MICs of penicillin (PG), EM, clindamycin (CM) and gentamicin (GM) were determined with Etest. PG MBCs were also determined by inactivating the drug in MIC plates using beta-lactamase. EM resistant (R) isolates were tested by the CM + EM double disk to determine macrolide R phenotypes.

Results: All strains were susceptible (S) to PG and no tolerance was observed with MBCs falling within 2 dilutions of MICs. 19.2% of isolates were R to EM, with significantly more R isolates from adults (30.8%; p <0.01) and serotype V (46.8%; p <0.001). 80% had the MLS_B phenotype (R to EM and CM), 16 were constitutive and 12 inducible. The M phenotype (R to EM and S to CM) was seen in 7 (20%) of isolates. Less than 10% of isolates were inhibited by GM MIC of <=64 mg/L. 83.6% by 128-256 mg/L and 2.9% by >/=512 mg/L. Non typable strains were more R to GM (p <0.01).

Conclusions: 1) PG remained active against all isolates and no tolerance was seen. 2) Prevalence of R to macrolides had increased since 1999, particularly in adult isolates and serotype V. 3) Intermediate to high level R to GM was seen and potential synergy of PG + GM should be investigated. 4) R surveillance is mandatory to guide prophylaxis and treatment of serious GBS infections.

BACKGROUND

Group B streptococcus (GBS) or *Streptococcus agalactiae* continue to be a major cause of life-threatening infections, sepsis, pneumonia and meningitis in neonates. GBS have been also recognized as an important cause of invasive disease in pregnant and non-pregnant adults.
Empiric therapy is usually started before susceptibility results are available. Based on accurate susceptibility surveillance data, penicillin G, for its bactericidal activity and narrow spectrum, remains the agent of choice for intrapartum prophylaxis to prevent early neonatal diseases. Several years ago, the penicillin tolerance phenomenon (MBC/MIC >16) was reported, but nothing has been documented in defined studies. In the penicillin allergic patients, clindamycin or erythromycin have been recommended as alternative drugs. However, probably as a consequence of the important use of erythromycin, macrolides and related drugs-resistance among streptococcal isolates is currently becoming recognized in many countries. In 1999, among clinically significant isolates of GBS collected in different Belgian areas, the prevalence of erythromycin resistance fluctuated between 10 and 20 %, while in the early 1990s only 3% were resistant.

OBJECTIVES

To monitor susceptibility profiles of GBS causing infections in different patients' age-groups or colonizing pregnant women.

- **Determination of distributions of MICs for penicillin G, erythromycin, clindamycin and gentamicin**
- **Determination of macrolide resistance phenotypes**
- **Investigation of tolerance to penicillin**

MATERIAL & METHODS

Strains

◆ A total of 187 clinical isolates collected in laboratories belonging to the national surveillance network, and sent to the Belgian reference laboratory for GBS between January 2001 and March 2003 (see table1). All strains are serotyped and stored in skimmed milk at -80℃.

Table 1: Description of the 187 clinical isolates of GBS : age groups and diagnostics

Patients	Diagnostic	Number (%)
Neonates	Early Onset Disease (EOD) Late Onset Disease (LOD)	73 (71.2) (28.8)
Pregnant and non-pegnant adults	Severe invasive infections	52
Pregnant women	Recto-vaginal colonization	62

Determination of MICs

◆ Etest® method (AB Biodisk, Sweden) with benzylpenicilline (PEN), erythromycin (ERY) , clindamycin (CL) and gentamicin (GN).
◆ Inoculum 0.5 McFarland on Mueller-Hinton agar + 5% sheep blood
◆ Incubation 18-24h at 35℃
◆ MIC resistance breakpoints according to NCCLS January 2002

Determination of macrolide resistance phenotypes

◆ ERY and CL double-disk and double-Etest® diffusion asays
◆ ERY (15 µg) and CL (2 µg) Disks (Becton Dickinson, USA) or Etest strips (AB Biodisk, Sweden) placed 15-20 mm apart on inoculated Mueller Hinton agar + 5% sheep blood ; 18-24 h incubation at 35℃.

Determination of MBCs to penicillin

◆ After reading the MIC (as above), a high concentration of β-lactamase suspension (β -lactamase de *B.cereus* BS3, ULg, Belgium) was sprayed twice onto PEN MIC plates.
◆ These plates were reincubated overnight before reading the MBC

RESULTS

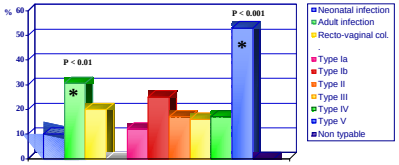
◆ Antimicrobial susceptibility profile

Table 2: Antimicrobial susceptibility profile of 187 clinical isolates of GBS among different groups of patients (January 2001 - March 2003)

Antimicrobial Agent	Percentage of Resistance				MIC90 (mg/L)	(range)
	Neonates	Adults	Vaginal colonization	Overall		
Penicillin	0	0	0	0	**0.06**	(0.03 - 0.09)
Erythromycin	11	30.8	19.3	19.2	**32**	(0.12 - >256)
Clindamycin	8.2	19.2	6.5	10.7	**0.75**	(0.094 - >256)
Gentamicin					**192**	(24 - 512)

◆ Erythromycin Resistance

Figure 1 : GBS Resistance to erythromycin among different groups of patients and per serotype



◆ Macrolide resistance phenotypes

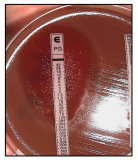
Table 3 : Distribution of macrolide resistance phenotypes among GBS resistant to ERY

Phenotype		Cross R with CL	% of ERY-R GBS
MLS	*Constitutive*	+	46
	Inducible	+	34
M		-	20

◆ GBS tolerance to penicillin

No tolerance was observed :

- 79.3 % of isolates had an MBC/MIC = 1
- 19 % of isolates had an MBC/MIC = 1.5
- 1.7 % of isolates had an MBC/MIC = 2



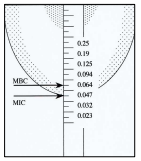


Figure 2 : Etest® assay for tolerance to penicillin

DISCUSSION AND CONCLUSION

- **As in the different surveillance studies of GBS antimicrobial susceptibilities, all isolates remain fully susceptible to Penicillin. Furthermore, with MBC/MIC ≤ 2, no tolerance phenomenon was observed. The Etest® assay combined with β-lactamase, a new procedure to investigate tolerance to β-lactams was easy to perform. With a low workload, it allowed testing of numerous isolates in a short time.**
- **The increase of resistance to macrolides becomes a relevant problem. In the 1990s, ERY-R increased in Belgium from 3 to 10%. For the last 2 years, R rate increased significantly, particularly in GBS serotype V and in isolates from adult infections. But serotype V isolates, in Belgium, are more frequent among isolates from adult. As already reported in the 1990s, most of ERY-R isolates had a MLS phenotype. Neither macrolides no lincosamides should be recommended anyway for empiric therapy of GBS infections.**
- **In 1998, Fleiter et al reported 8% of high level R (HLR) to aminoglycosides among clinical isolates of GBS. In Belgium, no HLR to GN was reported in the 1990s. In this study, some strains showed a relatively high resistance to gentamicin. Therefore, the potential synergy of PEN + GN should be investigated.**
- **Resistance surveillance to all classes of potentially useful antibiotics is mandatory to detect drifts in susceptibility and to constantly validate empiric regimens and for targeting individual therapy in severe GBS infections.**

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