PREVALENCE OF *ermB*, *ermTR* AND *mefA/B* GENE CLASSES AMONG ERYTHROMYCIN-RESISTANT GROUP B STREPTOCOCCUS (GBS) ISOLATES COLLECTED IN BELGIUM

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ABSTRACT

Background: Emergence of erythromycin (Er) and clindamycin (C) resistance (R) observed in GBS, is currently becoming recognized.

Methods: Clinical isolates were obtained from a Belgian surveillance for invasive GBS disease in newborns and adults in 1996-1998 (N1=235) and from consecutive specimens submitted, during 1999-2000, to the University hospital of Liege (N2=165). MICs of Er were determined buy using Etest® strip (interpretive criteria of NCCLS). Furthermore, for the ErR isolates, the inducible (iMLS), constitutive (cMLS) and M phenotypes were assessed by disk diffusion and by a double-disk test; the distribution of genes encoding RNA methylases and efflux pumps was investigated by PCR.

Results: Of the N1 and N2 isolates, 16 (6.8%) and 19 (11.5%) were respectively R to Er. Among these 35 ErR isolates, 21 (60%) exhibited the cMLS phenotype. They demonstrated a high level R to Er with MICs ranging from 16 to >256 mg/L. The *erm*B gene was harbored by 19/21 isolates, the *erm*TR gene by 1 isolate and both *erm*B and *erm*TR were present in another isolate. The iMLS phenotype was observed in 10 (29%) ErR isolates; the *erm*TR gene was present in all isolates except one harboring an *erm*TR gene. These strains demonstrated low level of R to Er, with MICs of 1-12 mg/L. All 4 isolates (11%) expressing an M phenotype, displayed low level R to Er alone (MICs, 2 mg/L) and were positive for the *mef*A/B gene.

Conclusion: In Belgium, by year 2000, prevalence of R to macrolide in GBS exceeded 10%. R was mainly caused by target-site modification (*erm*B, *erm*TR) mechanisms; efflux (mefA/B) R mechanism was also prevalent among the isolates tested. These results indicate the possibility of inappropriate prophylaxis or therapy using C or E as the recommended alternatives in penicillin-allergic patients.

BACKGROUND

Group B streptococci (GBS) or *Streptococcus agalactiae* are the leading bacterial cause of meningitis and sepsis in neonates. Penicillin is the treatment of choice for these infections or for intrapartum prophylaxis, but erythromycin and clindamycin are effective recommended alternatives in the penicillin allergic patients.

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 *S.agalactiae* collected in different areas of Belgium, the prevalence of erythromycin resistance fluctuated between 10 and 20 %.

Different known mechanisms account for acquired resistance to macrolides in streptococci as the target site modification by 23S rRNA methylases, encoded by *erm* genes. The Erm enzymes confer cross-resistance to macrolides, lincosamides and the streptogramin B compounds, so-called MLS_B phenotype. MLS-resistance may be inducible or constitutive. Another mechanism involving an active efflux, only affects 14- and 15-membered macrolides but not 16-membered macrolides, neither lincosamides or streptogramins (M phenotype) and is encoded by *mef* genes.

OBJECTIVES

To determine the distribution of macrolide resistance phenotypes and to identify the resistance genes, *erm*B, *erm*TR and *mef*A/B among erythromycin-resistant *S.agalactiae* isolated in Belgium.

MATERIAL & METHODS

Strains

Clinical isolates: by comparison to the abstract, results presented have been extended to a larger population of strains.

Description of 561 strains of GBS from which Erythromycin-resistant isolates were selected

	Collection 1	Collection 2
Period	1996-1998	01.1999-06.2001
Number	235	326
Laboratory	Belgian GBS reference laboratory	University Hospital of Liege
Origin	Early or late onset neonatal diseases and adult invasive diseases	Clinically significant isolates from consecutive specimens, neonates excluded
Screening method for erythromycin resistance	Dilution in agar/NCCLS	Broth microdilution by Vitek (bioMérieux) / NCCLS interpretative criteria

For all isolates categorized resistant to erythromycin, MICs were further determined as described below.

Reference strains for specific genes, GBS previously characterized, belonging to the collection of the Belgian GBS reference laboratory: N° 1546 and 1628 for *erm*B gene, N° 97123 for *erm*TR gene, N° 167 for *mef*A/B gene and N° 1732, 1734, 1747, 1745 and 1747 as negative controls.

Determination of erythromycin and clindamycin MICs

- Etest® method (AB Biodisk, Sweden).
- Inoculum 0.5 McFarland on Mueller-Hinton agar + 5 % sheep blood
- Incubation 18 h at 35°C
- MIC resistance breakpoints: ≥ 1 mg/L, NCCLS 2000.

Determination of resistance-phenotypes

- Double-disk and double- Etest® diffusion assays
- Erythromycin 15 µg paper disks, clindamycin 2 µg paper discs or Etest® strips (BBL, Becton Dickinson and Company, NN USA and AB Biodisk, Sweden).
- Disks or Etest® strips placed 15-20 mm apart on inoculated Mueller-Hinton agar + 5 % sheep blood ; 18-24 h incubation at 35°C
- ***MLS_B phenotype:***
  - ***Inducible resistance (IR):*** blunting of the clindamycin zone of inhibition proximal to the erythromycin disk or Etest® strip, or "D shape"
  - ***Constitutive resistance (CR):*** resistance to clindamycin with no blunting of the clindamycin zones of inhibition
- ***M phenotype:*** susceptibility to clindamycin with no blunting of the clindamycin zones of inhibition

Identification of resistance-genes

Protocol of PCR using gene specific primers for known macrolide resistance markers

Targets (Oligonucleotide references)	Initial denaturation	Number of cycles	Denaturation	Annealing	Extension	Final extension	Product sizes (bp)
erm B (2)	At 94°C for 5 min	30	At 94°C for 60 s	At 54°C for 60 s	At 72°C for 60 s	At 72°C for 5 min	640
erm TR (3)		35	At 94°C for 30 s	At 45°C for 90 s			530
mef A/B (2)		30	At 94°C for 60 s	At 52°C for 60 s			348

Thermocycler : GeneAMP PCR system 2400 (Perkin Elmer).

Following amplification, the product was visualized by electrophoresis on 2% agarose gel by ethidium bromide staining.

RESULTS

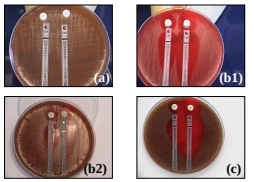
Respectively, 16/ 235 (6.8 %) and 34/326 (10.4 %) isolates from collections 1 and 2, were resistant to erythromycin.

MLS resistant mechanisms were phenotypically and genetically characterized for the 50 erythromycin resistant isolates.

Distribution of resistance phenotypes

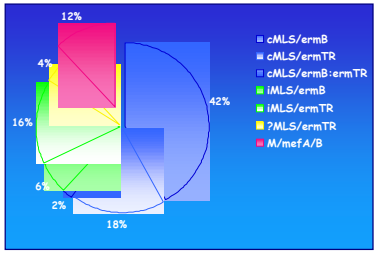
Phenotypes	*No (%) Isolates*	*Figures*
cMLS	31 (62)	(a)
iMLS	11 (22)	(b1, b2)
?MLS	2 (4)	
M	6 (12)	(c)

Erythromycin-R phenotypes of GBS isolates determined by double-disk test and with Etest strips. Clindamycin on the right and Erythromycin on the left. (a) constitutive resistance: cMLS, (b1) and (b2) inducible resistance: iMLS and (c) erythromycine resistance alone: M

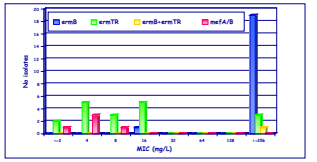


Distribution of resistance genes within the different phenotypes

Distribution of the ermTR, ermB and mefA/B genes within the different erythromycin-resistance phenotypes of 50 GBS isolates



Correlation of Erythromycin MICs with genotypes



CONCLUSIONS

- In Belgium by year 2000, prevalence of macrolide-resistance among *S.agalactiae* isolates exceeded 10 %.
- Resistance was mainly caused by target-site modification (*erm*B, *erm*TR) mechanisms. These isolates demonstrated MLS phenotypes, either constitutive or inducible.
- The presence of two *erm* genes could be demonstrated in one isolate.
- Efflux (*mef*A/B) resistance mechanism was also prevalent among *S.agalactiae* isolates. These isolates demonstrated a M phenotype.
- Erythromycin MICs were well correlated with genotypes. The high level of Erythromycin-resistance for some *erm*TR isolates could be due to an unknown resistance gene.
- These results indicate the possibility of inappropriate prophylaxis or empiric treatment using clindamycin or erythromycin as recommended alternatives in penicillin-allergic patients.
- Because of these data, routine susceptibility testing at least for erythromycin and continuing surveillance of *S.agalactiae* macrolide-R are advisable.

References

1. NCCLS, 2000 - Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - Fifth edition. NCCLS document M7-A5
2. Kataja J et al, 2000 - Erythromycin resistance genes in group A streptococci in Finland. AAC, 43: 48-52
3. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L, 1996 - Detection of erythromycin-resistant determinants by PCR. AAC, 40: 2562-6

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