SURVEILLANCE OF SEROTYPES AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE IN GROUP B STREPTOCOCCUS (GBS) IN BELGIUM

ICAAC 2013 53rd ICAAC | SEPT 10-13 | Denver, CO

Université de Liège

B-500

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REVISED ABSTRACT *(increase of studied population)*

Background Today GBS vaccines for prevention of severe neonatal disease through transplacental delivery of antibodies directly from immunized mothers are in advanced stage of development. For the introduction of any GBS vaccine there are urgent needs for pre and post vaccine enhanced surveillance studies of strains isolated from both neonatal diseases and vagino-rectal colonization of pregnant women. In Belgium, surveillance of invasive isolates is regularly done by the NRC. We report in this study a surveillance of colonizing isolates of GBS.

Methods In 2012, 382 GBS isolates were obtained from a Belgian surveillance for vagino-rectal colonization among pregnant women (max. 5 isolates/lab). Capsular types were determined by agglutination (Strep-B-latex, SSI, Denmark) and genotyping (PCR), and MICs by using a microdilution method (Sensititre) and Etest® (EUCAST interpretive criteria). Furthermore, for the erythromycin (E) resistant (R) isolates, the inducible (iMLS), constitutive (cMLS) and M phenotypes were assessed by a double-disk diffusion test.

Results Serotype III was the more common (29.3%) followed by V, Ia, II, Ib, IV, VI, VII and IX (19.9%, 17.8%, 17.8%, 8.1%, 5.2%, 0.3%, 0.3%, 0.3%) and 1% were non typable. All isolates were susceptible to penicillin ; 29% were R to E with a higher rate among serotypes IV and V ($p<0.05$). Among these E-R isolates, 92% exhibited the MLS phenotype (R to E and CC): 53% were cMLS with E MIC_{50}>256 mg/L and 39% iMLS with E MIC_{50}/MIC_{90} 2/>8 mg/L. The M phenotype (R to E and S to C) was expressed by 8% of E-R isolates with E MIC_{50}/MIC_{90} 2/4 mg/L.

Conclusions Compared with Belgian data relating to neonatal invasive strains (NRC reports) 1) Serotype V and II are more frequent and III less frequent among colonizing isolates 2) Prevalence of E-R is similar in percentage and phenotypes with the MLS R phenotype as major mechanism. Extended surveillance of both invasive and colonizing isolates is needed currently to prepare the follow-up in the future vaccine era..

BACKGROUND

From the 1990s to the present, where guidelines for prevention of perinatal GBS disease have been widely implemented, the incidence of neonatal early onset disease (EOD) has dramatically decreased to <0.5 cases per 1,000 live births but has not been eradicated and continues to be an important cause of neonatal sepsis and meningitis. There are numerous prevention strategies at this time but none are 100% effective in the eradication of neonatal GBS EOD and there are no preventive strategies for late onset disease (LOD). Development of a group B streptococcal vaccine is the most promising approach for the prevention of severe GBS neonatal disease through transplacental delivery of antibodies directly from immunized mothers. Pre and post vaccine enhanced surveillance studies of GBS strains isolated from both neonatal diseases and vagino-rectal colonization of pregnant women are of critical importance.

For the treatment of GBS infections or for intrapartum chemoprophylaxis penicillin is the first line antibiotic and for penicillin allergic patients clindamycin is an effective recommended alternative. Empiric therapy of severe GBS infections, initiated before availability of susceptibility results, and intrapartum chemoprophylaxis to prevent GBS EOD are based on accurate susceptibility surveillance data. But globally, resistance to macrolides and lincosamides has increased among GBS isolates over the last two decades: from less than 5% to common resistance of 20% to 30%. Different known mechanisms account for acquired resistance to macrolides in streptococci. The most prevalent of these is target site modification by 23S rRNA methylases. These enzymes confer resistance to macrolides and inducible or constitutive resistance to lincosamides and streptogramin B, so-called MLS_B phenotype. Another mechanism, involving active drug efflux, the Mef pump affects 14- and 15- membered ring macrolides but not 16-membered macrolides, neither lincosamides nor streptogramin B (M phenotype). Apart from these worrying resistances, another phenotype involving low-level of clindamycin resistance (with erythromycin remaining susceptible) in GBS isolates has recently been reported in many countries: the L phenotype.

In Belgium, the NRC routinely performs surveillance of invasive GBS isolates, but for strains isolated from colonized pregnant women, epidemiological surveys on strains isolated in labs from a national network or limited to regional area are organized periodically. We report in this study a surveillance of colonizing isolates of GBS: distribution of capsular serotypes and antimicrobial susceptibility profile.

METHODS

Clinical isolates from colonized pregnant women

In 2012, all Belgian laboratories were invited to send to the National Reference Centre (NRC) for GBS five consecutive *S.agalactiae* isolated from vagino-rectal specimens for further characterization and epidemiological purposes. A total of 382 isolates of GBS were included in this study.

Upon reception, strains were subcultured, identification was confirmed and they were stored in skimmed milk at -80°C before further testing.

Control strains

A set of GBS strains with characterized phenotypes and genetotypes, belonging to the collection of the NRC for GBS were used as reference strains for quality control.

- Ten reference GBS strains, one for each capsular types Ia, Ib-IX.
- Four GBS strains representing the different macrolide-lincosamide resistant phenotypes.

Determination of capsular types

Serotyping

All strains were submitted to serotyping by agglutination with Strep-B-Latex (SSI, Denmark) using a micro-method according to Afschar et al (2011).

Genotyping

All strains that were non-typable or had a weak positive reaction by the agglutination method were additionally tested using multiplex PCR based on the detection of GBS capsular polysaccharide genes according to Poyart (2007) and Kong (2008).

Determination of MICs

All isolates were submitted to determination of MICs by the microdilution method using Sensititre specific panels, customized for the Belgian NRC for GBS.

Interpretation of MICs according to "Breakpoints tables for interpretation of MICs and zone diameters" edited by EUCAST (V2.0, 2012).

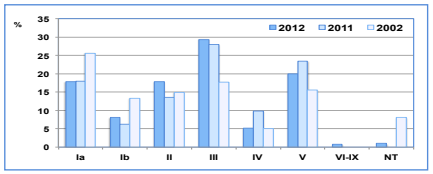
Determination of macrolide resistance phenotypes

- All strains showing resistance to erythromycin and/or clindamycin were submitted to a Dtest:
  - Erythromycin (15 mg) and clindamycin (2 mg) double-disk diffusion assay.
  - Disks placed 18 mm apart (edge to edge) on inoculated Mueller Hinton agar + 5% sheep blood ; 18-24 h incubation at 35°C.
  - Interpretation:
    - *MLS_B phenotype*
      - ***Inducible resistance*** *(iMLS)*: resistance to erythromycin and blunting of the clindamycin zone of inhibition proximal to the erythromycin disk or "D shaped zone"
      - ***Constitutive resistance*** *(cMLS)*: resistance to both erythromycin and clindamycin
    - *M phenotype*: resistance to erythromycin and susceptibility to clindamycin with no blunting of the clindamycin zone of inhibition.
    - *L phenotype*: susceptibility to erythromycin and resistance to clindamycin.

RESULTS AND DISCUSSION

Distribution (%) of capsular types of GBS

Figure 1: Distribution of types among 382 clinical isolates of GBS colonizing pregnant women (2012) compared to distribution of 111 (2011) and 322 (2002) GBS colonizing pregnant women.



The distribution observed in 2002 is based on serotyping alone while genotyping was added for strains collected in 2011 and 2012. Since 2002, the distribution has evolved with a decrease of the ratio of types Ia and Ib in favor of types III and V ($P<0.001$).

Antimicrobial susceptibility profile

Table 1: Antimicrobial susceptibility profile of 382 clinical isolates of GBS colonizing pregnant women (2012)

	% of Resistance	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC Range (mg/L)
Penicillin	**0**	**0.06**	**0.12**	**0.03 - 0.25**
Erythromycin	28.6	<0.06	128	<0.06 - >256
Clindamycin	26.7	<0.06	128	<0.06 - >256
Telithromycin	**2.6**	**<0.06**	**0.25**	**<0.06 - 8**
Levofloxacin	**2.0**	**1**	**1**	**<0.12 - 4**
Moxifloxacin	**2.6**	**0.25**	**0.25**	**<0.12 - 4**
Tetracycline	**86.9**	**32**	**64**	**<0.12 - 64**

All strains were susceptible to penicillin but the range of determined MICs extended up to 0.25 mg/L. Penicillin susceptibility requires specific attention and follow up to look for emergence of strains with reduced susceptibility.

For erythromycin/clindamycin, resistance was common and the observed rates of resistance, 28.6% and 26.7 %, are consistent with Belgian GBS data even if slightly lower than the 33% and 31.3 % reported for clinical GBS strains isolated from invasive diseases (Belgian Edition of the Sanford Guide 2012).

Resistance to fluoroquinolones was identified among 2-3% of GBS isolated from colonized pregnant women which is much lower than the Asian rates as the 38% reported in China (Wang et al, 2013).

The high resistance to tetracycline is stable since several decades as it is globally.

Macrolide resistance phenotypes

Table 2: Distribution of macrolide resistance phenotypes among 111 isolates of GBS resistant to erythromycin and/or resistant to clindamycin.

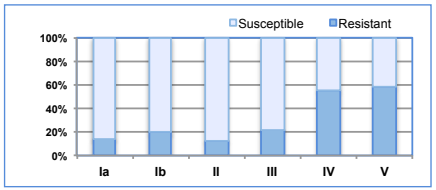
Phenotypes		%	Ery MIC_{50} / MIC_{90} (mg/L)
MLS	*Constitutive*	**52.2**	**128 / >128**
	Inducible	**37.8**	**4 / 128**
M		**8.1**	**2 / 4**
L		**1.8**	**0.12 / 0.12**

For erythromycin/clindamycin, the observed distribution of the different phenotypes of resistance among GBS isolated from colonized pregnant women is consistent with Belgian GBS data reported for clinical GBS strains isolated from invasive diseases (Belgian edition of the Sanford Guide 2012). The main mechanism is a target site modification, constitutive or inducible, among 90% of the resistant strains.

The L phenotype, described initially in South America, Australia and New Zealand, has been identified among 1.8% of isolates of this collection and has also been observed since 2010 in very few strains isolated from bacteriuria (CNR unpublished data).

Macrolide resistance among different capsular types

Figure 2: Rate of resistance to erythromycin among the 6 main capsular types of 364 isolates of GBS colonizing pregnant women (2012).



The observed rates of resistance to erythromycin showed relationships between resistance to erythromycin and capsular type. Erythromycin resistant strains were mostly types IV and V ($P<0.001$). Erythromycin resistance was 2.8 to 4.5 times more likely to occur in type IV and V isolates (55 and 58%) than in other serotypes.

OBJECTIVES

GBS vaccination era approaching, this study focusing on the distribution of circulating capsular serotypes among GBS isolated from colonized pregnant women and on their antimicrobial susceptibility patterns, was designed to determine a Belgian reference baseline. It should help to understand potential epidemiological changes among GBS isolated from severe neonatal diseases and from colonized pregnant women following GBS vaccine introduction.

CONCLUSION

- **Some evolution was showed in the distribution of capsular types of GBS : increase of types III and V.**
- **All tested strains of GBS isolated from pregnant women were susceptible to penicillin, the first line agent recommended for intrapartum prophylaxis (IAP) and therapy.**
- **On the other hand, prevalence of resistance to antibiotics commonly used for prophylaxis of GBS infection in penicillin allergic patients was found. GBS resistant to clindamycin and erythromycin was common.**
  - **High rates of erythromycin resistance are particularly associated with serotypes IV and V.**
  - **As previously reported in Belgium or in other European countries, most of erythromycin resistant isolates showed a MLS phenotype.**
  - **Clindamycin resistance without concurrent erythromycin resistance can be observed rarely.**
- **These findings are consistent with previous Belgian reports and support 2003 Belgian guidelines / CDC 2010 guidelines requiring susceptibility testing of isolates from IAP candidates with penicillin allergy and the need for regular epidemiological surveys, especially after introduction of GBS vaccines.**

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