Multiple-Clones of Group-B Streptococci Clinical Isolates with an Unusual Erythromycin-Susceptible and Clindamycin-Resistant Phenotype

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INTRODUCTION

Group-B Streptococci (GBS) is a common cause of neonatal diseases such as sepsis and meningitis. Macrolides are the recommended second-line agents and therapeutic alternative for mothers with a penicillin allergy.

Through the National Surveillance (WHO-ARG, 70 Hosp) we detected an increase in the resistance to erythromycin (ERY) and clindamycin (CLI) from 7.2% and 3% in 2005 to 12.8% and 5.7% in 2007.

Resistance to lincosamides in GBS is most commonly mediated by Erm-type methylases, but ribosomal mutations and Lnu-type nucleotidyl-transferases were also described. LnuB enzyme was only described in 3 GBS worldwide, 2 from Canada and 1 from the US.

During 2006-2008, six GBS expressing an unusual ERY susceptibility and CLI resistance (L-phenotype) by disc diffusion were submitted to the National Reference Laboratory (INEI) for molecular characterization.

OBJECTIVE

The objective of this work was to characterize the mechanism of resistance and to evaluate the relationship between these isolates.

MAT & METH

Clinical Strains. Six GBS from 3 hospitals (PYR, RAW and FER) from 3 different cities displaying an L-phenotype were recovered from recto-vaginal screening culture. Isolates were submitted to the National Reference Laboratory (INEI) for further molecular characterization.

Susceptibility assays. Disc diffusion and Minimal Inhibitory Concentrations by agar dilution method was performed and interpreted according CLSI guidelines.

PCR amplification. PCR was performed under standard conditions and detection of *ermA*, *ermB*, *lnuA*, *lnuB* and *lnuC* genes was carried out using primers described (Bozdogan B. AAC 43:925-9, 1999; Lina G. AAC 43:1062-6, 1999; Sutcliffe J. AAC 40:2562-6 1996).

DNA sequencing. *lnuB* gene was sequenced using BigDye terminator system.

Conjugation assay. *S. agalactiae* M6395 (susceptible to macrolide and lincosamides but resistant to fluoroquinolone) and *Staphylococcus aureus* RN4220 were used as recipient strains. A ratio 1:5 of donor:acceptor strains were assayed. Selection plates of BHI agar plus blood were incubated in CO_2 ambient.

Molecular typing. Genomic DNA was digested with *Apa*I enzyme and DNA fragments were separated on 1% agarose gel at 6 V/cm during 20 h and using 2 and 20 sec as initial and final switch time respectively. PFGE DNA patterns were analyzed using Tenover's criteria (Tenover F., et al. JCM (1995) 33:2233-9).

FIGURE 1.

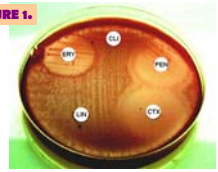

Figure 1. Disc diffusion assay of a representative SGB, M6642, showing susceptibility to PEN, CTX and ERY, and resistance to CLI and LIN.

TABLE.

Isolate	Disk Diffusion Interpretation									MIC (µg/ml)								Resistance Phenotype
	PEN	CTX	TET	OFX	LVX	VAN	ERY	CLI	LIN	ERY		AZM		LIN		CLI		
M6390	S	S	I	S	S	S	S	R	R	0,06	S	0,25	S	64	R	4	R	L
M6637	S	S	I	S	S	S	S	R	R	0,12	S	0,25	S	128	R	4	R	L
M6639	S	S	S	S	S	S	S	R	R	0,06	S	0,25	S	64	R	4	R	L
M6640	S	S	S	S	S	S	S	R	R	0,12	S	0,25	S	128	R	4	R	L
M6641	S	S	I	S	S	S	S	R	R	0,12	S	0,25	S	128	R	4	R	L
M6642	S	S	S	S	S	S	S	R	R	0,12	S	0,25	S	128	R	4	R	L

Antibiotics tested: penicillin (PEN); cefotaxime (CTX); tetracyclin (TET); ofloxacin (OFX); levofloxacin (LVX); vancomycin (VAN); erythromycin (ERY); azythromycin (AZM); clindamycin (CLI); lincomycin (LIN). L-Phenotype: ERY-susceptible and CLI-resistant.

RESULTS

- All six GBS were susceptible to β-lactams (penicillin and cefotaxime), fluoroquinolones (ofloxacin and levofloxacin), and vancomycin. Three isolates were intermediate to tetracyclin (Table).
- By disc diffusion the strains showed susceptibility to erythromycin but not zone for clindamycin and lincomycin (Figure 1).
- GBS isolates were susceptible to ERY (≤ 0.12 mg/L) and azithromycin (≤ 0.25 mg/L), and resistant to CLI (4 mg/L) and lincomycin (64-128 mg/L).
- All six isolates were positive for *lnu*B gene, and negative for Erm-methylases (*erm*A and *erm*B) and others *lnu* genes (*lnu*A and *lnu*C).
- *lnu*B sequence was confirmed by sequencing.
- Conjugation assays using *S. agalactiae* and *Stahpylococcus aureus* as recipient strains were unsuccessfull.
- Four clones were discriminated using *Apa*I-PFGE: Clone A (3 strains) was detected in FER hospital, and the remaining were from PYR (clone B), RAW (clone C) and FER (clone D) hospitals (Figure 2).

FIGURE 2.
ApaI-PFGE

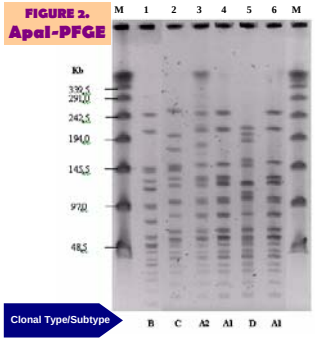

Figure 2. *Apa*I-PFGE of *S. agalactiae* genomic DNA. Lanes M, PFG marker; line 1, M6390; 2, M6637; 3, M6639; 4, M6640; 5, M6641; 6, M6642.

CONCLUSIONS

1) **The L-phenotype was associated with the presence of *lnu*B gene.**

2) **The emergence of SGB harboring the *lnu*B gene was polyclonal and was not transferable.**

3) **The continuous surveillance of the antibiotic susceptibility of GBS is necessary; not only to detect known resistance phenotypes, but also to identify newly acquired resistance mechanisms.**