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% in 2006 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 nucleotidylation-transferences 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 (NRL) for molecular characterization.

OBJECTIVE

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

RESULTS

- All six GBS were susceptible to β-lactams (penicillin and oxacillin), fluoroquinolones (doxycyclin and levofloxacin), and vancomycin. Three isolates were intermediate to tetracyclin (Table 1).
- 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 clindamycin (≤ 0.25 mg/L), and resistant to CLI (4 mg/L) and lincomycin (64-128 mg/L).
- All six isolates were positive for lnuB gene, and negative for Erm methylases (ermA and ermA) and others lincosamines (linS and linU).
- lnuB sequence was confirmed by sequencing.
- Conjugation assays using S. agalactiae were successful, but resistant to fluoroquinolones and streptothricin crude (RNGS) were used as recipient strains. A ratio of 1:5 of donor:recipient strains were selected. Selection plates of MRS agar plus blood were incubated in CO2 ambient.
- Conjugation transductants were diploid with Apal enzyme and DNA fragments were separated on 1% agarose gel 6.5 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) 33:2253-b).

TABLE 1

<table>
<thead>
<tr>
<th>Isolate</th>
<th>ERY</th>
<th>AZM</th>
<th>LIN</th>
<th>CLI</th>
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</tr>
<tr>
<td>M6641</td>
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- All six GBS were susceptible to β-lactams (penicillin and oxacillin), fluoroquinolones (doxycyclin and levofloxacin), and vancomycin. Three isolates were intermediate to tetracyclin (Table 1).

Conclusions

1. The L-phenotype was associated with the presence of lnuB gene.
2. The emergence of SGB harboring the lnuB gene was polyclonal and was not transferrable.
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.

Figure 1. Disc-diffusion patterns of a representative GBS, M6390 showing susceptibility to PEN, CTX and ERY, and resistance to CLI and LIN.

Figure 2. Apal-PFGE of S. agalactiae genomic DNA. Lanes M, PFGE marker, I: M6390; 2: M6367; 3: M6630; 4: M6640; 5: M6641; 6: M6642.