

Short Communication

Multiple-Clones of *Streptococcus agalactiae* harbouring *lnuB* gene

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Streptococcus agalactiae or Group B streptococci (GBS) usually colonizes gastrointestinal, respiratory, and urogenital human tracts causing diverse types of infections. Urogenital colonization of pregnant women with GBS is a critical risk factor for developing invasive neonatal disease, being the antimicrobial prophylaxis recommended during the delivery [1]. GBS is traditionally considered to be a neonatal pathogen although recently an increasing incidence of infections among American adults, especially those patients with underlying medical conditions, has been observed [2]. Macrolides and lincosamides are the recommended second-line agents and also a therapeutic alternative for patients with penicillin allergy. Resistance to lincosamides in *S. agalactiae* is commonly mediated by Erm-type methylases, which confer cross-resistance to macrolide, lincosamide and streptogramins B [3-5]. Recently, *LnuB* (also named as *LinB*) and *LnuC* lincosamide nucleotidyl-transferase enzymes were described, in three and one *S. agalactiae* isolates, respectively [3-5]. Erythromycin- and clindamycin-resistance in GBS increased from 7.2% and 3% in 2005 to 11.3% and 5.2% in 2007 according to the national surveillance performed by WHONET-Argentina (67 hospitals) [www.paho.org]. Susceptibility data from 2,187 *S. agalactiae* isolates were collected between September 2006 and July 2008; 16 (0,73%) displayed the L-phenotype (erythromycin susceptible but clindamycin resistant), and six isolates were submitted to the National

Reference Laboratory (INEI) for further characterization. The objective of this work was to determine the mechanism of lincosamide resistance in six GBS and to evaluate the relationship among them.

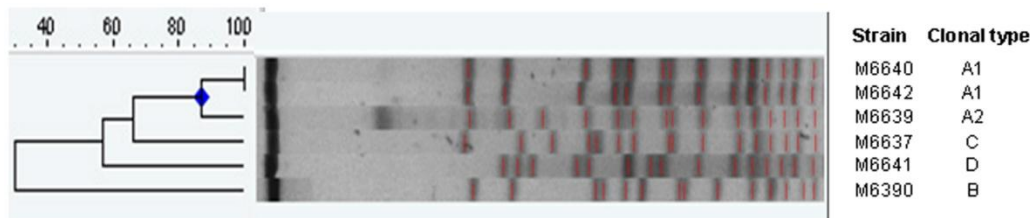
Six *S. agalactiae* were derived from three hospitals, namely Hospital Dr. Juan A. Fernandez (FER) (4), Hospital Dr. A. Piñeyro (PYR) (1) and Hospital Dr. G. Rawson (RAW) (1), and from three distant cities. The isolates displayed the L-phenotype and were recovered from prenatal recto-vaginal screening cultures (n = 5) and a urine sample (n = 1) (Table 1). Disk diffusion and minimal inhibitory concentration by agar dilution were performed according to CLSI guidelines [6]. Detection of *mefA*, *ermA*, *ermB*, *lnuA* and *lnuB* genes was performed by PCR, and DNA sequence was determined using the ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Clonal relationship was evaluated by *ApaI*-PFGE using the following conditions: initial and final pulsed times were 2 and 20 seconds, respectively, 1% agarose gel, 6 V/cm during 20 hs, and a CHEF-DRIII apparatus. DNA patterns were analyzed using Bionumerics software (Sint-Martens-Latem, Belgium) using Dice coefficient and 1% of tolerance, and the relationships were established according to Tenover's criteria [7]. Conjugation assays, 1:1 donor/acceptor ratio, were performed using *S. agalactiae* M6395 and *Staphylococcus aureus* RN4220 as recipient strains, both susceptible to macrolides and lincosamides, and

Table 1. Sources, susceptibility (MIC), mechanism of resistance and genetic relationship data of *S. agalactiae* isolates.

Isolate	Hospital	Date	Sample	MIC (mg/L)				Gene	ApaI-PFGE
				ERY	AZM	CLI	LIN		
M6390	PYR	08-Sep-06	Vaginal	0.06	0.25	4	64	<i>lnuB</i>	B
M6641	FER	21-Jan-07	Vaginal	0.12	0.25	4	128	<i>lnuB</i>	D
M6640	FER	27-Sep-07	Vaginal	0.12	0.25	4	128	<i>lnuB</i>	A1
M6642	FER	19-Nov-07	Vaginal	0.12	0.25	4	128	<i>lnuB</i>	A1
M6639	FER	05-Dec-07	Vaginal	0.06	0.25	4	64	<i>lnuB</i>	A2
M6637	RAW	04-Jul-08	Urine	0.12	0.25	4	128	<i>lnuB</i>	C

Abbreviation: MIC, minimal inhibitory concentration; PYR, HIGA Hospital "Dr. A. Piñeyro"; FER, Hospital "Dr. J. A. Fernandez"; RAW, Hospital "Dr. G. Rawson"; ERY, erythromycin; AZM, azithromycin; CLI, clindamycin; LIN, lincomycin.

Figure 1. Pulsed-field gel electrophoresis (PFGE) and genetic relation analysis of *S. agalactiae* isolates. Genetic relation analysis was performed using Dice algorithm and Bionumerics Software. Scale shows percentage of similarity only.



S. agalactiae M6390 as the donor strain. Brain heart infusion agar plates used for conjugation and selection were incubated at 35° C for 24 hours.

The six *S. agalactiae* isolates showing L-phenotype were susceptible to penicillin, cefotaxime, erythromycin, ofloxacin, levofloxacin and vancomycin, while three strains were resistant to tetracycline by disk diffusion. A modified triple disk-diffusion assay with clindamycin-erythromycin-lincomycin was performed [6]. Absences of inhibitory zones were observed around clindamycin and lincomycin disks; no inducible pattern was detected. Minimal inhibitory concentrations (MIC) of the six *S. agalactiae* isolates were susceptible to erythromycin, MIC = 0.06-0.12mg/L, and azithromycin, MIC = 0.25mg/L, but resistant to clindamycin, MIC = 4mg/L, and lincomycin, MIC = 64-128mg/L (Table 1). All isolates were PCR positive only for *lnuB* gene, and were confirmed by DNA sequencing (Accession number, HM209466). No mutation on L4 and L22 ribosomal proteins or 23S rRNA, domains II and V, were detected in the first recovered isolate (M6390), discarding additional mechanisms of resistance. Four clones were

discriminated by *ApaI*-PFGE (n): A (3), B (1), C (1) and D (1) (Figure 1). Domain V of a representative isolate of the other clonal types, A (M6640), C (M6637) and D (M6641), were also sequenced and no mutations were found. Clones A and D were detected in FER hospital, while clones B and C were from PYR and RAW hospitals, respectively (Table 1). Conjugation assays were unsuccessful under different experimental conditions and using two recipient strains. Although we can not discard methodological restrictions, the *lnuB* gene could be harboured in a cryptic plasmid or inserted in the chromosome as was described in *Enterococcus faecium* HM1025 [8]. In our country, the level of macrolide resistance is not only alarming in GBS, but also it has been increasing in invasive pediatric *S. pneumoniae* from 6% in the period of 1998-2001 to 16.4% in 2006, while no significant differences were observed in Group A streptococci during the last years, 4.7% in 2005 and 3.6% in 2007 [9; WHONET-Argentina unpublished data].

Although different authors described *S. agalactiae* clinical isolates expressing an L-phenotype in only three cases that the *lnuB* gene was detected, two strains were from Canada and one from

the US [3-5,10-12]. Moreover, recently we also detected the *lnuB* gene in a *Streptococcus infantarius* (formerly *Streptococcus bovis* II 1) isolate recovered from a blood sample from a 79-year-old man showing fever of unknown origin and suspected of endocarditis infection (personal communication). Additionally, *lnuB* gene was also described in seven strains of *Streptococcus dysgalactiae* ssp. *equisimilis* from pig samples and in four *Streptococcus uberis* recovered from milk samples [13,14]. These data suggest that animals could be a potential reservoir of the *lnuB* gene.

In conclusion, we describe for the first time the polyclonal emergence of *S. agalactiae* harbouring the *lnuB* gene in Latin-America. Fortunately, *S. agalactiae* isolates expressing *lnuB* gene were easily detected in the clinical laboratory applying disk diffusion or dilution methods. Continuous surveillance of antibiotic susceptibility is necessary not only to detect known resistance phenotypes, but also to identify newly acquired resistance mechanisms.

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