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Genetic Environment of the *lnu*(B) Gene in a *Streptococcus agalactiae* Clinical Isolate

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Specific resistance to lincosamides (L phenotype) is the result of modification and inactivation by lincosamide nucleotidyltransferase enzymes encoded by members of the *lnu* (previously *lin*) gene family (1, 2), of which six different types, *lnu*(A), *lnu*(B), *lnu*(C), *lnu*(D), *lnu*(E), and *lnu*(F), are currently recognized (1, 3–7). In *Streptococcus agalactiae*, the genetic environment in which the gene *lnu*(B) is located has not been explored; in contrast, a genetic element carrying *lnu*(C) has been reported in this species (1), as well as different elements harboring *lnu*(A) and *lnu*(B) in *Staphylococcus aureus* (8, 9), *lnu*(B) in *Enterococcus faecium* (10), *lnu*(C) in *Streptococcus anginosus* (11), and a truncated copy of *lnu*(E) in *Streptococcus suis* (7).

In 2008, an *S. agalactiae* strain (SGB76) was obtained from a pregnant female outpatient at Mater Dei Hospital in Buenos Aires. By Etest, the isolate showed susceptibility to erythromycin (MIC of 0.06 μg/ml) and resistance to clindamycin (MIC of 6 μg/ml). Total DNA was used as the template for PCR screening of *erm*(A), *erm*(B), *mef*(A), and *lnu*(B) genes. Of these, only *lnu*(B) was detected and confirmed by sequencing. To determine the genetic environment of the *lnu*(B) gene, two strategies were used, thermal asymmetric interlaced PCR (TAIL-PCR) in combination with PCR mapping using primers designed based on the previously reported structures from *Enterococcus faecalis* and *S. aureus* (GenBank accession numbers AF408195.1, JQ861958.1, and JX560992) (see Table S1 in the supplemental material).

The 12,076-bp *lnu*(B)-carrying fragment from strain SGB76 contained 11 open reading frames (ORFs) of at least 100 amino acids (accession number KF772204). Other resistance genes detected in this structure include *aadE* (streptomycin resistance), *spw* (spectinoycin resistance), and *lsa*(E) (pleuromutilin, lincosamide, and streptogramin A resistance) (Fig. 1). Basic Local Alignment Search Tool (BLAST) analysis revealed that this sequence exhibited similarity to the *lnu*(B)-containing structures previously identified in *S. aureus* (JQ861959 and JX560992) and *E. faecalis* (AF408195) (Fig. 1) (8, 9). An insertion sequence (IS*1216E*) is located at the left-hand end, similar to IS*1216* in the structure described in *S. aureus* (JX560992) and in a variant of the latter, IS*1216v*, in *E. faecalis*. However, two copies of IS*257* are flanking the *lnu*(B)-carrying element in the structure described in *S. aureus* by Lozano et al. (accession number JQ861959) (9). The region of 5,982 bp located upstream from the *lnu*(B) gene is 99%

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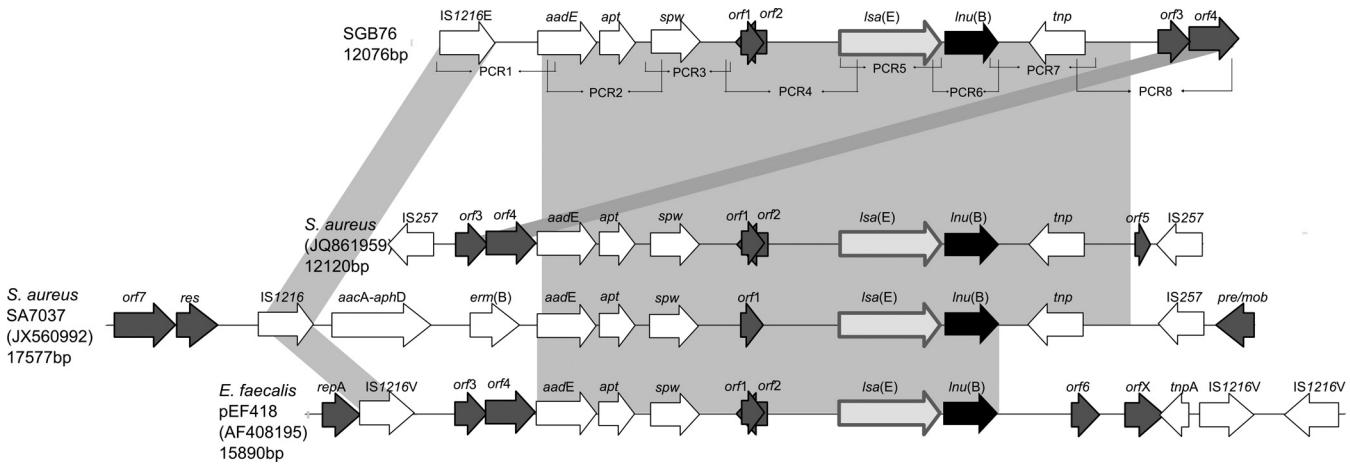

FIG 1 Genetic environment of the *lnu*(B) gene in *S. agalactiae* SGB76 (accession number KF772204) and structural comparison with the corresponding regions identified in *S. aureus* (accession numbers JQ861959 and JX560992) and *E. faecalis* pEF418 (accession number AF408195). Gray areas indicate regions with at least 99% nucleotide sequence identity. The arrows represent the position and orientation of the depicted genes as they were proposed in the original references. The amplicons of the 8 PCRs used to investigate the genetic environment of the *lnu*(B) gene in SGB76 are indicated below the top structure (see Table S1 in the supplemental material). *orf*1 to *orf7* represent open reading frames with either unknown or unconfirmed functions. *aadE*, aminoglycoside adenyltransferase E; *aacA-aphD*, aminoglycoside acetyltransferase A and aminoglycoside phosphotransferase D; *apt*, adenine phosphoribosyltransferase; *erm*(B), methyltransferase; IS, insertion sequence; *lnu*(B), lincosamide nucleotidyltransferase; *lsa*(E), ATP binding protein; *res* and *mob*, mobilization elements; *spw*, spectinomycin resistance gene; *tnp*, transposase.

identical to the structures described in *S. aureus* and *E. faecalis* (Fig. 1). The *lsa*(E) gene located immediately upstream from the *lnu*(B) gene encodes an ABC transporter involved in active efflux of lincosamides, streptogramins A, and pleuromutilins (10, 12). However, the region of 2,331 bp situated downstream from the *lnu*(B) gene showed 99% nucleotide identity to the *S. aureus* (but not to the *E. faecalis*) structure, which includes one copy of the gene encoding a putative transposase of the IS*L3* family (*tnp*) (Fig. 1). Besides the occurrence of this cluster in *E. faecalis* and *S. aureus*, a sequence from an *Enterococcus faecium* isolate of swine origin that was recently released (KF421157.1) (10) shows 99% nucleotide identity.

At the right-hand end, a region containing two genes (*orf3* and *orf4*) is 98% identical to the *orf3* and *orf4* region described in *S. aureus* (JQ861959); however, in the latter it is located at the left-hand end, associated with IS*257*. The description of this multiresistance cluster in *S. agalactiae* represents another example of resistance genes shared by enterococci, staphylococci, and streptococci (10).

Even if *S. agalactiae* SGB76 was susceptible to quinupristin-dalfopristin, as susceptibility to pleuromutilins and streptogramin A could not be assayed, a possible contribution of the *lsa*(E) gene to clindamycin resistance, as was described in *S. aureus*, could not be disregarded (8, 12). Further experiments will assess the contribution of this resistance marker in *S. agalactiae*.

Nucleotide sequence accession number. Sequence data were deposited in the GenBank/EMBL nucleotide databases under accession number KF772204.

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