Characterization of *ermA* in Macrolide-Susceptible Strains of Methicillin-Resistant *Staphylococcus aureus*^V

Two gene classes are responsible for macrolide resistance in *Staphylococcus aureus*. The erythromycin ribosome methylase (*erm*) genes confer resistance to macrolide, lincosamide, and streptogramin B antibiotics (MLS_B phenotype), while the macrolide streptogramin resistance (*msr*) drug efflux mechanism yields an MS_B phenotype (2–4). In methicillin-resistant *S. aureus* (MRSA), *ermA* resides on Tn554, located in staphylococcal cassette chromosome *mec* (SCC*mec*) types II and III (1).

The Canadian Nosocomial Infection Surveillance Program (CNISP) has been characterizing MRSA isolates collected from 49 sentinel Canadian hospitals since 1995 (9). We recently identified two isolates that carried *ermA* yet were susceptible to erythromycin and clindamycin while characterizing the macrolide and lincosamide resistance mechanisms in a subset of CNISP isolates (5). A retrospective examination of the susceptibility and molecular typing data between 1995 and 2007 was performed to identify any additional isolates with these characteristics. A total of 13 SCC*mec* II MRSA isolates were found that were susceptible to erythromycin and clindamycin (MICs of 0.25 and 0.5 µg/ml, respectively). These isolates, belonging to CMRSA2 (USA100) and *spa* type 2 (TJMBMDMGMK), were identified from three provinces: British Columbia (2007 [*n* = 1]), Manitoba (2007 [*n* = 4]), and Ontario (2004 [*n* = 2], 2005 [*n* = 1], and 2007 [*n* = 5]). The presence of the *ermA* coding sequence was confirmed by PCR (data not shown) (8). However, amplification with primers that span a 196-bp region upstream of the translation initiation site and the entire coding sequence yielded a smaller amplicon

from both genomic DNA and reverse-transcribed cDNA (data not shown). DNA sequence analysis revealed that 48 bp (nucleotides 5,277 to 5,324) of Tn554 (GenBank accession no. X03216) were missing in these isolates (Fig. 1).

The translation attenuation for *ermA* parallels that for *ermC* (10). The *ermA* attenuation sequence is composed of at least a 19-mer peptide coding sequence and two pairs of inverted repeats (IR1 and -2 and IR3 and -4) located immediately upstream of the AUG start codon for ErmA. In the absence of inducers, *ermA* mRNA forms two stable hairpin structures (IR1:IR2 and IR3:IR4), resulting in the sequestration of the ErmA Shine-Dalgarno sequence and start codon within IR3:IR4. ErmA translation occurs when an inducing macrolide binds and stalls the ribosome during translation of the 19-mer peptide that overlaps IR1 (Fig. 1). The sequestration of IR1 by the stalled ribosome elicits hairpin formation between IR2 and IR3, thereby freeing the translation initiation elements downstream of IR3. In the 13 erythromycin- and clindamycin-susceptible isolates, no ErmA protein could be produced because the 48-bp deletion had removed its translation initiation elements. In contrast to previous reports of *ermA* alterations that resulted in constitutive erythromycin resistance (6, 7, 11), the 48-bp deletion in the *ermA* attenuation sequence represents a novel event that confers an erythromycin- and clindamycin-susceptible phenotype. Although this deletion event appears to be rare, ongoing monitoring is needed to determine its frequency over time.

FIG. 1. Secondary structure of *ermA* mRNA permissible for translation. The region spanning positions 5,399 to 5,262 of Tn554 encodes a 19-mer peptide and IR1 to IR4 (arrows). Sequestration of IR1 by macrolide-bound ribosomes (conjoined ovals) leads to IR2:IR3 formation and the liberation of the Shine-Dalgarno sequence (small letters) and AUG start codon (bold) for translation initiation. In the absence of the 48-bp region that encompasses IR3 and IR4 (shown in box), translation attenuation is disrupted and the translation initiation elements are absent.

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