Characterization of a Novel lsa(E)- and lnu(B)-Carrying Structure Located in the Chromosome of a Staphylococcus aureus Sequence Type 398 Strain

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LETTER TO THE EDITOR

Characterization of a Novel *lsa*(E)- and *lnu*(B)-Carrying Structure Located in the Chromosome of a *Staphylococcus aureus* Sequence Type 398 Strain

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Lincosamides are extensively used in human and veterinary medicine in the treatment of staphylococcal infections (1). The major mechanisms of lincosamide resistance among Gram-positive cocci are the modification of the ribosomal target by the *erm*-encoded adenine-N6-methyltransferases (2) and the active drug efflux by ABC transporters encoded by *lsa*, *vga*, *eat*(A)$_v$, *and sal*(A) genes (3, 4, 5, 6, 7). Less-frequent resistance mechanisms in staphylococci such as enzymatic inactivation by the *lnu*(A)- and *lnu*(B)-encoded O-nucleotidyltransferases have also been described (8, 9). *lsa*(E)- and *lnu*(B) [*lsa*(E)/*lnu*(B)]-positive *Staphylococcus aureus* strains belonging to sequence type 9 (ST9), ST125, and ST398 have been sporadically isolated from humans and animals (10–13). Thus far, the respective genes occur as parts of similar structures resembling the *lsa*(E)/*lnu*(B)-carrying sequences from *Streptococcus agalactiae* (14) and *Enterococcus faecalis* (15), suggesting a common origin (Fig. 1A).

In a previous surveillance conducted in Greece, we identified for the first time a *lsa*(E)/*lnu*(B)-positive, methicillin-susceptible *S. aureus* (LAR2682) strain belonging to ST398 (*spa* type t034; *agr* type group 1) (16). The strain was penicillin resistant (MIC: 8 mg/liter), clindamycin resistant (MIC: 8 mg/liter), tetracycline resistant (MIC: 16 mg/liter), and trimethoprim-sulfamethoxazole resistant (MIC: 64 mg/liter), while it was intermediate-resistant to quinupristin-dalfopristin (MIC: 2 mg/liter) and susceptible to oxacillin (MIC: 0.5 mg/liter), erythromycin (MIC: 0.25 mg/liter), vancomycin (MIC: 0.5 mg/liter), teicoplanin (MIC: 0.5 mg/liter), linezolid (MIC: 2 mg/liter), daptomycin (MIC: 0.094 mg/liter), and tigecycline (MIC: 0.12 mg/liter). The level of its resistance to lincosamides correlated well with the copresence of *lsa*(E)/*lnu*(B) genes (17). According to demographic data, LAR2682 was recovered in March 2012 from a pus specimen from a 43-year-old male patient who presented at the Department of Vascular Surgery of the University Hospital of Larissa with a dried ulcer of the second toe of his left foot. We show here that LAR2682 contains a novel *lsa*(E)/*lnu*(B)-carrying structure distinct from those mentioned above.

To assess the localization of *lnu*(B) and *lsa*(E), genomic DNA was treated with either S1 nuclease or I-CeuI and separated by pulsed-field gel electrophoresis (PFGE) as described previously (18, 19), transferred to Hybond-N membranes, and hybridized with digoxigenin (DIG)-dUTP-labeled probes [for the 16S rRNA gene, nucleotides (nt) 515 to 1188 (J01859); for *lnu*(B), nt 15436 to 16286 (CP006623); and for *lsa*(E), nt 16859 to 17559 (CP006623)]. Detection was carried out using a DIG-DNA detection kit (Roche Diagnostics). An I-CeuI fragment of 1.08 Mb cohybridized with the 16S rRNA gene probe and both the *lnu*(B)- and *lsa*(E)-specific probes, indicating that the genes were chromosomally located.

To map the genetic context of *lsa*(E)/*lnu*(B), three approaches were used: (i) PCR mapping using primers based on previously reported sequences (GenBank accession no. AF408195, JX560992, JQ861959, KF772204, and CP006623), (ii) inverse PCR of genomic DNA digested by HindIII, and (iii) cloning of fragments of genomic DNA digested with HincII and EcoRV into pJET1.2/blunt cloning vector (20). Sequencing of amplicons and insertions was performed by primer walking. DNA Laser Gene (DNASTAR), BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and IS Finder (https://www-is.biotoul.fr/) were used for sequence analysis and annotation.

Sequencing data showed that the *lsa*(E)/*lnu*(B) genes were included in a novel structure (10,247 bp) bracketed by IS*Ssu5* insertion sequences (IS) (21) in parallel orientation (Fig. 1B). Downstream of the left-hand IS*Ssu5* was a segment of 6,243 bp (nt 818 to 7060) comprising six open reading frames (ORFs) [corresponding to *lsa*(E), *lnu*(B), resolvase, toxin/antitoxin, nucleotidyltransferase, and methyltransferase] and exhibiting 99% identity to the respective region of the p3 plasmid of *Enterococcus faecium* Aus0085 (GenBank accession no. CP006623). Adjacent to the latter part was an *aadE* gene showing 87% identity to that found in *E. faecium* (GenBank accession no. JQ861959) and showing identity to the respective genes in structures from *S. aureus*, *E. faecalis*, *S. agalactiae*, and *Streptococcus suis* (GenBank accession no. JX560992, AF408195, KF772204, and CP000407). The latter gene was followed by *apt* encoding an adenine phosphoribosyltransferase. In contrast to the previously described *lsa*(E)/*lnu*(B)-carrying structures, the *aadE*/*apt* region was located downstream of *lnu*(B). The right-hand IS*Ssu5* was adjacent to a 2,213-bp *S. aureus*-derived sequence, indicating the site of integration of this novel structure into the chromosome of LAR2682. Notably, the segment of 5,913 bp (from the resolvase gene to the right-hand IS*Ssu5*) was 99% identical to a region from *S. suis* (GenBank accession no. CP000407); however, the latter does not carry *lsa*(E)/*lnu*(B) (Fig. 1B).

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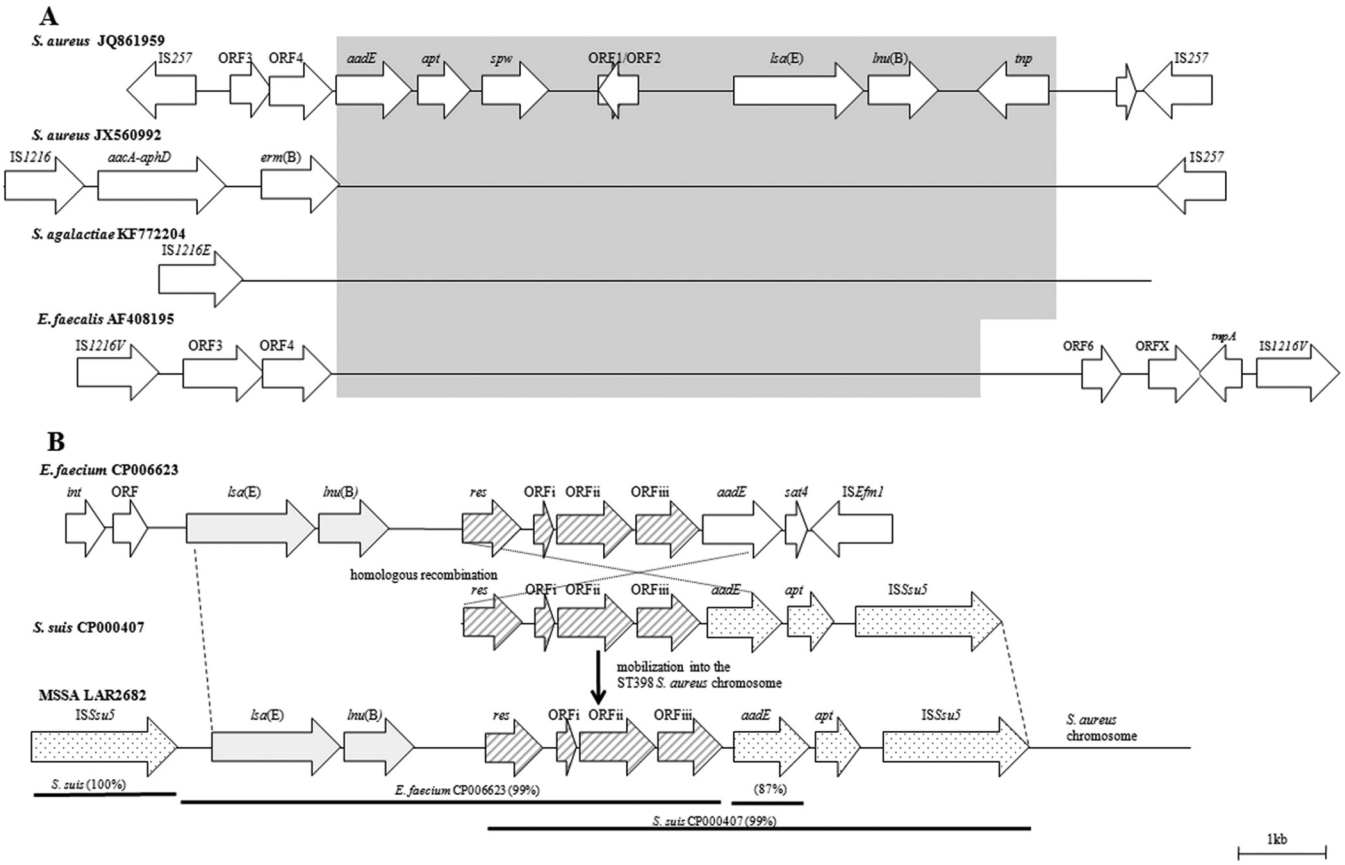

FIG 1 (A) Genetic environment of the *lnu*(B) gene in previously described structures from various Gram-positive species. Gray-shaded areas indicate similar sequences. (B) Structure of the novel *lnu*(B)-carrying transposon identified in this study (lower part of panel). Genes of *E. faecium* and *S. suis* exhibiting high similarity to the transposon gene from methicillin-susceptible *S. aureus* (MSSA) LAR2682, genes of *E. faecium* origin, and genes of *S. suis* origin are indicated by arrows containing diagonal lines, light gray shading, and dots, respectively (upper part of panel). Formation of the composite transposon by homologous recombination and integration in *S. aureus* chromosome is indicated.

Available data are not sufficient to reconstruct the steps leading to the formation of this novel structure. Yet it could be hypothesized that a recombination event within the *res*-toxin/antitoxin-nucleotidyltransferase-methyltransferase-*aadE* region present in both *E. faecium* and *S. suis* structures has replaced the right-hand end of *E. faecium* structure by the respective part downstream of the *aadE* gene from *S. suis*, resulting in the shaping of the final mosaic sequence (Fig. 1B). This event was probably followed by IS-mediated mobilization into the *S. aureus* chromosome.

The nucleotide sequence reported in this work has been deposited in the GenBank database under accession no. KP998101.

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