Analysis of a novel erm(T)- and cadDX-carrying plasmid from methicillin-susceptible Staphylococcus aureus ST398-t571 of human origin

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Sir,
The major mechanism of resistance to macrolides, lincosamides and streptogramin B antibiotics (MLSB) is the methylation of the adenine at position A2058 in domain V of 23S rRNA. Of the rRNA methylase genes so far detected in staphylococci, erm(T) has rarely been identified.1 It was described for the first time in staphylococci on plasmid pKKS25 in a porcine livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) ST398 isolate.2 Recent studies also revealed its presence in the chromosomal DNA of methicillin-susceptible S. aureus (MSSA) ST398 isolates in humans.3,4 In this study, we investigated a previously identified erm(T)-positive MSSA ST398NM01 isolate C3912 from a healthy human to gain insight into the genetic environment of erm(T), its possible association with other resistance genes and its plasmid location.

The MSSA ST398-t571 isolate C3912 was obtained in 2011 from the nasal swab of a healthy human.5 This isolate showed only inducible MLS8 resistance. Plasmids were prepared and transformed into S. aureus RN4220 with subsequent selection on medium containing erythromycin (15 mg/L). A single plasmid, designated pUR3912, was identified and shown to confer the aforementioned resistance phenotype. Plasmid pUR3912 was linearized by EcoRI, cloned into the plasmid vector pBlueScript II SK+ (Stratagene) and the recombinant plasmid was transformed into Escherichia coli JM101. The complete plasmid pUR3912 was sequenced by primer walking on both strands starting with M13 universal and reverse primers. A schematic map of the 6182 bp plasmid pUR3912 is shown in Figure 1. The nucleotide sequence of plasmid pUR3912 determined in this study has been deposited in the EMBL database under accession number HE805623.

The erm(T) gene encoded a 244 amino acid rRNA methylase which was indistinguishable from the recently described chromosomal Erm(T) of MSSA ST398 strain ST398NM01,3 and shared 98.9% identity (99.6% similarity) with Erm(T) of plasmid pKKS25 from LA-MRSA ST398.2 A comparison between the sequenced part of pKKS25 and pUR3912 revealed that the erm(T) gene was the only common feature between both plasmids (Figure 1a). A complete translational attenuator, which consisted of two pairs of inverted repeat sequences of 12 bp each and a reading frame for a regulatory peptide of 19 amino acids, was identified immediately upstream of the erm(T) gene (Figure 1b). Detailed analysis of the erm(T) region of pUR3912 showed that the erm(T) gene was flanked by two identical copies of the insertion sequence IS431 [named IS431L and IS431R based on their positions with respect to erm(T)], both located in the same orientation. A 709 bp region immediately downstream of IS431L showed 92.1% identity to the corresponding region of plasmid pSSP1 of Staphylococcus saprophyticus ATCC 15305 and included 120 bp of a truncated rep gene.5 A complete rep gene encoding a 206 amino acid replication initiation protein and a cadmium resistance operon were detected adjacent to this region. The rep gene showed 96.6% identity to that of pSSP1. The cadD gene encodes a 206 amino acid P-type metal efflux ATPase protein involved in cadmium resistance and cadX for a protein of 116 amino acids that serves as a transcriptional regulator of the cadmium resistance operon.6,7,8 Both microdilution assays revealed that the S. aureus RN4220 transformant carrying pUR3912 exhibited a 128-fold increase in the MIC of CdSO4 (128 mg/L) as compared with S. aureus RN4220 (MIC CdSO4 1 mg/L), and thus confirmed the functionality of the cadDX operon.

The comparison shown in Figure 1 suggested that pUR3912-like plasmid has been incorporated into the chromosomal DNA of MSSA ST398NM01 and that insertion elements of the type IS431 were most likely involved in this process.3,5,9 Three structural differences were noted between pUR3912 and the pUR3912-like plasmid in the chromosomal DNA of MSSA ST398NM01 (Figure 1): (i) two direct repeats of 64 bp are present between cadX and rep in MSSA ST398NM01, while only one of these sequences is present in pUR3912; (ii) an additional IS431 copy, named IS431c, was present in pUR3912—the detection of the 8 bp direct repeats immediately up- and downstream of the IS431, in pUR3912 strongly suggested that the integration of this insertion sequence into a pUR3912 precursor was an independent process; and (iii) the 709 bp plasmid pSSP1-like segment is missing in the chromosomally integrated plasmid, although the 9 bp (5'-AAAAATTTG-3') corresponding to the 5' terminus of the truncated rep gene are present. It is unknown when and by which way this 709 bp segment became part of pUR3912, but
the presence of several copies of IS431 in pSSP1 may suggest a potential involvement.

To determine whether pUR3912 is present in other S. aureus ST398 strains, eight recently described erm(T)-positive strains from our strain collection (two MSSA ST398 and two LA-MRSA ST398 of human origin and four LA-MRSA ST398 of porcine origin) were investigated. 10–12 Plasmid pUR3912 appeared to be present in the two unrelated MSSA ST398 strains of human origin. In these strains, a plasmid of the expected size was detected that carried the erm(T) and cadX genes at the expected distance—as confirmed by PCR analysis 

\[ \text{erm(T)-fw} + \text{tet(L)-fw} \] 

CATTGCTCTATGGCATGCG. These observations suggest that plasmid pUR3912 may occur more

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**Figure 1.** (a) Comparison of the complete plasmid pUR3912 (accession number HE805623) identified in this study with the erm(T)-cadDX chromosomal segment of MSSA strain ST398NM01 (accession number CP003045) and the sequenced part of plasmid pKKS25 of LA-MRSA ST398 (accession number FN390947). The arrows indicate the extents and directions of transcription of the genes erm(T) (combined resistance to MLSB), rep (plasmid replication), cadX (transcriptional regulator), cadD (P-ATPase metal efflux), dfrK (trimethoprim resistance) and tet(L) (tetracycline resistance). IS431 (including IS431L and IS431R), IS431L and the truncated IS element \( \Delta \text{tnp} \) are shown as dark grey, black and light grey boxes, respectively, with the white arrow indicating the transposase gene \( \text{tnp} \). The EcoRI cleavage site (E) is indicated. The 8 bp direct repeats at the IS431 integration sites within a transposase gene—related to the transposase gene of IS712G—of the chromosomal DNA of ST398NM01 as well as the 8 bp direct repeats upstream and downstream of the IS431L and IS431R within pUR3912 are shown in boxes. The 9 bp \( 5'\text{-AAAAATTTG}-3' \) of ST398NM01 identical to the last 9 bp of the 709 bp region of plasmid pSSP1 from S. saprophyticus ATCC 15305 in pUR3912 are indicated in a dashed box. The regions of similarity between pUR3912 and the segments of ST398NM01 and pKKS25 are indicated by grey shading. An additional copy of the 64 bp sequence present in ST398NM01 is shown in the stippled box. A size scale in kilobase pairs (kb) is given below each map. (b) Comparison of the complete \( \text{erm(T)} \) regulatory region of pUR3912 with the truncated pKKS25. The 55 bp deletion in pKKS25 is indicated by dashes. Vertical bars indicate bases that are identical in the two sequences while dots reflect the presence of different bases. The two pairs of inverted repeat sequences, IR1–IR2 and IR3–IR4, are marked by arrows, the ribosomal binding sites RBS 1 and RBS 2 are underlined and the reading frames for the 19 amino acid regulatory peptide and the 5′-terminal part of the \( \text{erm(T)} \) gene are indicated by grey shading.
often in the animal-independent MSSA ST398, whereas plasmid pKK25 or other larger plasmids seem to occur preferentially in LA-MRSA ST398. However, the small number of erm(T)-positive S. aureus strains does not allow reliable conclusions to be drawn.

This is the first description of an erm(T)-harbouring plasmid that also carries a cadmium resistance operon. The observation that erm(T) is present either on a small plasmid, such as pUR3912, or on larger plasmids that also carry tet(L), such as pKK25, or that it is located in the chromosomal DNA of MSSA ST398 strain does not allow reliable conclusions to be drawn.

The physical linkage of antibiotic resistance genes and genes that confer resistance to heavy metals may facilitate their persistence and dissemination under the selective pressure imposed by any of the involved agents.

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The enterococcal ABC transporter gene lsa(E) confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant Staphylococcus aureus

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Sir,

In recent years, combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in staphylococci has been attributed to ABC transporters of the Vga type. Besides variants of the vga(A) gene,4 novel genes such as vga(C) and vga(E) have