

often in the animal-independent MSSA ST398, whereas plasmid pKKS25 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 carried 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 pKKS25, or that it is located in the chromosomal DNA of MSSA ST398NM01 suggests that the *erm(T)* gene has been acquired at independent occasions by MSSA and MRSA ST398 strains that are adapted to different hosts.¹³ The physical linkage of antimicrobial 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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Transparency declarations

None to declare.

References

- Kadlec K, Feßler AT, Hauschild T et al. Novel and uncommon antimicrobial resistance genes in livestock-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2012; **18**: 745–55.
- Kadlec K, Schwarz S. Identification of a plasmid-borne resistance gene cluster comprising the resistance genes *erm(T)*, *dfrK*, and *tet(L)* in a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* 2010; **54**: 915–8.
- Uhlemann AC, Porcella SF, Trivedi S et al. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. *mBio* 2012; **3**: e00027–12.
- Vandendriessche S, Kadlec K, Schwarz S et al. Methicillin-susceptible *Staphylococcus aureus* ST398-t571 harbouring the macrolide-lincosamide-streptogramin B resistance gene *erm(T)* in Belgian hospitals. *J Antimicrob Chemother* 2011; **66**: 2455–9.
- Gómez-Sanz E, Torres C, Lozano C et al. High diversity of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* lineages and toxigenic traits in healthy pet-owning household members. Understanding normal household contact? *Comp Immunol Microbiol Infect Dis* 2012; in press.
- Kuroda M, Yamashita A, Hirakawa H et al. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. *Proc Natl Acad Sci USA* 2005; **102**: 13272–7.
- Chen YY, Feng CW, Chiu CF et al. *cadDX* operon of *Streptococcus salivarius* 57.1. *Appl Environ Microbiol* 2008; **74**: 1642–5.
- Crupper SS, Worrell V, Stewart GC et al. Cloning and expression of *cadD*, a new cadmium resistance gene of *Staphylococcus aureus*. *J Bacteriol* 1999; **181**: 4071–5.
- Schwarz S, Feßler AT, Hauschild T et al. Plasmid-mediated resistance to protein biosynthesis inhibitors in staphylococci. *Ann N Y Acad Sci* 2011; **1241**: 82–103.
- Lozano C, Gómez-Sanz E, Benito D et al. *Staphylococcus aureus* nasal carriage, virulence traits, antibiotic resistance mechanisms, and genetic lineages in healthy humans in Spain, with detection of CC398 and CC97 strains. *Int J Med Microbiol* 2011; **301**: 500–5.
- Lozano C, Rezusta A, Gómez P et al. High prevalence of *spa* types associated with the clonal lineage CC398 among tetracycline-resistant methicillin-resistant *Staphylococcus aureus* strains in a Spanish hospital. *J Antimicrob Chemother* 2012; **67**: 330–4.
- Gómez-Sanz E, Torres C, Lozano C et al. Detection, molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus* CC398 and CC97 in Spanish slaughter pigs of different age groups. *Foodborne Pathog Dis* 2010; **7**: 1269–77.
- Price LB, Stegger M, Hasman H et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *mBio* 2012; **3**: e00305–11.

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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,¹ novel genes such as *vga(C)* and *vga(E)* have

(a)

Walker A

MSLINVSNLTFSEYGSYDNIFENVSFQIDTDWKLGFIGRNGRGT - 45

TFLNLLLKGAYASGNISSTVKFEYFPYDVEDKSLYTIEMKSICT - 90

ABC signature

ECMDWEIFREISLLDVQEDALYRPFNTLSNGEOTKVLAAFLTA - 135

Walker B

SCFLIIDIEPTNHLDDIDARNVVQNYLKRKKGFIIVSHDRSLDQCV - 180

DHILSINKTNIETIQKGNFTSWENKTLQDNFELAENKLLKEIGR - 225

LSYAAKRSSNWSNKVEKSKYGTTSNGSKLDKGYVGHKAAMKRA - 270

KNIESRHQEAVLQKSELLHNIEQYDDLKISPLEFHKECLIEANDL - 315

Walker A

SLSYGDKEVCSNLNFRVNIQDRVAIIKNGSGKSSILKLINGDDI - 360

KFTGNFMLASGLKISYISQDTSYLKGNLSEFAYNNKIDETLFKTI - 405

ABC signature Walker B

LRKLDNFNEQFDKNMVDLSAGQKKKVLIAKSLCESAHLIYIWDI - 450

NYIDIFSRIQIEKMILEYCPDLLFVEHDDAFCNNICTKNINLGL - 494

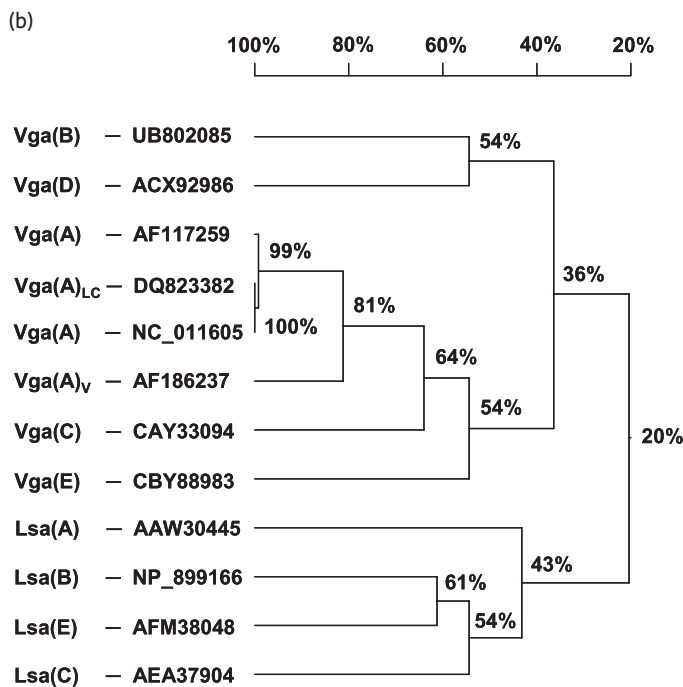


Figure 1. (a) Amino acid sequence of the Lsa(E) protein in which the Walker A, Walker B and the ABC signature motifs are shown in bold and underlined. (b) Homology tree of the currently known Vga and Lsa proteins with a confirmed role in antimicrobial resistance. The GenBank accession numbers of the various Vga and Lsa proteins are indicated. The branching order follows the amino acid exchanges observed in a multisequence alignment. Of the numerous closely related Vga(A) proteins, only the original Vga(A) (AF117259) and two variants, which exhibited slightly different substrate spectra, Vga(A)_{LC} (streptogramin A and lincosamides; DQ823382) and Vga(A) (streptogramin A, lincosamides and pleuromutilins; NC_011605),¹ were included for reasons of clarity and, of the numerous closely related Lsa(A) proteins from *E. faecalis*, only Lsa(A) (AAW30445)⁶ was included for reasons of clarity. The percentages of amino acid identity are rounded up or down to the nearest integral per cent value.

been detected in methicillin-resistant *Staphylococcus aureus* (MRSA) of clonal complex (CC) 398 of swine, cattle and poultry origin and shown to confer this resistance phenotype.²⁻⁵ In *Enterococcus faecalis* and *Streptococcus agalactiae*, the genes *lsa(A)* and *lsa(C)*, respectively, which also encode ABC transporters, mediate a similar resistance phenotype.^{6,7} In a recent study by Lozano and co-workers,⁸ a 12120 bp segment was sequenced (GenBank accession no. JQ861959) to gain insight into the genetic environment of the lincosamide nucleotidyltransferase gene *lnu(B)* in one MRSA isolate of sequence type (ST) 398 and two methicillin-susceptible *S. aureus* (MSSA) ST9 isolates of human origin. A gene coding for a putative ABC transporter of 494 amino acids was identified immediately upstream of *lnu(B)*. Database searches identified proteins with the same amino acid sequence in *E. faecalis* strains 418 (GenBank accession no. AAL05553), D6 (GenBank accession no. EEU82260), E1071 (GenBank accession no. EFF20876) and TX2173 (GenBank accession no. EFT38229). However, no function has been assigned to this putative ABC transporter so far.

Detailed analysis of this ABC transporter revealed two ATP-binding domains, each containing the Walker A and B motifs as well as the conserved ABC signature motif,⁹ but like other known Vga and Lsa proteins it lacked membrane-spanning domains (Figure 1a). In a multisequence alignment, it was more closely related to Lsa proteins (44.4%–61.8% amino acid identity) than to Vga proteins (13.7%–22.9% amino acid identity) (Figure 1b). The next most closely related Lsa proteins were Lsa(B) proteins from *Staphylococcus warneri* (61.8% amino acid identity; GenBank accession no. CAL64018) and *Staphylococcus sciuri* (61.4% amino acid identity; GenBank accession no. NP_899166). As a consequence, this ABC transporter protein recently received the designation Lsa(E) and the corresponding gene the designation *lsa(E)* from the macrolide–lincosamide–streptogramin (MLS) resistance gene nomenclature centre (<http://faculty.washington.edu/marilynr/>). Since, for all three staphylococcal isolates, all of which harboured the *lsa(E)* gene, the MICs of tiamulin and virginiamycin (a compound consisting of streptogramin A + streptogramin B moieties)⁸ were high (>128 mg/L and 2 mg/L, respectively), a finding which could not be explained by the other resistance genes detected in these strains, we sought to determine whether the Lsa(E) protein might play a role in conferring resistance to these compounds.

A PCR assay was developed (forward primer: 5'-CGGCTATAG AACGTTTGTTTT-3'; reverse primer: 5'-AGTTATTGTGGCAACT CAAAATC-3'; annealing temperature 52°C) that amplified the entire *lsa(E)* gene including 310 bp in the upstream region and 24 bp in the downstream region. This 1819 bp amplicon, obtained from the MRSA ST398 isolate, was first cloned into the pCR[®]-Blunt II-TOPO[®] vector and transformed into the *Escherichia coli* TOP10 strain using the Zero Blunt[®] TOPO[®] PCR Cloning Kit (Invitrogen, Groningen, the Netherlands). In a second step, the insert was cut from this vector by EcoRI digestion and inserted into the single EcoRI site of the *E. coli*-*S. aureus* shuttle vector pLI50.¹⁰ The recombinant shuttle vector was then transferred by electrotransformation into the recipient strain, *S. aureus* RN4220. Susceptibility testing was conducted by broth microdilution for the pleuromutilin tiamulin, the lincosamides clindamycin and pirlimycin and the streptogramin A+B

combination quinupristin/dalfopristin and by broth macrodilution for the lincosamide lincomycin and the streptogramin A virginiamycin M1 according to the recommendations given in the CLSI document M31-A3.¹¹ *S. aureus* ATCC[®] 29213 served as a quality control strain.

In comparison with *S. aureus* RN4220 and *S. aureus* RN4220 carrying the shuttle vector pLI50, for *S. aureus* RN4220 transformants carrying the *Isa(E)* gene cloned into pLI50 the MIC of tiamulin was increased 64-fold (MIC 32 mg/L), and those of pirlimycin (MIC 2 mg/L), lincomycin (MIC 8 mg/L) and clindamycin (8 mg/L) were increased 4-fold, 16-fold and 64-fold respectively. In addition, the MIC of quinupristin/dalfopristin was increased 8-fold (MIC 2 mg/L) and the MIC of virginiamycin M1 was increased ≥ 32 -fold (MIC ≥ 64 mg/L) (Table S1, available as Supplementary data at JAC Online). These data confirmed that, in the presence of *Isa(E)*, the MICs for *S. aureus* RN4220 of the three lincosamides tested, as well as of the streptogramin A antibiotic virginiamycin M1 and the pleuromutilin tiamulin, are distinctly elevated. As such, *Isa(E)* is the first *Isa* gene in staphylococci demonstrated to confer this resistance phenotype.

The detection of *Isa(E)* (which most likely originated from *E. faecalis*) in MRSA ST398 and MSSA ST9 isolates confirms that there is a gene flux between *E. faecalis* and *S. aureus*, as has been observed previously with other plasmid- or transposon-borne resistance genes including the *vanA* gene cluster,¹² the tetracycline resistance gene *tet(L)*, the trimethoprim resistance gene *dfrk*¹³ and the multiresistance gene *cfr*.¹⁴ Further analysis of the sequence described by Lozano *et al.*⁸ revealed that an 8550 bp segment on which *Isa(E)* is located in the three staphylococcal strains showed 98.7% nucleotide sequence identity to the sequence of the *E. faecalis* plasmid pEF418 (GenBank accession no. AF408195). This finding is in agreement with the aforementioned observations and supports the assumption that plasmids may play a role in the inter-genus transfer of resistance genes between *Enterococcus* and *Staphylococcus*. Further work is warranted to determine how widespread the *Isa(E)* gene is in staphylococci.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

References

- Gentry DR, McCloskey L, Gwynn MN *et al.* Genetic characterization of Vga ABC proteins conferring reduced susceptibility to pleuromutilins in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2008; **52**: 4507–9.
- Kadlec K, Schwarz S. Identification of a novel ABC transporter gene, *vga(C)*, located on a multiresistance plasmid from a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* 2009; **53**: 3589–91.
- Kadlec K, Pomba CF, Couto N *et al.* Small plasmids carrying *vga(A)* or *vga(C)* genes mediate resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-resistant *Staphylococcus aureus* ST398 from swine. *J Antimicrob Chemother* 2010; **65**: 2692–3.
- Schwendener S, Perreten V. New transposon Tn6133 in MRSA ST398 contains *vga(E)*, a novel streptogramin A-, pleuromutilin-, and lincosamide-resistance gene. *Antimicrob Agents Chemother* 2011; **55**: 4900–4.
- Hauschild T, Feßler AT, Kadlec K *et al.* Detection of the novel *vga(E)* gene in methicillin-resistant *Staphylococcus aureus* CC398 isolates from cattle and poultry. *J Antimicrob Chemother* 2012; **67**: 503–4.
- Singh KV, Murray BE. Differences in the *Enterococcus faecalis* *Isa* locus that influence susceptibility to quinupristin-dalfopristin and clindamycin. *Antimicrob Agents Chemother* 2005; **49**: 32–9.
- Malbruny B, Werno AM, Murdoch DR *et al.* Cross-resistance to lincosamides, streptogramins A, and pleuromutilins due to the *Isa(C)* gene in *Streptococcus agalactiae* UCN70. *Antimicrob Agents Chemother* 2011; **55**: 1470–4.
- Lozano C, Aspiroz C, Sáenz Y *et al.* Genetic environment and location of the *Inu(A)* and *Inu(B)* genes in methicillin-resistant *Staphylococcus aureus* and other staphylococci of animal and human origin. *J Antimicrob Chemother* 2012; **67**: 2804–8.
- Schneider E, Hunke S. ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS Microbiol Rev* 1998; **22**: 1–20.
- Lee CY, Buranen SL, Ye Z-H. Construction of single-copy integration vectors for *Staphylococcus aureus*. *Gene* 1991; **103**: 101–5.
- Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals—Third Edition: Approved Standard M31-A3*. CLSI, Wayne, PA, USA, 2008.
- Noble WC, Virani Z, Cree RG. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett* 1992; **72**: 195–8.
- López M, Kadlec K, Schwarz S *et al.* First detection of the staphylococcal trimethoprim resistance gene *dfrk* and the *dfrk*-carrying transposon Tn559 in enterococci. *Microb Drug Resist* 2012; **18**: 13–8.
- Liu Y, Wang Y, Wu C *et al.* First report of the multidrug resistance gene *cfr* in *Enterococcus faecalis* of animal origin. *Antimicrob Agents Chemother* 2012; **56**: 1650–4.