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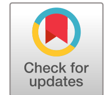

A Novel *erm*(44) Gene Variant from a Human *Staphylococcus saprophyticus* Isolate Confers Resistance to Macrolides and Lincosamides but Not Streptogramins

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ABSTRACT A novel *erm*(44) gene variant, *erm*(44)$_v$, has been identified by whole-genome sequencing in a *Staphylococcus saprophyticus* isolate from the skin of a healthy person. It has the particularity to confer resistance to macrolides and lincosamides but not to streptogramin B when expressed in *S. aureus*. The *erm*(44)$_v$ gene resides on a 19,400-bp genomic island which contains phage-associated proteins and is integrated into the chromosome of *S. saprophyticus*.

KEYWORDS MLS$_B$, antibiotic resistance, phages, coagulase-negative staphylococci, 23S RNA methylase, Staphylococcus, macrolides-lincosamides-streptogramin B

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Staphylococcus saprophyticus is a bacterium which is widespread in the environment and in animals and may also occur on the skin of humans. It is known as a major cause of urinary tract infection and cystitis in humans (1). Although macrolides and lincosamides are not used for the treatment of urinary tract infections, they are among the antibiotics of choice for the treatment of other infectious diseases, such as pulmonary infection, and their use may contribute to the selection of resistance in bacteria of the normal human flora, including staphylococci (2). Resistance to macrolide, lincosamide, and streptogramin (MLS$_B$) antibiotics in staphylococci has been associated with erythromycin ribosome methylase (*erm*) genes (Fig. 1) which methylate the 23S rRNA at position A2058, preventing binding of the MLS$_B$ antibiotics (3). The *erm*(44) gene, originally found in *Staphylococcus xylosus* from bovine mastitis milk (4), has also been recently identified in a *S. saprophyticus* isolate from river water (5) and has now been identified in *S. saprophyticus* from human skin.

Three of 10 healthy human volunteers who did not receive MLS$_B$ antibiotics and who were participating to a large project aiming at determining the effects of antibiotic administration on the emergence and persistence of antibiotic-resistant bacteria in humans (ANTIRESDEV project [www.ucl.ac.uk/antiresdev]; UK ethics approval number EC 10/H0806/12) were found to harbor *Staphylococcus saprophyticus* on the skin. The strains were isolated on sheep blood agar plates and identified using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) (microflex LT; Bruker Daltonic GmbH, Bremen, Germany). MICs of MLS$_B$ antibiotics erythromycin, clindamycin, virginiamycin S1, and pristinamycin 1A were determined by the microdilution method in Mueller-Hinton broth, and one strain (N041) showed resistance to erythromycin and clindamycin according to the EUCAST interpretation criteria (6). As this strain did not contain any known *erm* genes as determined using a microarray (7), whole-genome sequencing was performed at the UZH/ETH Functional Genomics Center (Zurich, Switzerland) by Life Technologies Ion Torrent semiconductor sequencing using a

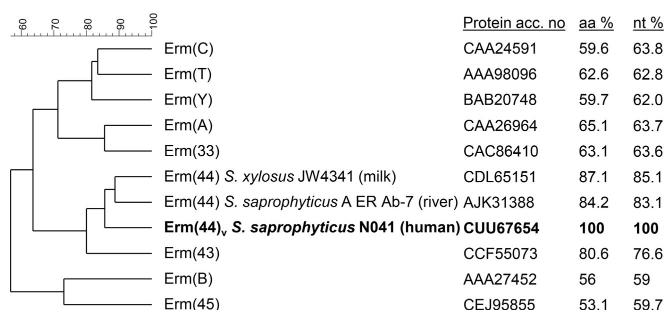

FIG 1 Relationship tree of erythromycin resistance methylases (Erm) detected in different *Staphylococcus* species. Amino acid (aa) identity and nucleotide (nt) identity were obtained by sequence alignment and clustering with BioNumerics 7.6 (Applied Maths). Comparison settings were as follows: standard algorithm for pairwise alignment; open gap penalty, 100%; unit gap penalty, 0%; and unweighted pair group method using average linkages (UPGMA). Methylase genes that were detected in *Staphylococcus* only by PCR and/or hybridization and whose sequences are not available [e.g., *erm*(F), *erm*(G), *erm*(Q)] were not included (http://faculty.washington.edu/marilynr/).

400-bp library on a 314v2 chip. Comparisons of all contigs with currently annotated *erm* genes using BLAST identified an *erm* gene which showed the closest relatedness to *erm*(44) from *S. xylosus* JW4341 with 81% amino acid (aa) and 85% DNA identity and to *erm*(44) from *S. saprophyticus* A ER Ab-7 with 84% aa sequence identity and 83% DNA identity (Fig. 1). The newly detected *erm* gene encodes a 243-aa protein containing an rRNA adenine dimethylase signature (PS01131) as found in other *erm* methylases (8). It was not preceded by any intact leader peptides, neither by a complete IFVI motif nor by inverted repeat sequences, which are essential for induction and translational attenuation of *erm* genes (3, 9–11), likely explaining constitutive expression of this *erm* gene as determined by MIC analysis (Table 1). Putative −10 (TTTTAAAAT) and −35 (TTGCCT) promoter sequences were found 27 bp and 48 bp upstream of the start codon, respectively.

TABLE 1 MIC of erythromycin, clindamycin, pristinamycin Ia, and virginiamycin S1 for different *Staphylococcus* strains, as determined by broth microdilution

Strain	Characteristic(s) or origin	Reference or source	Antibiotic resistance gene(s)[a]	MIC (μg/ml)[b]						
				ERY	CLI	iCLI	PIA	iPIA	VS1	iVS1
S. saprophyticus										
N041	Human nose skin sample	This study	*erm*(44)$_v$	128	>256	>256	32	32	32	32
S. aureus										
RN4220	Recipient strain for electrotransformation, plasmid free	18		<0.5	<0.25	NA	4	NA	8	NA
RN4220/pBUS1-HC	RN4220 with cloning vector pBUS1-HC	12	*tet*(L)	<0.5	<0.25	NA	4	NA	8	NA
RN4220/pBUS1-P$_{cap}$-HC[c]	RN4220 with pBUS1-HC containing *cap* promoter	12	*tet*(L)	<0.5	<0.25	NA	2	NA	8	NA
RN4220/pBJW13	RN4220 with *erm*(44) from *S. xylosus* JW4341 cloned into pBUS1-P$_{cap}$	4	*tet*(L), *erm*(44)	>256	>256	>256	8	8	16	32
RN4220/pLI50-*erm*(44)	RN4220 with *erm*(44) from *S. saprophyticus* A ER Ab-7 cloned into pLI50	5	*bla*$_{TEM-1}$, *cat*$_{pC194}$, *erm*(44)	>256	<0.25	256	4	64	32	128
RN4220/pBCS0714	RN4220 with *erm*(44)$_v$ from *S. saprophyticus* N041 and its regulatory region cloned into pBUS1-HC	This study	*tet*(L), *erm*(44)$_v$	16	>256	>256	2	1	8	4
RN4220/pBCS0814	RN4220 with *erm*(44)$_v$ from *S. saprophyticus* N041 cloned into pBUS1-P$_{cap}$-HC	This study	*tet*(L), *erm*(44)$_v$	16	>256	>256	2	1	8	4

[a]Antibiotic resistance genes and functions: *bla*$_{TEM-1}$, β-lactamase; *cat*$_{pC194}$, chloramphenicol acetyltransferase; *tet*(L), tetracycline efflux; *erm*(44) and *erm*(44)$_v$, 23S rRNA methylase.

[b]Abbreviations: ERY, erythromycin; CLI, clindamycin; PIA, pristinamycin IA; VS1, virginiamycin S1; iCLI, iPIA, and iVS1, 2 μg/ml erythromycin added to the broth for the detection of inducible resistance to clindamycin (iCLI), pristinamycin IA (iPIA), and virginiamycin S1 (iVS1); NA, not applicable.

[c]Vector pBUS1-P$_{cap}$-HC is a pBUS1-HC derivate that harbors the *cap* promoter of the *S. aureus* type 1 capsular polysaccharide biosynthesis gene cluster.

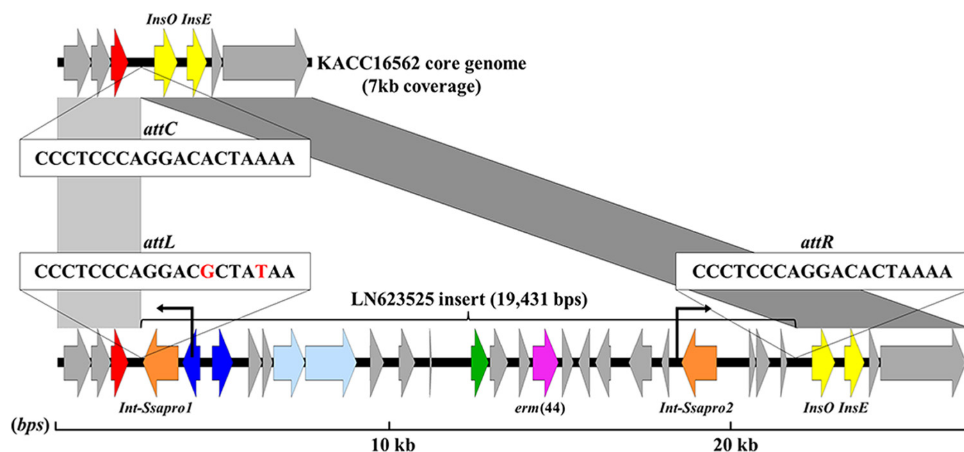

FIG 2 Insertion site of genomic island in *S. saprophyticus* N041 (GenBank accession no. LN623525) and core genome of *S. saprophyticus* KACC16562 (GenBank accession no. NZ_AHKB00000000.1). Gray areas represent high similarity at the nucleotide level (>98%). Arrows represent positions and orientations of open reading frames (ORFs). New ML resistance gene *erm*(44)$_v$ is shown in pink. The 19-bp putative insertion site *attC* and the duplicated sites *attL* and *attR* in the N041 genome are shown. Two transposases of the core genome (*InsO_Ssapro* and *InsE_Ssapro* [abbreviated as *InsO* and *InsE*]) are indicated in yellow, the metal-dependent phosphodiesterase in red, and the two flanking integrases of the genomic island (*Int-Ssapro1* and *Int-Ssapro2*) in orange. Additional genes are colored according to sequence and function: transcription regulators are dark blue; replication genes (including the primase gene) are light blue; the terminase gene is green; genes encoding hypothetical proteins are gray. Primers for the circular form test are indicated with a black arrow.

The functionality of the *erm* gene of strain N041 was assessed after cloning into the shuttle vector pBUS1-HC was performed (12), generating plasmid pBSC0714, where the gene was expressed with its own promoter. The presence of pBCS0714 in *S. aureus* RN4220 led to an increase of the MIC of erythromycin to 16 μg/ml and of clindamycin to ≥256 μg/ml, while the MICs for the streptogramins pristinamycin Ia and virginiamycin S1 did not increase compared to those seen with the *S. aureus* RN4220 recipient strain alone and a RN4220 strain harboring pBUS1-HC or pBUS1-P$_{cap}$-HC. To verify this uncommon phenotype, the *erm* gene was placed under the control of a strong *cap* promoter in plasmid pBSC0814, confirming both the erythromycin and clindamycin phenotype and the absence of increased MICs for streptogramin B pristinamycin and virginiamycin in RN4220 (Table 1), in contrast to the results seen with the closely related *erm*(44) from *S. xylosus* JW4341 and that from *S. saprophyticus* A ER Ab-7 (4, 5). Due to the sequence identity being above the 80% threshold for a new *erm* determinant and to an altered phenotype compared to that seen with the original *erm*(44) from *S. xylosus* when expressed in *S. aureus*, the *erm* gene identified in *S. saprophyticus* N041 was assigned the name *erm*(44)$_v$ according to the nomenclature of the MLS$_B$ resistance genes (http://faculty.washington.edu/marilynr/) (13). However, the possibility cannot be excluded that *erm*(44)$_v$ might confer resistance to streptogramin B in *S. saprophyticus* due to the presence of a specific inducer which is absent in *S. aureus* RN4220.

The *erm*(44)$_v$ gene was located on a putative 19,400-bp genomic island (GenBank accession no. LN623525) which is absent in the MLS$_B$-susceptible strain *S. saprophyticus* KACC16562 (GenBank accession no. AHKB01; Fig. 2). In contrast to *erm*(44) from *S. xylosus* JW4341, which is situated on a prophage ΦJW4341-pro (4), the genomic composition of the island described here shows a rather heterogeneous composition of open reading frames (ORFs) remotely resembling that of a temperate siphoviral bacteriophage, SaPImw2, with the common presence of one terminase, two primases, two transcriptional regulators, and an integrase belonging to the tyrosine type of bacterial phage integrases (*Int-Ssapro1*; NCBI conserved domain number cd01189) (Fig. 2) (14). The genomic island contains an additional integrase of the same type (*Int-Ssapro2*; NCBI conserved domain number cd01189) at its distal end which potentially played a role in the integration and recombination of the genomic island into the *S. saprophyticus* genome. However, no conjugal transfer of macrolide resistance into *S. aureus* 80Cr5

(rifampin resistant [Rif^r]) (15) and *S. saprophyticus* 7108R (a rifampin-resistant mutant of 7108) (16) was observed by filter mating (17) using different donor-recipient ratios (10^6:10^8, 10^8:10^8, and 10^8:10^6 cells/ml) and 10 μg/ml erythromycin and 100 μg/ml rifampin in the brain heart infusion (BHI) agar selective plates. No circular form could be observed by PCR using GoTaq polymerase (Promega) and plasmid DNA (NucleoBond PC 100; Macherey-Nagel) as the template and using primer1 (5′-CCCGTTGTTACGGGG TTTCT) and primer2 (5′-GCGATAAAGAGCATTTTGATTTTCC) (annealing temperature, 55°C; extension time, 2 min), reading outward of the genomic island (Fig. 2).

Analysis of *Staphylococcus* whole-genome sequences using a MaGe microscope platform (https://www.genoscope.cns.fr/agc/microscope/home/) revealed that the genetic island containing *erm*(44)$_v$ inserted into a chromosomal hot spot, as most strains annotated in MaGe show large sequence variation at this specific locus. The genomic island integrated at a specific 19-bp integration site (*attC* [CCCTCCCAGGACACTAAAA]) situated between a metal-dependent phosphodiesterase and two tandem transposases (*InsO_Ssapro* and *InsE_Ssapro*; NCBI conserved protein family numbers COG2801 and COG2963) (Fig. 2). Attachment site *attC* was duplicated in the N041 strain with one perfect copy downstream (*attR*) and one imperfect copy upstream (*attL*) of the genomic island (Fig. 2).

This report describes an *erm*(44) gene variant, *erm*(44)$_v$, in a human isolate of *S. saprophyticus* which does not confer decreased susceptibility to streptogramin B in *S. aureus*, in contrast to the *erm*(44) gene from *S. xylosus* from milk and from *S. saprophyticus* from river water. However, besides this uncommon phenotype, the *erm*(44)$_v$ was found, like *erm*(44) from *S. xylosus* (4), on an element containing genes associated with phages, indicating that phage-associated elements may play a role in the spread of MLS_B resistance.

Accession number(s). The *erm*(44)$_v$-containing element and its insertion region in *S. saprophyticus* N041 have been deposited in the DDBJ/ENA/GenBank database under accession number LN623525.

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