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BACTERIOLOGY (N BOREL, SECTION EDITOR)

Discovery of Novel MLS_B Resistance Methylase Genes and Their Associated Genetic Elements in Staphylococci

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Abstract Erythromycin resistance methylases (Erm) confer resistance to three classes of clinically important antibiotics, the macrolides, the lincosamides, and the streptogramins B (MLS_B). Erm methylases are located on acquired genetic elements and are widespread in *Staphylococcus* and other bacterial species. The latest *erm* determinants have been identified predominantly in coagulase-negative staphylococci using whole-genome sequencing (WGS) and the subsequent search for sequence similarity to *erm* methylases and specific amino acid motifs. This review presents workflows facilitating the identification and characterization of novel methylase genes, as well as the current status of dissemination of *erm* genes and their associated genetic elements in staphylococci. Discovery of novel antibiotic resistance genes is necessary to give new insight into molecular epidemiology of antibiotic resistance, for the establishment of better identification and surveillance systems, as well as to continuously improve molecular diagnostic of antibiotic resistance.

Keywords Whole-genome sequencing (WGS) · *Staphylococcus* · *erm* · Mobile genetic elements · Macrolide · Lincosamide

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Introduction

The erythromycin resistance methylase (Erm) ribosomal RNA methylases are a class of enzymes which confer resistance to macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics in bacteria. Macrolides are bacteriostatic agents, first introduced in the 1950s in the form of erythromycin, produced by the bacterium *Saccharopolyspora erythrea* [1]. Macrolides have a 12- to 16-membered macrolactone ring which targets the bacterial ribosome mainly through its sugar substitutions [2–4]. Binding of the antibiotic to the bacterial ribosome obstructs the elongation of the nascent peptide chain and subsequently inhibits protein translation. Inhibition of protein synthesis is a mechanism also used by the structurally distinct antibiotic classes lincosamides and streptogramin B [2]. All MLS_B antibiotics possess overlapping binding sites and contact the adenine 2058 (A2058, *Escherichia coli* numbering) of the 23S rRNA, a key nucleotide for drug–ribosome interaction [2, 5]. Generally, MLS_B antibiotics are used to treat Gram-positive bacterial infections in both humans and animals [6–8]. Newer semisynthetic macrolide compounds have a wider range of action also targeting Gram-negative species like *Bartonella*, *Bordetella*, *Borrelia*, *Campylobacter*, *Chlamydia*, *Haemophilus*, *Helicobacter*, *Legionella*, *Neisseria*, *Moraxella*, and *Shigella* and some species of the Gram-positive related *Mycoplasma*, *Ureaplasma*, and *Mycobacterium* [9]. In addition, macrolides are used for their prokinetic and anti-inflammatory properties in human and veterinary medicine [10–12]. Lincosamides such as clindamycin and lincomycin are used against Gram-positive infections, as well as in the treatment of anaerobes, while streptogramin B antibiotics like virginiamycin S1, quinupristin, or pristinamycin 1A have gained importance due to their activity against vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-resistant *Enterococcus* (VRE) [6, 13]. In

addition, individual MLS_B compounds are extensively used as herd/flock medication to prevent or treat infectious diseases in food-producing animals [7, 13]. The World Health Organization (WHO) has classified macrolides as the highest-priority critically important antibiotics for human medicine and both lincosamides and streptogramins as highly important drug classes for human medicine [14]. The widespread use of MLS_B antibiotics jeopardizes their effectiveness by increasing the risk of selecting bacteria with acquired resistance to these classes of drugs [15–17].

One of the most common mechanism of resistance to MLS_B antibiotics is the chemical modification of the bacterial 23S rRNA by monomethylation or dimethylation of A2058 by Erm rRNA methylases [18]. Erm methylases add up to three methyl groups to nucleotides at positions 2057, 2058, and/or 2059 of the 23S rRNA [19, 20]. However, dimethylation of A2058 is the most common mechanism found in bacteria and efficiently prevents the binding of MLS_B antibiotics, including the macrolide derivates ketolides (e.g., telithromycin) [16, 18, 20, 21]. Erm methylases are encoded by *erm* genes which are either constitutively expressed or induced in the presence of 14- and 15-membered macrolides [22]. Inducible MLS_B resistance is regulated by a region upstream of the *erm* mRNA start codon. In staphylococci, this regulatory region consists of open reading frames (*orf*s) coding for either one or two leader peptides, of which one harbors the conserved IVFI or MRNVD amino acid motif [23–26]. The secondary structure of this leader mRNA embeds the ribosomal binding site and start codon of the *erm* gene in a hairpin structure, which prevents its expression. Binding of inducer macrolides such as erythromycin to the ribosome results in attenuated translation of the leader peptide mRNA, subsequently causing the ribosome to stall [26]. Translational attenuation causes a rearrangement of the mRNA hairpin secondary structure, releasing the ribosomal binding site and the start codon of the *erm* gene thus allowing translation of the methylase [27, 28]. As a result, isolates expressing an inducible phenotype can remain susceptible to lincosamides, streptogramin B, 16-membered macrolides, or ketolides if no macrolide is present [25]. However, selection of mutants constitutively expressing the *erm* gene may rapidly occur when lincosamides, streptogramin B, or ketolides are being used [9, 22]. Other mechanisms conferring resistance to all or individual classes of MLS_B antibiotics are listed in Table 1 and were not taken into consideration in this review.

The genus *Staphylococcus* comprises more than 50 species which are subdivided into two groups depending on their ability to coagulate plasma [29, 30]. The coagulase-positive staphylococci include *S. aureus*, *Staphylococcus argenteus*, *Staphylococcus delphini*, *Staphylococcus hyicus*, *Staphylococcus intermedius*, *Staphylococcus lutrae*, *Staphylococcus pseudintermedius*, *Staphylococcus schleiferi* subsp. *coagulans* and *Staphylococcus schweitzeri*, while all other species belong to the group of the coagulase-negative staphylococci (CoNS). Staphylococci are naturally found on the skin and mucous membranes of mammals, as well as in diverse environmental sources [30, 31]. They can turn into major opportunistic pathogens in humans and animals and cause a wide variety of infections ranging from skin and soft tissue infections to bacteremia [30, 32, 33]. In general, *S. aureus* and other coagulase-positive staphylococci are the main staphylococcal pathogens [33]. However, during recent years, some of the CoNS have emerged as an important cause of nosocomial infections in human medicine as well as in veterinary medicine where they have been increasingly associated with bovine mastitis, as well as with several types of infections in companion animals [33–36]. Due to the acquisition of multiple resistance mechanisms, antimicrobial therapy became limited with some staphylococcal species representing a serious threat for human and animal health [23, 36–41]. As β-lactams and other critically important classes turned out to be increasingly ineffective in the treatment of staphylococcal infections, macrolide and lincosamide classes gained importance as alternative antimicrobial treatment options [42].

Due to the alarming increase of antibiotic resistance in bacteria over the last decades, the WHO has declared the current state of antimicrobial resistance a global health security threat [43]. Worldwide surveillance of antibiotic-resistant bacteria in humans, animals, and the food chain, and the reduction of inappropriate use of antimicrobial drugs are the essential proposed actions to control the spread of antimicrobial resistance [43]. To successfully monitor resistance, it is crucial to know all mediators and reservoirs of antibiotic resistance. In the last 5 years, novel MLS_B resistance genes have been mostly detected in bacteria of animal origin [44–47]. These novel genes consisted of *erm*(43), *erm*(44), and *erm*(45) from different *Staphylococcus* species and *erm*(46) from *Rhodococcus equi* and were all detected using whole-genome sequencing (WGS) and subsequent sequence analysis.

Possible workflows useful for the discovery and characterization of novel *erm* genes using WGS are described in this review. Furthermore, the current state of distribution of *erm* genes in bacteria, as well as their location on different genetic elements in *Staphylococcus* sp., is presented, offering an updated baseline for future characterization of novel *erm* genes and of *erm*-containing genetic elements.

Search for Novel *erm* Methylases

Phenotypic Criteria The likelihood of the presence of an *erm* gene can be evaluated by the phenotypic observation of either resistance to both erythromycin and clindamycin or resistance to erythromycin and inducible resistance to clindamycin. An

Table 1 Mechanisms conferring resistance to macrolides, lincosamides and streptogramin B, as well as to other antibiotics

Mechanism	Mutation/gene family[a]	Resistance phenotype[b]	Selected references
Mutation	60S ribosomal protein [proteins L4 and L22]	**MLS**	[54, 88]
	23S rRNA [around adenine 2058]	**MLS$_B$**	[19, 54]
Efflux by ATP-binding-cassette (ABC) family transporters	*car*	**M**	[89]
	eat	**LS$_A$**P	[90]
	lsa	**LS$_A$**P	[90]
	msr	**MS$_B$**	[91, 92]
	ole	**M**	[93]
	sal	**LS$_A$**	[94]
	srm	**M**	[89, 95]
	tlc	**M**	[89, 96]
	vga	**LS$_A$**P	[71, 97]
Efflux by major facilitator superfamily (MFS) transporters	*cmr*	**M**RTON	[98]
	lmr	**L**	[99]
	mdf	**M**RTPhAF	[100]
	mdt	**MLS**T	[101]
	mef	**M**	[102]
	mre	**M**	[103]
Esterases	*ere*	**M**	[104]
Lyases	*vgb*	**S$_B$**	[105]
Phosphorylases	*mph*	**M**	[106]
Transferases	*lnu*	**L**	[107, 108]
	vat	**S$_A$**	[109, 110]
RNA methyltransferases	*cfr*	**LS$_A$**POPh	[111]
	erm	**MLS$_B$**	[70]

[a] Information on novel MLS resistance genes can be found in the nomenclature center (http://faculty.washington.edu/marilynr/), which is regularly updated by Dr. Marylin C. Roberts, University of Washington, Seattle, WA

[b] MLS antibiotics are represented in bold. M, macrolides; L, lincosamides; S, streptogramins; S_A, streptogramin A; S_B, streptogramin B; P, pleuromutilins; R, rifampicins; T, tetracyclines; N, novobiocin; Ph, phenicols; A, aminoglycosides; F, fluoroquinolones; O, oxazolidinones

inducible MLS_B phenotype can be visualized in vitro by performing *D*-Test or by MIC determination of clindamycin in the presence of 4 μg/ml of erythromycin [48, 49]. In the presence of an inducible phenotype, it can be assumed that the strain contains an *erm* gene, whereas a constitutive resistance phenotype may also be associated with other resistance mechanisms (Table 1).

Genetic Approach to Identify an *erm* Determinant Whole-genome sequencing has become more accessible during recent years due to increased user friendliness, efficiency, cost-effectiveness, and an increase in the number of facilities offering WGS services [50–52]. Compared to the classical shotgun cloning and subsequent phenotypic screening for the identification of candidate genes, WGS is significantly faster and therefore became the method of choice to identify novel MLS_B candidate genes in bacterial genomes. However, rapid preliminary screening of bacteria for the presence of already known resistance mechanisms should still be considered. In general, the detection of acquired MLS_B resistance is easier than that of resistance conferred by ribosomal mutations. Identification of ribosomal mutations calls for specific detection methods, since several operons of ribosomal genes are dispersed through the bacterial genome (Table 1)[53, 54]. Currently, microarrays represent an efficient and affordable tool to assign an acquired phenotype to known resistance genes [55, 56]. The recently described microarray of Strauss et al. is among the most suitable for allocating acquired resistance, as it can detect all currently known MLS_B methylase genes in *Staphylococcus* as well as most other genes associated with MLS_B resistance in Gram-positive bacteria [55]. If no association with a known resistance genotype can be made using such a microarray, there is a strong suspicion that the investigated strain contains a novel resistance mechanism and WGS becomes inevitable.

Nowadays, several WGS technologies for bacterial whole-genome sequencing are available including Illumina®MiSeq (Illumina, San Diego, CA), IonTorrent™ PGM (Life Technologies, Guilford, CT), PacBio® RSII (Pacific Biosciences, Menlo Park, CA), and MinION™ (Oxford Nanopore Technologies, Oxford, UK) [57, 58]. In general, techniques generating long read lengths, such as IonTorrent and PacBio, are recommended for de novo sequencing of new *erm* determinants which can be located on genetic elements that may contain several copies of repeat elements, such as insertion sequences (*IS*) and extensive direct or tandem repeats [59].

Subsequent screening of the generated sequence contigs for putative *erm* genes using bioinformatic tools is straightforward. By current nomenclature, Erm methylases are defined novel if they have ≤79 % amino acid homology with any known methylase (http://faculty.washington.edu/marilynr/MLSnomenclatureCenter.pdf) [8]. Novel Erm methylases can be detected by BLAST alignment of either their amino acid or nucleotide sequence with known *erm* determinants [60]. Several freely available online tools, such as ResFinder and ARG-ANNOT, are also useful to detect novel resistance genes based on DNA/amino acid homology [61]. Searching for a specific conserved amino acid motif is an alternative to the detection of putative Erm homologies. The majority of the *erm* methylases contain the PROSITE rRNA adenine dimethylase signature pattern PS01131 (Fig. 1) [44]. Exceptions to this rule are Erm(I), Erm(N), Erm(Z), Erm(32) , Erm(37), and Erm(41), which are found in *Streptomyces* and *Mycobacteria* and are suspected to be monomethylases, rather than dimethylases [20, 62]. To also include these additional *erm* methylases, a modified version of the PS01131 signature has been proposed by Schwendener and Perreten, allowing the detection of all known Erm methylases except Erm(32) (Fig. 1). Bioinformatic tools such as Scan Prosite (http://prosite.expasy.org/scanprosite/) or MOTIF (http://www.genome.jp/tools/motif/) can be used to search for such a specific Erm signature pattern. However, DNA sequences of entire contigs have to be first translated into all six amino acid reading frames and each frame must be screened individually.

Proof of Functionality The function of MLS_B resistance can only be attributed to a newly detected methylase gene after demonstration of an association between the gene and the phenotype. To demonstrate its expression in vitro, the gene can either be inactivated in the original strain generating a MLS_B susceptible knockout mutant or transformed into a MLS_B susceptible host generating a MLS_B-resistant transformant. Replacement of genes in field strains of *Staphylococcus* can be impaired by a low transformation efficiency and/or the presence of restriction modification systems; therefore, expression in a laboratory strain like *S. aureus* RN4220 is more frequently used [63, 64]. Recently, *S. aureus–E. coli* shuttle vectors pBUS1-HC and pBUS1-P_{cap}-HC have been shown to be suitable for expression of *erm* genes under control of their own promoter and the strong constitutive promoter of *S. aureus* type 1 capsule gene 1A [45, 46, 65]. Of note, inducibility will be kept only if the *erm* gene is still under control of its own regulatory sequence including the leader peptide region. On the other hand, the *erm* gene will be expressed constitutively in the absence of the leader peptide region [45, 46].

Identification of Regions Flanking *erm* Genes WGS provides further information on the regions flanking a novel gene and facilitates the characterization of the acquired genetic element carrying the new *erm* gene. For instance, plasmids can be identified by the presence of *rep*-genes, circularized sequence, or by a higher coverage [66]. Elements integrated into the bacterial chromosome can be identified by alignment of the *erm*-containing sequence region with that of a MLS_B-susceptible strain of the same species. This allows identification of both the integrated element and the integration site, as well as the adjacent housekeeping genes. PCR with primers designed from the putative flanking housekeeping genes can be used to identify additional susceptible strains lacking the integrated element. Such PCR experiments will further assist the identification of the chromosomal integration site in MLS_B-susceptible strains for which no whole-genome sequence is available. The presence of inverted and direct repeats, which are directly linked to transposases and integrases, is a further indication of foreign element integration.

Distribution of *erm* Genes and Their Association With Acquired Genetic Elements

The *erm* genes are widely distributed in Gram-positive, Gram-negative, aerobic, and anaerobic genera (Fig. 1) [8, 16]. Among the 38 currently described *erm* genes in bacteria, 12 have been identified in the genus *Staphylococcus* using PCR, hybridization, or sequence analysis. They consist of *erm*(A), *erm*(B), *erm*(C), *erm*(F), *erm*(G), *erm*(Q), *erm*(T), *erm*(Y), *erm*(33), *erm*(43), *erm*(44), and *erm*(45) [39, 45, 46] (Fig. 1 and Table 2). To date, the presence of all *erm* genes in *Staphylococcus* has been confirmed by sequence analysis, except for *erm*(F), *erm*(G), and *erm*(Q) [37, 67, 68]. Of note, the Erm determinants confirmed to be present in staphylococci by sequence analysis all clustered into the same branch of the Erm dendrogram (Fig. 1). Searching the GenBank and literature revealed that *erm*(A), *erm*(B), and *erm*(C) are the most widespread *erm* genes in the different staphylococcal species, as well as in a multitude of other bacterial genera (Table 2). The broad distribution of *erm*(A), *erm*(B), and *erm*(C) can be explained by their association with mobile genetic elements with a high potential for dissemination, like broad-host-range

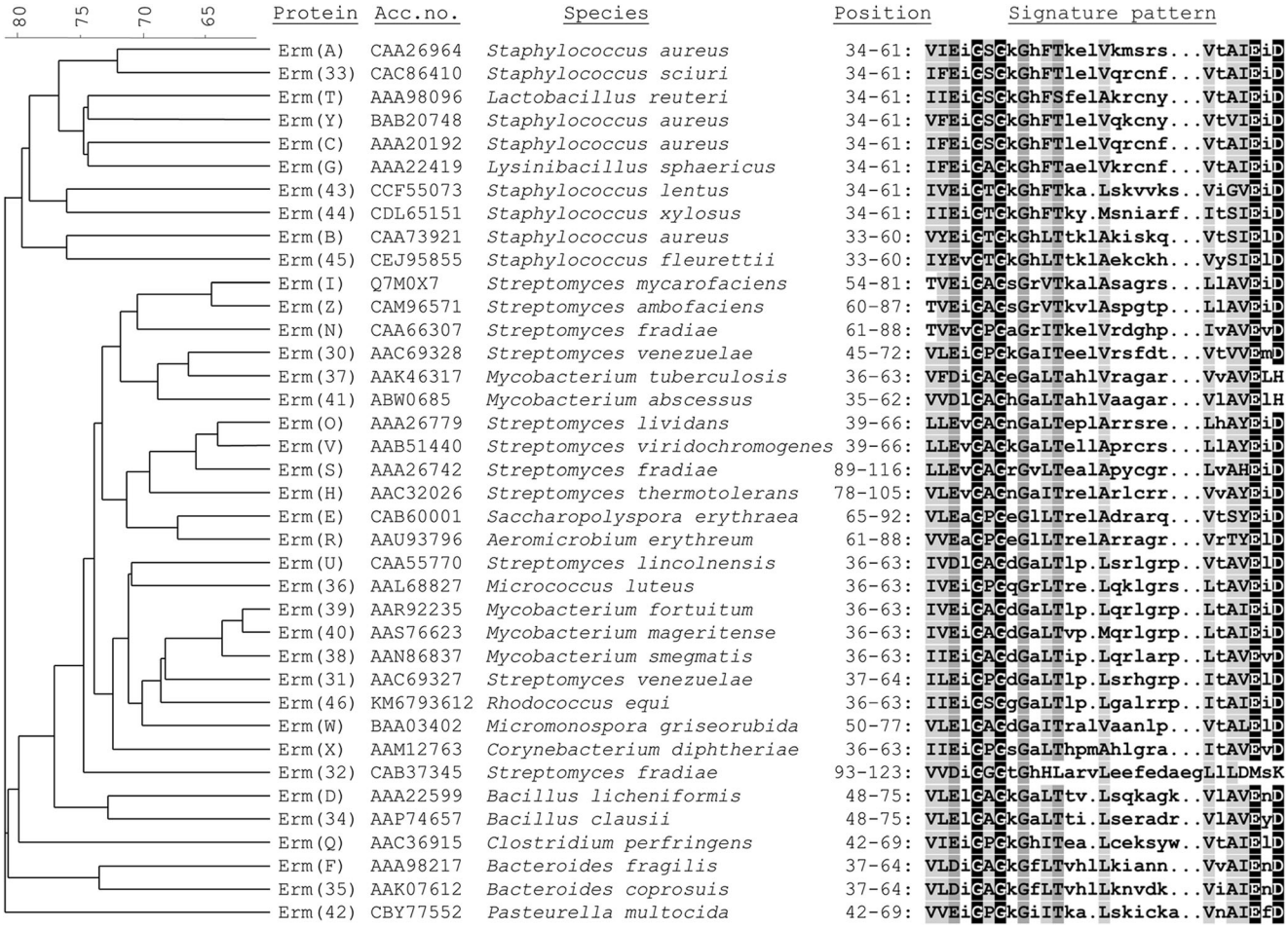

Fig. 1 Phylogenetic relatedness and alignment of the ribosomal RNA adenine dimethylase signature (PROSITE pattern PS01131) of all 38 Erm methylases (modified from Schwendener et al. [44]). Complete amino acid sequence (Acc. no.) of Erm methylases were used for dendrogram construction and were chosen from the species for which the protein was initially described (species). Clustering of Erm amino acid sequences was performed by BioNumerics 7.5 (Applied Maths). The comparison settings were standard algorithm for pairwise alignment, open gap penalty 100 %, unit gap penalty 0 %, and the unweighted-pair group method using average linkages. The PS01131 signature pattern, with amino acids acceptable for one given position listed between square brackets and x for any amino acid followed by the possible repetition range between parentheses, is defined as [LIVMAC]-[LIVMFYWT]-[DE]-x-G-[STAPVLCG]-G-x-[GAS]-x-[LIVMF]-[ST]-x(2,3)-[LIVMA]-x(5,8)-[LIVMYF]-x-[STAGVLC]-[LIVMFYHCS]-E-x-D. In the figure, invariant amino acids are shaded *black*, highly conserved amino acids *dark grey*, and less conserved positions *light grey*. A modified PS0113 signature to recognize most Erm methylases would be [LIVMACT]-[LIVMFYWT]-[DE]-x-G-[STAPVLCG]-G-x-[GAS]-x-[LIVMF]-[ST]-x(2,3)-[LIVMA]-x(5,8)-[LIVMYF]-x-[STAGVLC]-[LIVMFYHCS]-E-x-[DH]. Erm(32) is listed but does not contain a complete PS01131 signature

plasmids and transposons, whereas other *erm* genes are located on specific elements integrated into the chromosome. These integrated elements seem to be rather species specific or have lost their transfer machineries.

The *erm*(A) gene has been identified in a multitude of *S. aureus* strains and in CoNS (Table 2). The spread of *erm*(A) in *Staphylococcus* is mediated by transposon Tn*554* and its relatives such as Tn*6133*, which belong to the high-frequency and site-specific transposable elements [69–71]. Tn*554*-like transposons integrate into a specific chromosomal attachment site but may also use alternative integration sites, such as those present on acquired genetic elements like SCC*mec* elements and plasmids [72, 73].

Erm(B) is the most widespread methylase in different bacterial genera and is also common in a multitude of staphylococcal species (Table 2). Spread of *erm*(B) is mediated by Tn*917*/Tn*551* and Tn*5405*-like transposons [40, 74]. Those non-site-specific transposons easily conglomerate into larger transposal structures and promiscuously integrate into the chromosome and plasmids, offering further vessels for transportation and broad host distribution of *erm*(B) [75–78].

The *erm*(C) gene which is the most frequently annotated among staphylococcal species is commonly found on small plasmids (Table 2). Although these small plasmids are not self-transmissible, they contain mobilizing genes which contribute to their dissemination [79].

Table 2 Macrolide-lincosamide-streptogramin B resistance genes identified in staphylococci and their dissemination in other bacterial genera

Gene	Distribution in *Staphylococcus* sp. (Reference and /or GenBank accession number)[a,b]	Distribution in other bacterial genera (Reference and /or GenBank accession number)[a]
erm(A)	*S. aureus* (X03216) [112], *S. epidermidis* (CP000029) [113], *S. hyicus* [114], *S. hominis* [115], *S. sciuri* [142], *S. lentus* [116], *S. cohnii* [117], *S. capitis* [118], *S. warneri* [118], *S. simulans* [118], *S. rostri* [128]	*Actinobacillus*, *Streptococcus* [16], *Bacteroides*, *Helcococcus*, *Peptostreptococcus*, *Prevotella* [8], *Listeria* (AGN12846), *Lactobacillus* (WP_002360844), *Bacillus* (WP_041902801), *Gracilibacillus* (WP_018930853), *Aerococcus* (CP002512), *Nocarida* (KM194593), *Oceanobacillus* (BA000028)
erm(B)	*S. aureus* (Y13600) [119], *S. hyicus* (HE662694) [120], *S. lentus* (U35228) [121], *S. (pseud)intermedius* (AF299292) [122], *S. sciuri* (NG_041678) [123], *S. epidermidis*, *S. haemolyticus* [124], *S. hominis* [115], *S. cohnii* [123], *S. chromogenes*, *S. warneri* [125], *S. schleiferi* [116], *S. simulans* [126], *S. xylosus* [127], *S. equorum* [118], *S. fleurettii* [129]	*Actinobacillus*, *Clostridium*, *Escherichia*, *Enterococcus*, *Klebsiella*, *Neisseria*, *Pediococcus*, *Streptococcus*, *Wolinella* [16], *Acinetobacter*, *Aerococcus*, *Arcanobacterium*, *Bacillus*, *Bacteroides*, *Citrobacter*, *Corynebacterium*, *Enterobacter*, *Eubacterium*, *Fusobacterium*, *Gemella*, *Haemophilus*, *Lactobacillus*, *Micrococcus*, *Pantonea*, *Peptostreptococcus*, *Porphyromonas*, *Proteus*, *Pseudomonas*, *Ruminococcus*, *Rothia*, *Serratia*, *Treponema* [8], *Lactococcus* (AB290882), *Macrococcus* (AP009486), *Eggerthella* (AP012211), *Campylobacter* (KC876752), *Salmonella* (KR091911), *Listeria* (JX535233), *Nocardia* (KM194594), *Weissella* (KF245590)
erm(C)	*S. aureus* (V01278) [130], *S. chromogenes* (U82607) [131], *S. haemolyticus* (Y09002) [132], *S. simulans* (AF019140) [133], *S. epidermidis* (M12730) [134], *S. hominis* (Y09001) [132], *S. saprophyticus* (AM159501) [135], *S. equorum* (X82668) [132], *S. arlettae* (JF834911),*S. cohnii* (JQ219851) [123], *S. hycicus* (JF968543) [136], *S. lentus* (AJ888003) [137] *S. sciuri*, *S. warneri* [132],*S. capitis* [118],*S. (pseud)intermedius* [138], *S. gallinarium*, *S. xylosus* [132]	*Actinobacillus*, *Bacillus*, *Eubacterium*, *Lactobacillus*, *Neisseria*, *Streptococcus*, *Wolinella* [16], *Actinomyces, Bacteroides, Corynebacterium, Enterococcus, Haemophilus, Micrococcus, Prevotella, Peptostreptococcus* [8], *Capnocytophaga* (JQ886176)
erm(T)	*S. aureus* (FN390947) [139]	*Lactobacillus* [16], *Streptococcus* [8], *Erysipelothrix* (KM576795), *Haemophilus* (KC405064), *Bacillus* (AHN52258)
erm(F)	*S. aureus, S. haemolyticus*, *S. (pseud)intermedius*, *S. lentus*, *S. sciuri* [67]	
erm(Y)	*S. aureus* (AB014481) [140]	
erm(33)	*S. sciuri* (AJ313523) [141]	
erm(43)	*S. lentus* (HE650138) [44]	
erm(44)	*S. xylosusx* (HG796218) [45], *S. saprophyticus* (KJ728533) [86], (LN623525)	
erm(45)	*S. fleurettii* (LN680996) [46]	

[a] GenBank search was performed September 2015

[b] Genes identified by sequencing are shown in bold, while genes detected by PCR only are shown in regular typeface

The remaining *erm* genes detected in *Staphylococcus* sp. have been mainly associated with single species. For instance, *erm*(T) and *erm*(Y) were found in *S. aureus*, *erm*(33) in *Staphylococcus sciuri*, *erm*(43) in *Staphylococcus lentus*, and *erm*(45) in *Staphylococcus fleurettii* (Table 2). The only exception is *erm*(44), which has been detected in *Staphylococcus xylosus* and *Staphylococcus saprophyticus* (Table 2). However, one *erm*(44) gene from a *S. saprophyticus* of human origin and one *erm*(44) from a *S. saprophyticus* isolated from sewage were found to share 77 % amino acid homology with each other and 81 and 84 % homology to the original *erm*(44), respectively. This suggests a different epidemiological origin [80]. The *erm*(T), *erm*(Y), and *erm*(33) genes are all located on plasmids between 12 and 20 kb in size, and *erm*(T) has also been found integrated into the chromosome (Table 2) [81]. The *erm*(33) is additionally linked to Tn*554* located on a plasmid, most likely due to its relation to *erm*(A) [82].

The more recently described *erm* genes *erm*(43), *erm*(44), and *erm*(45) were found on so far unknown integrated elements. The *erm*(43) gene is located on an acquired fragment flanked by long direct repeats at a specific chromosomal site in *S. lentus* [44]. Structures like the *erm*(43)-carrying fragment arguably form a novel group of mobile genetic elements, which are also present in other species and associated with different resistance genes [83].

The recently discovered *erm*(44) was located in the genome of an integrated prophage in *S. xylosus* [45].

This prophage ΦJW4341-pro was the first to be fully sequenced and has been found to have naturally incorporated a resistance gene in *Staphylococcus*. The integration of bacterial DNA into phage genomes can lead to selective advantages for the phage host and was extensively described for virulence and other fitness-enhancing genes [84, 85]. With the detection of *erm*(44), the phenomenon of spreading genetic information via bacteriophages has gained in importance in the field of antimicrobial resistance dissemination. Although no active transduction of ΦJW4341-pro was observed, the occurrence of *erm*(44) in different *S. xylosus* from bovine mastitis suggests spreading of this gene by phage ΦJW4341-pro. Recently, *erm*(44) has also been identified on acquired chromosomal fragments in a human and environmental *S. saprophyticus* isolate using WGS (Table 2) [86]. The diverse flanking regions of the genetically distinct *erm*(44) genes support the suggested individual acquisition of those genes without direct transfer between *S. xylosus* and *S. saprophyticus* (Table 2) [86].

The *erm*(45) gene is another novel methylase gene detected on a phage related fragment [46]. It has been identified in a *S. fleurettii* strain isolated from bovine milk using WGS. This *erm*(45)-containing genetic island consists of open reading frames and a structure similar to those found in phage or the phage-associated *S. aureus* pathogenicity islands (SaPIs) [87]. Although capable of circularization, this island was not observed to be mobilized by either conjugation or transformation into *S. aureus* and its ability to disseminate is still unknown.

Conclusions

The recent discoveries of novel *erm* genes emphasize the role of staphylococci as a large reservoir of MLS_B resistance genes. Staphylococci seem to have a particular ability to acquire new genes through multifaceted mechanisms, including plasmids, transposons, genomic islands, and bacteriophage or bacteriophage-related elements. WGS has already widely contributed to the detection of so far unknown and large mobile genetic elements in staphylococci and other bacteria. Considering the ability of bacteria to rapidly adapt to foreign environments such as antimicrobial selective pressure, it is crucial to have tools for the rapid identification of new emerging resistance genes. Rapid detection of novel resistance genes from different ecological and clinical niches is of major importance for the continuous improvement of antibiotic resistance surveillance programs and diagnostics, as well as for well-targeted and prudent use of antibiotics.

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Compliance with Ethical Standards

Conflict of Interest The authors have no conflict of interest.

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