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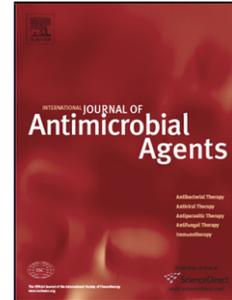
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Molecular basis of resistance to macrolides, lincosamides and streptogramins in *Staphylococcus saprophyticus* clinical isolates

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ABSTRACT

The aim of this study was to evaluate the prevalence of resistance to macrolide–lincosamide–streptogramin (MLS) antibiotics as well as to assess the molecular basis of this resistance among 72 *Staphylococcus saprophyticus* urinary isolates collected from 2005 to 2009 in University Hospital of Caen (France). Of the 72 strains studied, 33 (45.8%) were resistant to at least one MLS antibiotic, including 24 (72.7%) with an M phenotype, 5 (15.2%) with an inducible MLS_B phenotype, 3 (9.1%) with a combined M+L phenotype and 1 (3.0%) with an L phenotype. All isolates were susceptible to the combination of streptogramins A and B. The resistance genes *erm(A)*, *erm(B)*, *erm(C)*, *msr(A)* and *Inu(A)* were detected alone in 0, 0, 5 (15.2%), 24 (72.7%) and 1 (3.0%) of the 33 MLS-resistant isolates, respectively, whereas 2 strains (6.1%) were positive for both *msr(A)* and *Inu(A)*. All *msr(A)*-positive isolates exhibited an M phenotype, whereas all five *erm(C)*-positive and all three *Inu(A)*-positive strains displayed, respectively, an inducible MLS_B phenotype and an L phenotype with a positive Hodge test. Plasmid analysis indicated that *erm(C)* and *Inu(A)* genes were borne by small-size plasmids (ca. 2.5 kb), whereas larger plasmids (30–90 kb) harboured *msr(A)*. In conclusion, these findings show a high prevalence of MLS resistance in *S. saprophyticus*, which was mainly associated with the presence of the *msr(A)* gene. Since *S. saprophyticus* colonises the gastrointestinal tract, it may constitute an unexpected reservoir for MLS resistance genes, in particular *msr(A)*, among coagulase-negative staphylococci.

1. Introduction

Staphylococcus saprophyticus, a member of the coagulase-negative staphylococci (CoNS), is a common cause of acute uncomplicated urinary tract infection (UTI) in young, sexually active female outpatients, accounting for up to 42% of UTIs in this population [1]. More severe complications have also been reported, including acute pyelonephritis, septicaemia, nephrolithiasis and endocarditis, as well as infections in males such as urethritis, prostatitis and nephrolithiasis [2]. Except for fosfomycin, *S. saprophyticus* is susceptible to many antimicrobial agents, including macrolide–lincosamide–streptogramin (MLS) antibiotics [3–6]. However, since MLS antibiotics are excreted primarily in bile, they are not used for the treatment of UTIs. As a consequence, resistance to these antibiotics has been poorly documented in *S. saprophyticus* in comparison with other CoNS. Although *S. saprophyticus* appears to be an exclusive uropathogen, its major reservoir is the gastrointestinal tract, with the most common site being the rectum [7], and its mode of transmission may be through consumption of contaminated animal food products [8]. An study in the UK [9] as well as preliminary results from University Hospital of Caen (France) (unpublished data) showed a high prevalence of MLS resistance in *S. saprophyticus*, indicating that this species might be a potential reservoir of MLS resistance genes. However, molecular characterisation of resistance has not been yet investigated.

Although MLS antibiotics are chemically distinct, they are classified in the same group owing to their similar mechanism of action and spectrum of activity [10]. Macrolides are classified according to the number of atoms forming the lactone ring, i.e. 14-membered

(e.g. erythromycin), 15-membered (e.g. azithromycin) or 16-membered (e.g. spiramycin). Lincomycin and clindamycin belong to the lincosamides, and streptogramins correspond to a mixture of two compounds that act synergistically, i.e. streptogramins A (e.g. dalfopristin) and streptogramins B (e.g. quinupristin). MLS antibiotics act by inhibiting protein synthesis following binding to the 50S subunit (23S rRNA) of the bacterial ribosome [10].

In staphylococci, MLS resistance is mediated by three major mechanisms, namely target site modification, active efflux and drug inactivation [10]. Ribosomal alteration is mediated by a methyltransferase, encoded by *erm* genes (erythromycin ribosome methylase), which methylates the A2058 residue in domain V of the 23S rRNA. Although nearly 40 *erm* genes have been reported so far (<http://faculty.washington.edu/marilynr/>), *erm(A)*, *erm(B)* and *erm(C)* genes are the most frequently detected in staphylococci. This methylation results in the so-called MLS_B phenotype, which can be expressed either inducibly or constitutively. Inducible expression is characterised by unique resistance to 14- and 15-membered ring macrolides, which are inducers. Constitutive expression is characterised by resistance to all macrolides, lincosamides and streptogramins B. Mutations in 23S rRNA (A2058 and A2059) and in ribosomal proteins L4 and L22 (encoded by *rpID* and *rpIV* genes, respectively) have been infrequently described [11–13]. Active efflux is mediated by the *msr(A)* gene that codes for a putative efflux pump and is responsible for the so-called M phenotype characterised by unique resistance to 14- and 15-membered ring macrolides. The L phenotype is related to acquisition of the *Inu(A)* gene (formerly known

as *linA* or *linA*¹) that encodes 3-lincomycin, 4-clindamycin O-nucleotidyltransferase, which only inactivates lincosamides.

Since molecular characterisation of MLS resistance has not been yet undertaken in *S. saprophyticus*, the aim of this study was to evaluate the prevalence of MLS resistance among a collection of *S. saprophyticus* clinical isolates recovered from a French hospital and to identify genes associated with this resistance as well as their genetic supports.

2. Materials and methods

2.1. Bacterial isolates

From 2005 to 2009, a total of 72 *S. saprophyticus* clinical isolates were recovered from urine specimens (pure culture $\geq 10^5$ colony-forming units/mL) from the University Hospital of Caen (France). Isolates were identified to species level using a VITEK 2 ID-GPC card (bioMérieux, Marcy l'Etoile, France) in accordance with the manufacturer's instructions. Sequencing of the *sodA* gene was performed as described previously for 14 isolates because biochemical identification was equivocal [14].

Staphylococcus aureus ATCC 25922, *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as controls for antimicrobial susceptibility testing. *Staphylococcus aureus* HM290, *Streptococcus pneumoniae* HM28, *S. aureus* HM1054R, *S. aureus* RN4220(pUL5054) and *Staphylococcus haemolyticus* BM4610

were used as *erm(A)*-, *erm(B)*-, *erm(C)*-, *msr(A)*- and *lnu(A)*-positive controls, respectively. *Micrococcus luteus* ATCC 9341 and streptomycin-resistant *S. aureus* 80CR5 were used as indicator organism for the Hodge test and as recipient in conjugation experiments, respectively.

2.2. Antimicrobial susceptibility testing

In vitro susceptibility was determined for all 72 isolates by disk diffusion on Mueller–Hinton agar (disks and medium supplied by Bio-Rad Laboratories, Marnes-la-Coquette, France) according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (<http://www.sfm.asso.fr>). The following antibiotics were tested: cefoxitin (30 µg); kanamycin (30 µg); tobramycin (10 µg); gentamicin (15 µg); erythromycin (15 IU); lincomycin (15 µg); pristinamycin (15 µg); linezolid (30 µg); ofloxacin (5 µg); vancomycin (30 µg); teicoplanin (30 µg); fusidic acid (10 µg); rifampicin (30 µg); and trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 µg). Interpretation was performed in accordance with CA-SFM recommendations.

For MLS-resistant isolates, the minimum inhibitory concentrations (MICs) of erythromycin, spiramycin, lincomycin, clindamycin, quinupristin, dalfopristin and the combination quinupristin/dalfopristin (Q/ D) were determined by the broth microdilution technique (medium supplied by Bio-Rad) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.escmid.org/research_projects/eucast/).

A double-disk diffusion test (D-test) was performed with erythromycin and clindamycin disks applied 20 mm apart (edge-to-edge). Blunting of the zone of inhibition around the clindamycin disk was considered positive for inducible MLS_B resistance. The ability of the MLS-resistant strains to inactivate lincomycin and/or clindamycin was detected by the disk and radial streak test method (called the Hodge test [15]) in *M. luteus* ATCC 9341-seeded Mueller–Hinton agar. Distortion of the susceptibility zone of *M. luteus* along the line of inoculation of the test strain was considered positive, indicating antibiotic inactivation.

2.3. Polymerase chain reaction (PCR) amplification and sequencing

Bacterial genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France). Detection of *erm(A)*, *erm(B)*, *erm(C)*, *msr(A)* and *Inu(A)* genes was performed for MLS-resistant *S. saprophyticus* isolates. PCR experiments were carried out according to standard conditions using primers synthesised by Eurogentec France SAS (Angers, France) (Table 1). Briefly, 2 µL of total DNA was subjected to PCR in a 50 µL reaction mixture containing 1× PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl], 1.5 mM of MgCl₂, 200 µM of each deoxynucleotide triphosphate, 0.5 µM of each primer and 1.25 U of GoTaq[®] Flexi DNA Polymerase (Promega, Charbonnières-les-Bains, France). PCR amplifications were performed using a Mastercycler[®] gradient thermal cycler (Eppendorf, Le Pecq, France) as follows: (i) initial denaturation step of 5 min at 95 °C; (ii) 30 cycles consisting of denaturation for 30 s at 95 °C, annealing for 30 s at 50–58 °C (Table 1) and extension for 30 s at 72 °C; and (iii) a final extension step of 5 min at 72 °C.

To detect putative mutations responsible target for site modification, a portion of the *rrl* gene for domain V of the 23S rRNA gene as well as the entire sequence of the *rpID*, *rpIV* and *rpIC* genes (coding for L4, L22 and L3 ribosomal proteins, respectively) were amplified by PCR using the conditions described above and specific primers (Table 1). Purified PCR products were then directly sequenced with the same sets of primers in both directions by GATC Biotech (Konstanz, Germany).

2.4. Plasmid analysis and conjugation

Plasmid DNA of MLS-resistant strains was prepared using a modification of the Kieser technique [19]. Lysostaphin was substituted for lysozyme and was used at a final concentration of 200 mg/L. Plasmid DNA was analysed by agarose gel electrophoresis analysis, then by a Southern blot hybridisation assay with *erm(C)*-, *msr(A)*- and *Inu(A)*-specific probes labelled with digoxigenin–dUTP (DIG DNA Labelling Kit; Roche, Meylan, France) according to the manufacturer's instructions. Briefly, plasmid DNA inside the gel was denatured and was then transferred onto a HybondTM-N+ nylon membrane (Amersham, Courtaboeuf, France) for 2 h under vacuum. Following pre-hybridisation (1 h at 42 °C), the membrane was hybridised overnight at 42 °C and washed twice in 0.1% sodium dodecyl sulphate (SDS)–2× standard sodium citrate (SSC) for 5 min and then twice in 0.1% SDS–0.1× SSC for 15 min at 42 °C. Hybridisation reactions were visualised colorimetrically with an NBT/BCIP Nucleic Acid Detection Kit (Roche) according to the manufacturer's instructions.

Conjugation experiments using the streptomycin-resistant *S. aureus* 80CR5 strain as recipient were performed in solid culture media [20]. Transconjugants were selected on brain–heart infusion agar plates containing streptomycin (20 mg/L) and erythromycin (2 mg/L).

3. Results

3.1. Antimicrobial susceptibility

All 72 *S. saprophyticus* clinical isolates studied were susceptible to cefoxitin, aminoglycosides, glycopeptides, ofloxacin, linezolid, fusidic acid, rifampicin and SXT. However, 33 isolates (45.8%) were resistant to at least one of the MLS antibiotics, comprising 29 isolates (40.3%) resistant to erythromycin only, 3 isolates (4.2%) resistant to both erythromycin and lincomycin and 1 isolate (1.4%) resistant to lincomycin only. No resistance to pristinamycin or Q/D was observed.

For the 33 MLS-resistant strains, MIC ranges were as follows: erythromycin, 0.12 mg/L to >256 mg/L; spiramycin, 0.5–128 mg/L; lincomycin, 0.12 mg/L to >256 mg/L; clindamycin, 0.03 mg/L to >256 mg/L; dalfopristin, 0.12–16 mg/L; quinupristin, 1–8 mg/L; and Q/D, 0.12–0.5 mg/L (Table 2). The MICs for 50% and 90% of the organisms (MIC₅₀ and MIC₉₀ values) were, respectively: erythromycin, 32 mg/L and >256 mg/L; spiramycin, 4 mg/L and 8 mg/L; lincomycin, 0.25 mg/L and 8 mg/L; clindamycin, 0.03 mg/L and 0.06 mg/L; dalfopristin, 2 mg/L and 4 mg/L; quinupristin, 4 mg/L and 8 mg/L; and Q/D, 0.25 mg/L and 0.5 mg/L.

3.2. *MLS resistance phenotypes*

Four phenotypes were observed among the 33 MLS-resistant isolates. A large majority of them (24 isolates; 72.7%) displayed an M phenotype, whilst 5 isolates (15.2%) exhibited inducible MLS_B resistance (Table 2). Three isolates (9.1%) displayed an ML phenotype with low quinupristin MICs, which is not consistent with constitutive MLS_B resistance and would probably result from a combination of M and L phenotypes. A single isolate (3.0%) had an L phenotype (Table 2). Only three isolates presented a positive Hodge test; strain no. 15 was negative for this test.

3.3. *Characterisation of MLS resistance genes*

The most prevalent resistance determinant was *msr(A)*, which was detected alone in 24 strains (72.7%), followed by *erm(C)* and *Inu(A)* detected alone in 5 strains (15.2%) and 1 strain (3.0%), respectively (Table 2). Two strains (6.1%) were positive for both *msr(A)* and *Inu(A)* genes, whereas a single strain (strain no. 15) was negative for all screened genes. No *erm(A)* and *erm(B)* genes were detected. All isolates harbouring the *msr(A)* gene alone exhibited an M phenotype with a moderate level of resistance to erythromycin (MIC = 4–128 mg/L) and susceptibility to other MLS antibiotics (Table 2). All five *erm(C)*-positive strains presented an inducible MLS_B phenotype, with high-level resistance to erythromycin (MIC > 256 mg/L) in four of them as well as susceptibility to other MLS antibiotics for all five (Table 2). Finally, the three *Inu(A)*-positive strains were

resistant to lincomycin (MIC = 8–128 mg/L) but remained susceptible to clindamycin (MIC = 0.03–0.12 mg/L).

Isolate no. 15, which was negative for all five resistance genes, displayed a combined ML phenotype with resistance to erythromycin (MIC = 16 mg/L), spiramycin (MIC = 128 mg/L) and lincosamides (MIC > 256 mg/L), but susceptibility to streptogramins A and B. Since this phenotype did not correspond to any known resistance gene(s), the presence of chromosomal mutations in domestic genes was evaluated. No mutation was detected in *rrl*, *rpID* and *rpIV* genes, whereas a unique mutation (A103G) leading to an isofunctional substitution (I35V) was found in the *rpIC* gene. Therefore, the mechanism of MLS resistance in this isolate remains unknown.

3.4. Genetic support of MLS resistance genes

All 33 MLS-resistant *S. saprophyticus* strains contained at least one plasmid [Fig. 1(A1),(A2)]. Southern blot experiments showed that all detected resistance genes were located on plasmids. The size of *msr(A)*-containing plasmids varied from ca. 30 kb to 90 kb [Fig. 1(A1),(A2)], whereas that of *erm(C)*- and *lnu(A)*-containing plasmids was much smaller, ca. 2.5 kb [Fig. 1(B),(C)].

Since *erm(C)*- and *lnu(A)*-containing plasmids were, in theory, too small to be self-transferable or mobilisable, conjugation experiments were carried out only with several isolates carrying *msr(A)*-containing plasmids. Unfortunately, it was not possible to transfer the *msr(A)* gene from five different strains, for which different sizes of plasmids

were observed (strains no. 4, 7, 17, 18 and 26), to *S. aureus* 80CR5 recipient despite repeated attempts.

4. Discussion

In this study, *S. saprophyticus* clinical isolates were susceptible to almost all antimicrobial agents. These findings are in agreement with previously reported data where the prevalence of acquired resistance is generally <5% [3–6,9,21,22], confirming that *S. saprophyticus* is a multisusceptible species. Although all of the studied strains were susceptible to ceftiofur, methicillin resistance by acquisition of the *mecA* gene has been rarely reported [23,24].

Surprisingly, the prevalence of MLS resistance, particularly to erythromycin, is much higher than previously reported. According to studies conducted in the early 1980s, the prevalence of resistance to erythromycin and clindamycin varied from 8% to 16% and 0% to 6%, respectively [3–6]. Only a single study conducted in the UK in the late 1990s reported a higher prevalence (25.1%) of resistance to erythromycin, whilst the prevalence of resistance to clindamycin was only 1.5% [9]. A more recent study on antimicrobial susceptibility of *S. saprophyticus* clinical isolates recovered in Japan in 2003 showed that only 10 isolates (9.9%) were highly resistant to erythromycin (MIC \geq 64 mg/L) [23]. The high incidence of MLS resistance in the collection of isolates studied here might be the reflection of a particular situation in our hospital or a global evolution towards increased resistance. Finally, no *S. saprophyticus* resistant to the combination

of streptogramins A and B was found in this study; this resistance has not been yet reported in this species.

No data are available regarding the distribution of MLS resistance genes in *S. saprophyticus*. This study showed that the distribution of these genes is different from that generally reported for CoNS, mostly *Staphylococcus epidermidis*, *S. haemolyticus* and *Staphylococcus hominis* [17,25-27]. In these species, the most prevalent gene is generally *erm(C)* with a prevalence ranging from 44% to 78%, followed by the *erm(A)* gene with a prevalence ranging from 5% to 32%. Efflux of macrolides due to *msr(A)* is a mechanism found in only a minority of CoNS, representing 11–24% of erythromycin resistance [17,25–27]. In contrast, in this study MLS resistance in *S. saprophyticus* was mainly due to the presence of the *msr(A)* gene, as previously described in *S. haemolyticus* [28]. The *erm(B)* gene was not detected in this study, however this is not surprising since this resistance gene has been seldom described in staphylococci previously. Little is known about the prevalence of the *Inu(A)* gene in CoNS. A study reporting a prevalence of 5% identified a frequent association with *msr(A)* or an *erm* gene [17]. Note that a single study contrasts with data from other studies, reporting a high occurrence of *msr(A)* (53%) and *Inu(A)* (30%) genes among a collection of 98 CoNS comprising mostly *S. haemolyticus* ($n = 62$) and *S. epidermidis* ($n = 27$) [29].

The genetic support of MLS resistance in *S. saprophyticus* has been poorly studied. The current findings are similar to those previously reported for *S. aureus* and CoNS. *erm(C)* genes are usually located on small, structurally related, multicopy plasmids

ranging in size between 2.3 kb and 2.5 kb, and more rarely on structurally diverse larger plasmids of 3.7–4 kb [30]. To date, only one *erm(C)*-carrying plasmid of 4 kb has been characterised from *S. saprophyticus* [31]. *Inu(A)* genes are also located on small, structurally different plasmids of 2.3–3.8 kb [32,33], whereas the *msr(A)* gene was located on larger plasmids of ca. 20–40 kb [30,34]. Results of conjugation experiments suggest that these *msr(A)*-containing plasmids do not encode their own transfer function. However, they might be mobilised by other mobile elements, which remain to be evidenced. Finally, exchange of *erm(C)*- and *Inu(A)*-containing plasmids might be due to generalised transduction mediated by many bacteriophages known to be present in staphylococci [35].

For *S. saprophyticus* strain no. 15, only a single point mutation has been identified in the *rplC* gene coding for the L3 ribosomal protein. Various mutations in this gene have been only associated with resistance to oxazolidinones [36,37] and pleuromutilins [38], but at different positions. However, strain no. 15 was entirely susceptible to linezolid (an oxazolidinone) and tiamulin (a pleuromutilin) (data not shown). The involvement of this mutation in putative MLS resistance should be confirmed by site-directed mutagenesis. In addition, this strain harboured at least two plasmids (sizes ca. 60 kb and 80 kb) [Fig. 1(A1)] and further investigations regarding the transferability of the resistance are in progress in addition to cloning experiments.

This is the first description of the distribution of MLS resistance genes in *S. saprophyticus* showing that, in contrast to other CoNS, MLS resistance in *S.*

saprophyticus is mainly due to the presence of the plasmid-mediated *msr(A)* gene and that this species may be a reservoir of these resistance genes among staphylococci.

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Competing interests

None declared.

Ethical approval

Not required.

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Fig. 1. Plasmid content analysis of *Staphylococcus saprophyticus* isolates. Ethidium bromide-stained electrophoresis pattern of undigested plasmids (left) and corresponding Southern hybridisation assay (right) using a specific probe for (A1/A2) *msr(A)*, (B) *erm(C)* or (C) *Inu(A)*. Lanes 1–33, refer to strains described in Table 2; M, *Escherichia coli* NCTC 50192 (used as reference for plasmid sizes).

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Table 1

Oligonucleotide primers used in this study

Target gene	Primer ^a	Sequence (5'→3')	Amplicon size (bp)	Annealing temperature (°C)	Source
<i>erm(A)</i>	<i>ermA-F</i>	TCTAAAAAGCATGTAAAAGAAA	533	52	[16]
	<i>ermA-R</i>	CGATACTTTTTGTAGTCCTTC			
<i>erm(B)</i>	<i>ermB-F</i>	CCGTTTACGAAATTGGAACAGGTAAAGGGC	359	55	[17]
	<i>ermB-R</i>	GAATCGAGACTTGAGTGTGC			
<i>erm(C)</i>	<i>ermC-F</i>	GCTAATATTGTTTAAATCGTCAATTCC	572	52	[17]
	<i>ermC-R</i>	GGATCAGGAAAAGGACATTTTAC			
<i>msr(A)</i>	<i>msrA-F</i>	TGCTGACACAATTTGGGAT	278	54	This study
	<i>msrA-R</i>	GAGCAGCCTTCTCAACC			
<i>Inu(A)</i>	<i>linA-F</i>	GGTGGCTGGGGGGTAGATGTATTA ACTGG	323	57	[17]
	<i>linA-R</i>	GCTTCTTTTCAAATACATGGTATTTTTTCGA			
<i>rrl</i>	23SCPU8	GACCCGCACGAAAGGCG	942	50	[18]
	23SCPL10	ATAAGTCCTCGAGCGATTAG			
<i>rplD</i>	L4F	GACGTATGGGCGGTAACAC	1007	52	This study
	L4R	CCTTGGTAACGGCCCATAC			
<i>rplV</i>	L22F	CGTAACAGAAGATATGGTTG	636	52	This study
	L22R	CGTAACAGAAGATATGGTTG			

<i>rpl</i>	L3F	ACTGTTATTCGTGCCGTGC	936	58	This
	L3R	CGCTTAGTTCAACTGAACCT			study

^a F, sense primer; R, antisense primer.

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Table 2

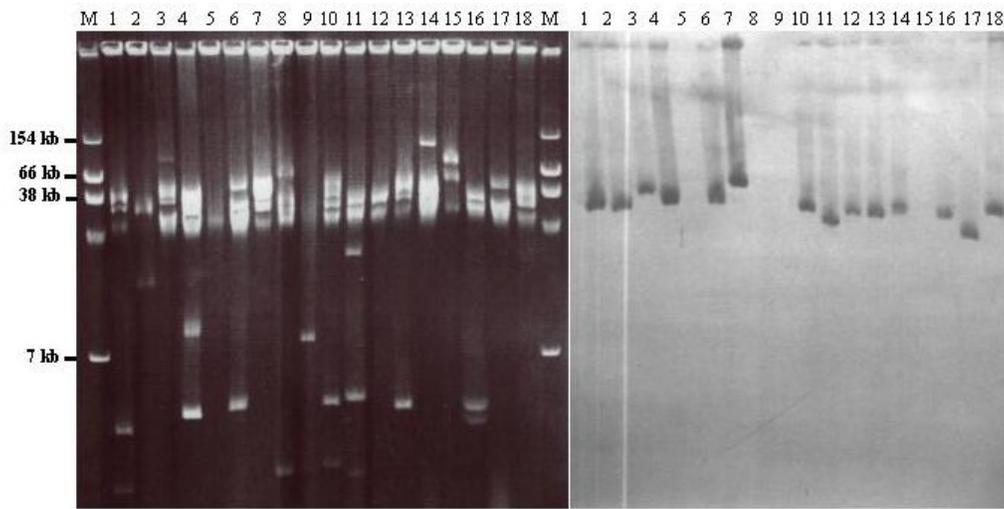
Susceptibility profiles to macrolide–lincosamide–streptogramin (MLS) antibiotics and corresponding resistance genotypes of 33 MLS-resistant *Staphylococcus saprophyticus* clinical isolates

Strain	MIC (mg/L)							D-test	Hodge test	PCR detection of:				
	ERY	SPI	LIN	CLI	DAL	QUI	Q/D			<i>erm(A)</i>	<i>erm(B)</i>	<i>erm(C)</i>	<i>msr(A)</i>	<i>Inu(A)</i>
1	64	4	0.12	0.03	4	4	0.25	–	–	–	–	–	+	–
2	64	4	0.12	0.03	4	4	0.25	–	–	–	–	–	+	–
3	64	8	0.5	0.06	4	2	0.25	–	–	–	–	–	+	–
4	64	4	0.5	0.06	2	4	0.12	–	–	–	–	–	+	–
5	16	4	0.12	0.03	4	4	0.25	–	–	–	–	–	+	–
6	32	1	0.12	0.03	4	4	0.25	–	–	–	–	–	+	–
7	128	8	0.5	0.06	4	8	0.5	–	–	–	–	–	+	–
8	>256	4	0.5	0.06	2	8	0.25	+	–	–	–	+	–	–
9	8	1	0.12	0.03	0.5	1	0.12	+	–	–	–	+	–	–
10	32	2	0.25	0.03	2	4	0.25	–	–	–	–	–	+	–
11	4	2	64	0.06	4	2	0.25	–	+	–	–	–	+	+
12	16	4	0.25	0.03	2	4	0.25	–	–	–	–	–	+	–
13	128	8	0.12	0.06	4	8	0.5	–	–	–	–	–	+	–
14	128	4	0.12	0.03	4	8	0.5	–	–	–	–	–	+	–
15	16	128	>256	>256	1	8	0.25	–	–	–	–	–	–	–
16	16	2	0.12	0.03	1	4	0.25	–	–	–	–	–	+	–

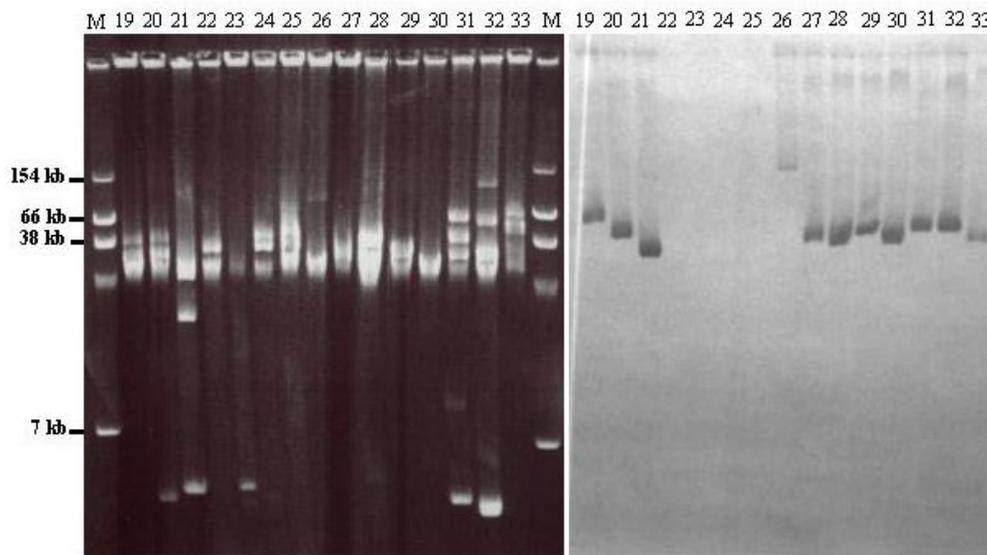
17	8	4	0.5	0.03	2	4	0.25	-	-	-	-	-	-	+	-
18	16	1	0.12	0.03	4	4	0.25	-	-	-	-	-	-	+	-
19	32	1	0.12	0.03	0.5	8	0.25	-	-	-	-	-	-	+	-
20	16	2	0.5	0.03	2	8	0.25	-	-	-	-	-	-	+	-
21	16	4	0.5	0.03	1	8	0.25	-	-	-	-	-	-	+	-
22	0.12	1	8	0.03	1	2	0.25	-	+	-	-	-	-	-	+
23	>256	2	0.5	0.03	2	4	0.25	+	-	-	-	-	+	-	-
24	>256	4	0.25	0.03	4	2	0.25	+	-	-	-	-	+	-	-
25	>256	4	0.5	0.12	2	4	0.25	+	-	-	-	-	+	-	-
26	128	16	0.5	0.06	16	8	0.5	-	-	-	-	-	-	+	-
27	128	2	0.5	0.03	2	8	0.25	-	-	-	-	-	-	+	-
28	64	8	128	0.12	16	8	0.5	-	+	-	-	-	-	+	+
29	64	8	0.25	0.03	2	8	0.5	-	-	-	-	-	-	+	-
30	8	2	0.25	0.03	2	4	0.25	-	-	-	-	-	-	+	-
31	16	0.5	0.12	0.03	0.12	1	0.12	-	-	-	-	-	-	+	-
32	128	8	0.25	0.03	4	8	0.25	-	-	-	-	-	-	+	-
33	8	2	0.25	0.03	4	8	0.25	-	-	-	-	-	-	+	-

MIC, minimum inhibitory concentration; ERY, erythromycin; SPI, spiramycin; LIN, lincomycin; CLI, clindamycin; DAL, dalfopristin; QUI, quinupristin; Q/D, quinupristin/dalfopristin; D-test, double-disk diffusion test; PCR, polymerase chain reaction.

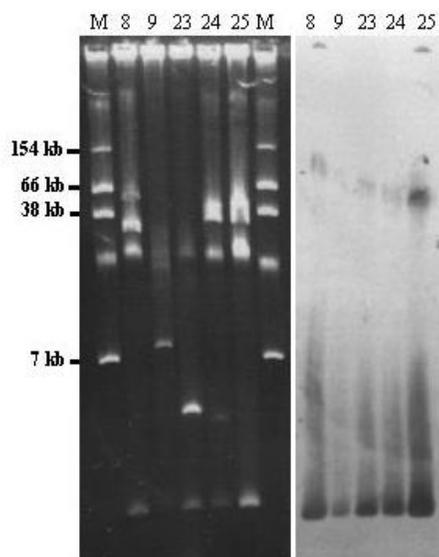
A1



A2



B



C

