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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 amongst 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 *lnu*(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 *lnu*(A). All *msr*(A)-positive isolates exhibited an M phenotype, whereas all five *erm*(C)-positive and all three *lnu*(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 *lnu*(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), amongst 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]. A 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

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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
erm(A)	ermA-F	TCTAAAAAGCATGTAAAAGAAA	533	52	[16]
	ermA-R	CGATACTTTTTGTAGTCCTTC			
erm(B)	ermB-F	CCGTTTACGAAATTGGAACAGGTAAAGGGC	359	55	[17]
	ermB-R	GAATCGAGACTTGAGTGTGC			
erm(C)	ermC-F	GCTAATATTGTTTAAATCGTCAATTCC	572	52	[17]
	ermC-R	GGATCAGGAAAAGGACATTTTAC			
msr(A)	msrA-F	TGCTGACACAATTTGGGAT	278	54	This study
	msrA-R	GAGCAGCCTTCTCAACC			
lnu(A)	linA-F	GGTGGCTGGGGGGTAGATGTATTAACTGG	323	57	[17]
	linA-R	GCTTCTTTTGAAATACATGGTATTTTTCGA			
rrl	23SCPU8	GACCCGCACGAAAGGCG	942	50	[18]
	23SCPL10	ATAAGTCCTCGAGCGATTAG			
rplD	L4F	GACGTATGGGCGGTAACAC	1007	52	This study
	L4R	CCTTGGTAACGGCCCATAC			
rplV	L22F	CGTAACAGAAGATATGGTTG	636	52	This study
	L22R	CGTAACAGAAGATATGGTTG			
rpl	L3F	ACTGTTATTCGTGCCGTGC	936	58	This study
	L3R	CGCTTAGTTCAACTGAACCT			

[a] F, sense primer; R, antisense primer.

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 *rplD* and *rplV* 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 *lnu*(A) gene (formerly known as *linA* or *linA′*) that encodes a 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 amongst 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. *Escherichia coli* NCTC 50192 was used as reference for plasmid sizes.

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 *lnu*(A) genes was performed for MLS-resistant *S. saprophyticus* isolates. PCR experiments were carried out according