



## Short Communication

## Distribution of Genes Encoding Resistance to Macrolides, Lincosamides and Streptogramins Among Clinical Staphylococcal Isolates in a Turkish University Hospital

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This study investigated the prevalence of genes encoding resistance to macrolides, lincosamides and streptogramins (MLS<sub>B</sub>) among staphylococci in a series of 301 erythromycin-resistant clinical isolates of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS). Erythromycin-resistance phenotypes were determined according to Clinical and Laboratory Standards Institute guidelines and specific resistance genes *erm*(A), *erm*(B), *erm*(C), *msr*(A) and *msr*(B) were identified using polymerase chain reaction. Two hundred of 301 (66.5%) erythromycin-resistant staphylococcal isolates exhibited resistance to MLS<sub>B</sub> antibiotics. Of these, 127 (63.5%) exhibited a cMLS<sub>B</sub> resistance phenotype (resistant to both erythromycin and clindamycin), whereas 73 (36.5%) expressed the iMLS<sub>B</sub> resistance phenotype (resistant to erythromycin and susceptible to clindamycin). The most prevalent resistance determinants were *erm*(A) (62%) among *S. aureus* and *erm*(C) (30%) among CoNS isolates. Combinations of resistance mechanisms were rarely seen, and occurred most often in oxacillin-resistant isolates. The results of the present study support the idea that there are geographical differences in the prevalence of erythromycin resistance mechanisms among staphylococci, therefore local surveillance studies are important tools for guiding therapy and in the promotion of judicious use of antimicrobial agents.

**KEYWORDS:** *erm* genes, MLS<sub>B</sub> resistance, staphylococci

### Introduction

Empirical outpatient treatment options for staphylococcal infections have become more limited as

methicillin resistance has increased. The efficacy of macrolide antibiotics in the treatment of infections caused by multi-resistant staphylococci makes them the most often prescribed drugs in clinical medicine. However, the expanded use of these antibiotics has been accompanied by increased resistance rates among staphylococci all over the world.<sup>1,2</sup>

Macrolide antibiotic resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) may be due to an active drug efflux mechanism encoded by *msrA* and *msrB* (conferring resistance to macrolides and type B streptogramins only) or may be the result of ribosomal target modification, mediated by the presence of *erm* genes [*erm*(A), *erm*(B) and *erm*(C)] conferring resistance to macrolides,

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lincosamides, and type B streptogramins (MLS<sub>B</sub> resistance).<sup>2,3</sup> When the underlying mechanism of resistance to erythromycin is an altered efflux system, expressed by the *msrA/msrB* gene, staphylococcal strains are susceptible to clindamycin, whereas ribosomal methylation expressed by the *erm* gene could render strains resistant to clindamycin. Phenotypic expression of MLS<sub>B</sub> resistance in staphylococci can be either inducible or constitutive. Strains with inducible MLS<sub>B</sub> (iMLS<sub>B</sub>) resistance demonstrate *in vitro* resistance to 14- and 15-member macrolides, but appear susceptible to 16-member macrolides, lincosamides and type B streptogramins. Strains with constitutive MLS<sub>B</sub> (cMLS<sub>B</sub>) resistance are considered resistant to all MLS<sub>B</sub>-type antibiotics, although streptogramin A-type antibiotics escape resistance.<sup>2</sup>

Although there is growing data regarding the prevalence of MLS<sub>B</sub> resistance phenotypes among staphylococcal isolates from different regions of Turkey,<sup>4-8</sup> data on the genetic determinants of MLS<sub>B</sub> resistance are limited.<sup>9,10</sup> The present study was undertaken to investigate the prevalence of genes encoding resistance to MLS<sub>B</sub> antibiotics in a series of 301 erythromycin-resistant clinical isolates of *S. aureus* and CoNS.

## Methods

### Bacterial isolates

This study included 301 erythromycin-resistant staphylococcal isolates consisting of 47 *S. aureus* [32 methicillin-resistant *S. aureus* (MRSA) and 15 methicillin-susceptible *S. aureus* (MSSA)] and 254 CoNS [161 methicillin-resistant CoNS (MRCoNS) and 93 methicillin-susceptible CoNS (MSCoNS)], collected between January 2006 and January 2007 in the Clinical Microbiology Laboratory of the Faculty of Medicine, Suleyman Demirel University (Isparta, Turkey). All staphylococcal isolates determined to be resistant to erythromycin and clinically significant based on species identification were included in the study. Duplicate isolates from the same patients were excluded. Isolates were characterized to the species level by Gram staining, catalase, slide and tube coagulase and by biochemical tests (API Staph; bioMerieux, Marcy l'Etoile, France).

### Susceptibility testing

Erythromycin and oxacillin susceptibilities of the strains were determined by the Kirby-Bauer disk diffusion

method following the criteria of the Clinical and Laboratory Standards Institute.<sup>11</sup> The resistance phenotypes of erythromycin-resistant isolates were determined by the double-disk test method in accordance with the recommendations of the Clinical and Laboratory Standards Institute. *S. aureus* ATCC BAA-977 was used as a positive control for iMLS<sub>B</sub> resistance and *S. aureus* ATCC 25923 was used as a control for the other tested antibiotics.

### Polymerase chain reaction amplification of MLS<sub>B</sub> resistance genes

Polymerase chain reaction (PCR) was carried out on the 301 staphylococcal strains displaying resistance to erythromycin. Genomic DNA was extracted from staphylococcal cultures using a genomic DNA purification kit (Fermentas Life Sciences) and used as a template for amplification. Extracted DNA was stored at -20°C until PCR was performed. The *erm(A)*, *erm(B)*, *erm(C)*, *msr(A)* and *msr(B)* genes were detected by PCR using the oligonucleotide primer pairs described by Lim et al<sup>1</sup> and Spiliopoulou et al.<sup>12</sup> These oligonucleotides were synthesized by Bio Basic Inc (Canada).

Five different PCRs were run for each isolate. Each reaction was carried out in a final volume of 50 µL and included 5 µL of 10× PCR buffer [100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl] (Fermentas), 2 mM MgCl<sub>2</sub> (Fermentas), 0.2 mM each of dATP, dTTP, dCTP and dGTP (Fermentas), 0.4 mM of the sense and anti-sense primers, 1.25 U *Taq* DNA polymerase (Fermentas) and 1 µL of extracted template DNA. The PCR were initialized by a denaturation step (5 min at 94°C) followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 54°C, 1 minute at 72°C; and a final extension step (5 min at 72°C). PCR products were detected by gel electrophoresis (100 V, 60 min) in 2% (w/v) agarose gels containing ethidium bromide. The sizes of the PCR products were estimated with standard molecular weight markers (1.5–100 kb DNA ladder; Bio Basic Inc., Ontario, Canada). Isolates were considered positive for *erm(A)*, *erm(B)*, *erm(C)*, *msr(A)* and *msr(B)* genes when the respective PCR products of the expected size could be visualized.

### Statistical analysis

Statistical analysis was conducted using Fischer's exact test and  $\chi^2$  test.

## Results

### *MLS<sub>B</sub> resistance phenotypes*

Two hundred (66%) of the 301 erythromycin-resistant staphylococcal isolates exhibited resistance to *MLS<sub>B</sub>* antibiotics; of these, 127 (64%) exhibited a *cMLS<sub>B</sub>* resistance phenotype, whereas 73 (37%) expressed the *iMLS<sub>B</sub>* resistance phenotype. It was observed that 40 (85%) of 47 *S. aureus* and 160 (63%) of 254 CoNS isolates exhibited *MLS<sub>B</sub>* resistance. Approximately 44% of CoNS isolates expressed the *cMLS<sub>B</sub>* phenotype, and 19% expressed the *iMLS<sub>B</sub>* phenotype whereas 51% of *S. aureus* isolates expressed *cMLS<sub>B</sub>* and 34% expressed the *iMLS<sub>B</sub>* phenotype. When the resistance rates were evaluated according to oxacillin resistance of the strains, 29 of 32 (91%) MRSA, 11 of 15 (73%) MSSA, 112 of 161 (70%) MRCoNS, and 48 of 93 (52%) MSCoNS displayed *MLS<sub>B</sub>* resistance. *cMLS<sub>B</sub>* was detected in 89 of 161 (55%) MRCoNS, 14 of 32 (44%) MRSA, 22 of 93 (24%) MSCoNS and 2 of 15 (13%) MSSA. *iMLS<sub>B</sub>* was detected in 9 of 15 (60%) MSSA, 15 of 32 (47%) MRSA, 26 of 93 (28%) MSCoNS, and 23 of 161 (14%) MRCoNS. *MLS<sub>B</sub>* resistance was more prevalent among *S. aureus* strains, and oxacillin-resistant strains exhibited significantly higher *MLS<sub>B</sub>* resistance rates compared with oxacillin-susceptible strains ( $p < 0.001$ ). The most frequently detected resistance phenotype among all erythromycin-resistant staphylococcal isolates was the constitutive type and this resistance phenotype was more frequently encountered among oxacillin-resistant strains. Additionally, the *iMLS<sub>B</sub>* resistance phenotype was significantly higher among *S. aureus* than CoNS strains ( $p < 0.001$ ).

### *MLS<sub>B</sub> resistance genotypes*

The distribution of resistance genes is presented in the Table. The most prevalent resistance determinant among *S. aureus* was *erm(A)*, which was detected in 62% (29/47) of the isolates. The *erm(C)* determinant was found as a single *MLS<sub>B</sub>* resistance gene in eight (17%) *S. aureus* isolates while three isolates (6%) contained both *erm(A)* and *erm(C)*. *msrA/B* genes were detected among 13 *S. aureus* isolates (27%). The most prevalent resistance determinant among CoNS was *erm(C)*, which was detected in 30% (77/254) of the isolates. The *erm(A)* determinant was found as a single *MLS<sub>B</sub>* resistance gene in 44 (17%) CoNS isolates while 11 isolates (4%) contained both *erm(A)* and *erm(C)*,

and *msrA/B* genes were detected among 81 CoNS isolates (32%). *Erm(B)* was detected only in three MRCoNS strains with the *cMLS<sub>B</sub>* resistance phenotype and one of these strains also contained *erm(C)*.

Combinations of resistance mechanisms were rarely seen, and occurred mainly in oxacillin-resistant isolates. Eighty-three of the erythromycin-resistant isolates did not harbor any of the tested resistance mechanisms. Seventy-seven of these isolates were CoNS, while only six were *S. aureus*.

## Discussion

While data on genetic determinants of *MLS* resistance from Turkey are limited to the reports of Aktas et al and Saribas et al,<sup>9,10</sup> other groups have studied the prevalence of *MLS* resistance using phenotypic methods in other hospitals from Turkey.<sup>4-8</sup> While the *MLS<sub>B</sub>* resistance rate determined in our hospital was higher than the rate reported by Tunckanat and Arikan,<sup>8</sup> it was lower than the rate reported by Delialioglu et al.<sup>6</sup> Overall, the most frequently determined genetic determinant responsible for *MLS<sub>B</sub>* resistance was *erm(C)*, with a constitutive resistance phenotype in our study. This finding was in accordance with previous reports from Turkey.<sup>9,10</sup> Aktas et al reported that 57.8% of CoNS isolates expressed the *cMLS<sub>B</sub>* phenotype and 20.6% expressed *iMLS<sub>B</sub>*. These rates were similar to the rates we detected (44% and 19%, respectively) for CoNS isolates. However, Aktas et al also reported that 78.2% of their CoNS isolates harbored the *erm(C)* gene and 8.9% had *erm(A)*. The prevalence rates we detected were 30% for *erm(C)* and 17% for *erm(A)*. Aktas et al reported that in *S. aureus*, *cMLS<sub>B</sub>* (58.3%) was more common than the inducible phenotype (20.8%) and *erm(A)* was detected in 50% and *erm(C)* in 62.5% of the isolates.<sup>9</sup> Saribas et al<sup>10</sup> reported that *cMLS<sub>B</sub>* was more common (64%) than the inducible phenotype (36%), and their findings were in accordance with Aktas et al. This contrasted with our study in which the most prevalent phenotype among *S. aureus* detected was the *iMLS<sub>B</sub>* type (51%) mediated by the *erm(A)* (62%) genes. While our findings for *S. aureus* isolates differ with the two studies from Turkey and from the results of Spiliopoulou et al<sup>12</sup> and Schmitz et al<sup>13</sup> (both of which also identified a predominance of the *erm(C)* gene among *S. aureus*), other published studies agreed with our finding

**Table.** Distribution of resistance genes among 301 erythromycin-resistant staphylococci isolates<sup>a</sup>

Isolate	Resistance type	<i>Erm A</i>	<i>Erm B</i>	<i>Erm C</i>	<i>Erm A+C</i>	<i>msrA/B</i>	<i>Erm+msr</i>	ND
<i>S. aureus</i> (n=47)	iMLS <sub>B</sub> (n=24)	14	0	6	1	7	5	3
	cMLS <sub>B</sub> (n=16)	15	0	2	2	2	1	0
	MLS <sub>B</sub> ∅ (n=7)	0	0	0	0	4	0	3
	Total (n= 47)	29 (62)	0	8 (17)	3 (6)	13 (27)	6 (13)	6 (13)
MRSA (n=32)	iMLS <sub>B</sub> (n=15)	9	0	4	1	2	2	3
	cMLS <sub>B</sub> (n=14)	13	0	2	2	2	1	0
	MLS <sub>B</sub> ∅ (n=3)	0	0	0	0	2	0	1
	Total (n=32)	22	0	6	3	6	3	4
MSSA (n=15)	iMLS <sub>B</sub> (n=9)	5	0	2	0	5	3	0
	cMLS <sub>B</sub> (n=2)	2	0	0	0	0	0	0
	MLS <sub>B</sub> ∅ (n=4)	0	0	0	0	2	0	2
	Total (n=15)	7	0	2	0	6	2	2
CoNS (n=254)	iMLS <sub>B</sub> (n=49)	11	0	21	3	6	3	17
	cMLS <sub>B</sub> (n=111)	33	3	56	8	28	13	13
	MLS <sub>B</sub> ∅ (n=94)	0	0	0	0	47	0	47
	Total (n=254)	44 (17)	3 (1)	77 (30)	11 (4)	81 (32)	16 (6)	77 (30)
MRCoNS (n=161)	iMLS <sub>B</sub> (n=23)	9	0	15	3	3	3	2
	cMLS <sub>B</sub> (n=89)	23	3	48	6	25	13	10
	MLS <sub>B</sub> ∅ (n=49)	0	0	0	0	29	0	20
	Total (n=161)	32	3	63	9	57	16	32
MSCoNS (n=93)	iMLS <sub>B</sub> (n=26)	2	0	6	0	3	0	15
	cMLS <sub>B</sub> (n=22)	10	0	8	2	3	0	3
	MLS <sub>B</sub> ∅ (n=45)	0	0	0	0	18	0	27
	Total (n=93)	12	0	14	2	24	0	45
Total (n=301)	iMLS <sub>B</sub> (n=73)	25	0	27	4	13	8	20
	cMLS <sub>B</sub> (n=127)	48	3	58	10	30	14	13
	MLS <sub>B</sub> ∅ (n=101)	0	0	0	0	51	0	50
	Total (n=301)	73 (24)	3 (1)	85 (28)	14 (5)	94 (31)	22 (7)	83 (28)

<sup>a</sup>Data presented as n (%). ND=Not detected; *S. aureus*=*Staphylococcus aureus*; iMLS=inducible macrolide, lincosamide and streptogramin resistance; cMLS=constitutive macrolide, lincosamide and streptogramin resistance; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*; CoNS=coagulase-negative staphylococci; MRCoNS=methicillin-resistant coagulase-negative staphylococci; MSCoNS=methicillin-susceptible coagulase-negative staphylococci.

that *erm(A)* was the most frequent genetic determinant among *S. aureus*.<sup>14-16</sup> The most prevalent resistance determinant was *erm(C)* in CoNS isolates in our study and these results are confirmed by those of Reyes et al, Chaib et al, Eady et al, and Gatermann et al, who documented the predominance of *erm(C)* in a large series of clinical and commensal CoNS isolates.<sup>16-19</sup> Thus, in conjunction with previous reports, the results of the present study support the hypothesis that there are geographical differences in

the prevalence of erythromycin resistance mechanisms among staphylococci.<sup>2</sup>

However, we must also mention the fact that since species identification was not performed for CoNS isolates, we cannot exclude the possibility that these differences might be due to variation in the distribution of different species. Most of the isolates from which we were not able to detect any resistance genes were CoNS, and this was in accordance with the previous reports that have found

unidentified resistance mechanisms in a considerable proportion of CoNS isolates.<sup>1</sup> It is likely that other erythromycin resistance genes such as *Ere A–B* or *mef*, which we did not include in our study, might be present among these isolates.<sup>3</sup>

The main problem among strains that have been detected as erythromycin resistant and clindamycin susceptible by standard antibiotic susceptibility tests is the possibility that these strains may be inducibly resistant to MLS<sub>B</sub> antibiotics, since this resistance phenotype can be underestimated if testing for inducible resistance is not performed. Although most erythromycin-resistant isolates in the present study showed constitutive expression of MLS<sub>B</sub> resistance, 24 (77%) of 31 *S. aureus* and 49 (34%) of 143 CoNS isolates that were erythromycin resistant and clindamycin susceptible were positive by the D-test. Therefore, we suggest that erythromycin-resistant isolates of *S. aureus* may also be considered to be clindamycin resistant in most cases. Although clindamycin is reported as one of the therapeutic options in the treatment of MRSA,<sup>20</sup> it seems that we cannot consider this antibiotic as useful in the treatment of hospital-associated MRSA infections. However, the lower positivity rate by the D-test among erythromycin-resistant CoNS made us think that clindamycin should not be considered to be ineffective in these species without testing for inducible resistance. It has been reported that resistance mechanisms other than methylation are more common among CoNS, the assumption being that methylases are responsible for erythromycin resistance in these isolates could lead to an unnecessary avoidance of lincosamides and an increased usage of glycopeptides.<sup>14</sup>

In conclusion, the most prevalent phenotype among *S. aureus* was the iMLS<sub>B</sub> type mediated by the *erm(A)* gene, while the most prevalent phenotype among CoNS was the cMLS<sub>B</sub> type mediated by *erm(C)* gene. These findings with significant geographical differences in resistance patterns and resistance rates make the results of local surveillance studies an important tool in guiding therapy and for judicious use of antimicrobial agents.

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