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RESEARCH NOTE

Prevalence of *erm* genes encoding macrolide-lincosamide-streptogramin (MLS) resistance among clinical isolates of *Staphylococcus aureus* in a Turkish university hospital

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ABSTRACT

This study investigated the prevalence of the *erm*(A), *erm*(B) and *erm*(C) genes among 122 MLS-resistant clinical isolates of *Staphylococcus aureus* from a Turkish university hospital. Of these isolates, 44 were inducibly resistant and 78 were constitutively resistant. The presence of one or more *erm* genes was demonstrated in 114 isolates; the *erm*(C) gene was detected in 97 isolates, and the *erm*(A) gene was detected in 96 isolates. Seventy-eight isolates harboured both *erm*(A) and *erm*(C). The combination of *erm*(A), *erm*(B) and *erm*(C) genes was detected in only one isolate.

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Macrolide (erythromycin), lincosamide (clindamycin) and streptogramin B (vernamycin Bα) antibiotics inhibit protein synthesis by binding to overlapping sites in the 50S ribosomal subunit [1]. Although they are chemically distinct antibiotics, they have a similar mode of action. Emergence of drug-resistant, especially methicillin-resistant, *Staphylococcus* strains, has led to the investigation of possible new antibiotics for the treatment of staphylococcal infections. Use of macrolide, lincosamide and streptogramin (MLS) antibiotics is limited for staphylococcal infections, but they are often considered as an alternative treatment regiment [2,3].

Three different resistance mechanisms for macrolide antibiotics have been described in staphylococci, but the main mechanism involves target-site modification following methylation of the ribosome. The methylase enzyme adds one or two methyl groups to the adenine residue in the 23S rRNA moiety, and thereby decreases the affinity of the ribosomal subunit for MLS antibiotics. Cross-resistance to these chemically unrelated antibiotics is observed since their binding sites overlap. The second mechanism of resistance involves an efflux system that results in resistance to macrolides and streptogramin B antibiotics. The third mechanism involves inactivation of antibiotics by the enzymes acetyltransferase, hydrolase, nucleotidyltranferase and phosphotransferase [4–6].

MLS resistance in staphylococci can be either constitutively or inducibly expressed. While isolates showing constitutive resistance are resistant to 14-membered (erythromycin, roxithromycin,

clarithromycin), 15-membered (azithromycin), 16-membered (spiramycin, josamycin, miomycin and midecamycin) macrolides and clindamycin, isolates showing inducible resistance are resistant to 14- and 15-membered macrolides only [6,7].

Three main *erm* (erythromycin ribosome methylation) genes, i.e., *erm*(A), *erm*(B) and *erm*(C), have been described in staphylococci. The *erm*(A) gene is located on transposon Tn*554*, which has insertion sites in the *Staphylococcus aureus* chromosome. Structural alterations in constitutively or inducibly expressed *erm*(A) genes have been demonstrated [6,8,9]. The *erm*(C) gene has been found on a 3.7-kb element on a plasmid, pE194, and has also been found on smaller plasmids. The *erm*(B) gene is located on transposon Tn*551* [6]. Other genes, such as *erm*(F) and *erm*(Y), may also be responsible for MLS resistance [10,11].

The prevalence of *erm* genes in MLS-resistant *S. aureus* isolates has been demonstrated previously [12–15]. As the prevalence of MLS resistance and *erm* genes varies between countries and individual hospitals, the present study investigated, for the first time, the prevalence of the *erm*(A), *erm*(B) and *erm*(C) genes in MLS-resistant clinical isolates of *S. aureus* from a university hospital in Turkey.

Hacettepe University Hospital is a 1150-bed hospital with 600 000 admissions annually. Antibiotic use is strictly controlled by the infectious diseases control committee of the hospital. Data concerning the prevalence of MLS resistance and resistance phenotypes in staphylococci in this hospital have been reported previously [16]. In this previous study, 500 consecutive clinical isolates of staphylococci were collected from different adult inpatients between June 1996 and June 1998. Of the 500 isolates, 132 (26.4%) were resistant to MLS antibiotics, with 91 (18.2%) being constitutively resistant, and 40 (8%) being inducibly resistant. The MS phenotype (resistance to macrolides and lincosamides only) was detected in only one isolate. Of the 132 resistant isolates, 122 were *S. aureus* and ten were coagulase-negative staphylococci.

In the present study, 122 MLS-resistant *S. aureus* isolates, 104 of which were methicillin-resistant and 18 were methicillin-susceptible, were retested for MLS resistance by the disk-diffusion method described by Jenssen *et al*. [17]. The absence of inhibition zones around the antibiotic disks was considered to be indicative of constitutive resistance. Flattening or blunting of the shape of the clindamycin and vernamycin Bα inhibition zones, adjacent to the erythromycin disk, was considered to be indicative of inducible resistance [7,17]. Methicillin resistance was determined by the broth macrodilution test [18].

Total DNA of all isolates was prepared by the boiling method [19]. The primers described by Lina *et al*. [13] were used for amplification of the *erm*(A), *erm*(B) and *erm*(C) genes [13]. Amplification products were visualised following electrophoresis on agarose 1.5% w/v gels. Table 1 summarises the distribution of the *erm* genes, grouped according to the methicillin resistance of the isolates. The ratio of constitutively MLS-resistant isolates to inducibly-resistant isolates (approximately 2 : 1) may reflect the more frequent use of non-inducing MLS antibiotics in the hospital studied.

Constitutively-resistant isolates have been reported to be the predominant form of resistance in some previous studies, whereas others have found that the inducible resistance pattern is more frequent [13,15,17]. In the present study, 78 isolates harboured both the *erm*(A) and *erm*(C) genes. Similarly, in the study by Melter *et al*. [12], 64 of 100 isolates contained both *erm*(A) and *erm*(C), which was the predominant pattern of resistance. All except one of the methicillin-susceptible *S. aureus* (MSSA) isolates in the present study were inducibly MLS-resistant, and the *erm*(C) gene was the predominant gene expressed. Lina *et al*. [13] also reported

Table 1. Distribution of *erm* genes among macrolide-lincosamide-streptogramin (MLS)-resistant isolates of *Staphylococcus aureus*

	erm(A)	*erm*(C)	*erm*(A) + *erm*(C)	*erm*(A) + *erm*(B) + *erm*(C)	No *erm* gene detected
Inducible MLS (n = 44)					
MSSA (n = 17)	1	9	7	–	–
MRSA (n = 27)	11	2	12	–	2
Constitutive MLS (n = 78)					
MSSA (n = 1)	–	–	1	–	–
MRSA (n = 77)	5	7	58	1	6
Total (n = 122)	17	18	78	1	8

that the inducible phenotype was predominant among MSSA, with 25% of isolates having *erm*(C) as a single MLS resistance gene. In the study by Spiliopoulou *et al*. [15], *erm*(C) was the most frequent gene among constitutively MLS-resistant methicillin-resistant *S. aureus* isolates. As in previous studies of clinical isolates of staphylococci, *erm*(B) was rare, being detected in only one isolate in the present study. However, *erm*(B) has been detected in isolates of *Staphylococcus intermedius*, *Staphylococcus xylosus* and *Staphylococcus hyicus* [20]. Future studies should investigate the possible presence of other *erm* genes and ribosomal mutations that have been defined recently [4,10,11].

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