

Short Communication

Inducible Clindamycin Resistance in Staphylococci Isolated from Clinical Samples

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SUMMARY: This study aimed to determine the levels of the macrolides-lincosamides-streptogramins B (MLS_B) resistance phenotype in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) isolates from clinical samples. A total of 521 strains of staphylococci, comprising 230 *S. aureus* and 291 CNS isolates from various clinical samples, were identified by conventional methods. The double-disc test was applied by placing erythromycin and clindamycin discs on these isolates to investigate the inducible and constitutive MLS_B resistance phenotypes and MS phenotype. Among the *S. aureus* strains, 24.3% showed the constitutive and 7.8% the inducible phenotype, while there was no MS phenotype. In the CNS strains, 40.2% showed the constitutive and 14.7% the inducible MLS_B resistance phenotype, and 18.2% had the MS phenotype. In both *S. aureus* and CNS strains, the constitutive MLS_B resistance rate was found to be higher than the rate of inducible resistance. By applying double-disc tests on a routine basis to detect inducible MLS_B resistance, clindamycin can be effectively used on staphylococcal infections. Additionally, it can be used to survey the MLS_B resistance of staphylococci strains from specific geographical regions or hospitals.

The macrolide, lincosamide, and streptogramin (MLS) antibiotics have similar inhibitory effects on bacterial protein synthesis, but they are chemically distinct. In the treatment of Gram-positive infections, MLS antibiotics are used widely. However, this widespread use has led to an increase in the number of staphylococci strains resistant to MLS antibiotics (1-3). Macrolide antibiotic resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) may be due to an active efflux mechanism encoded by *msrA* (conferring resistance to macrolides and type B streptogramins only) or may be due to ribosomal target modification affecting macrolides, lincosamides, and type B streptogramins (MLS_B resistance). *erm* genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA, thereby reducing binding by MLS agents to the ribosome (4-6). The *msrA* gene confers the so-called MS phenotype (resistance to erythromycin, inducible resistance to streptogramin B, and susceptibility to clindamycin) by efflux (2,6,7). Strains with inducible MLS_B resistance demonstrate in vitro resistance to 14- and 15-member macrolides (e.g., erythromycin), while appearing susceptible to 16-member macrolides, lincosamides, and type B streptogramins; strains with constitutive MLS_B resistance show in vitro resistance to all the agents (2,4,5,8). Inducible resistance is expressed in the presence of strong inducers of methylase synthesis, such as 14- and 15-member macrolides (3,9). Inducible MLS_B resistance can be detected by a disc diffusion induction test by placing erythromycin and clindamycin discs in adjacent positions (4).

In this study, the aim was to determine the incidence of MLS_B resistance among staphylococci isolates from various clinical samples and to detect inducible MLS_B resistant strains.

We also compared our results with those of other hospitals and regions.

A group of 230 isolates of *S. aureus* comprising 128 methicillin-resistant *S. aureus* (MRSA) and 102 methicillin-susceptible *S. aureus* (MSSA) isolates and 291 isolates of CNS comprising 180 methicillin-resistant CNS (MRCNS) and 111 methicillin-susceptible CNS (MSCNS) isolates, were collected from recent (September 2003 to June 2004) clinical isolates recovered from the Microbiology Laboratory of the Faculty of Medicine, Mersin University. Duplicate isolates from the same patient were not included. Isolated microorganisms were identified by using conventional methods (colony morphology, Gram stain, catalase, slide and tube coagulase test and DNase test) (10). Antibiotic susceptibilities were studied by disc diffusion methods based on the guidelines from the National Committee for Clinical Laboratory Standards (11).

To detect inducible clindamycin resistance, 15 µg erythromycin and 2 µg clindamycin discs (Bioanalyse, Ankara, Turkey) were placed at a distance of 15-20 mm for *S. aureus* strains or 20-26 mm for CNS strains (4). *S. aureus* ATCC 25923 was used as a control for these tests.

Of the *S. aureus* isolates, 66.9% (154) were susceptible to erythromycin and 76% (175) to clindamycin. Of the CNS isolates, 26.8% (78) were susceptible to erythromycin and 58.7% (171) to clindamycin. Two isolates of *S. aureus* were intermediate to erythromycin and two isolates of CNS were intermediate to clindamycin (Table 1).

In the double-disc test, if there is inducible clindamycin resistance, the erythromycin will diffuse throughout the agar, and resistance to lincosamide will be induced, resulting in a flattening or blunting of the lincosamide zone of inhibition adjacent to the erythromycin disc, and giving a D shape to the zone (4,6). Strains that were resistant to both erythromycin and clindamycin were defined as showing constitutive MLS_B resistance, those showing flattening of the clindamycin zone adjacent to the erythromycin disc were defined as having

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Table 1. Erythromycin and clindamycin susceptibilities in *S. aureus* and CNS

	Erythromycin <i>n</i> (%)	Clindamycin <i>n</i> (%)
MSSA (102)	91 (89.2)	102 (100.0)
MRSA (128)	63 (49.2)	73 (57.0)
<i>S. aureus</i> (230)	154 (66.9)	175 (76.0)
MSCNS (111)	45 (40.5)	89 (80.1)
MRCNS (180)	33 (18.3)	82 (45.5)
CNS (291)	78 (26.8)	171 (58.7)

CNS, coagulase-negative staphylococci; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*.

inducible MLS_B resistance, and those that were resistant to erythromycin and sensitive to clindamycin (no induction) were defined as showing the MS phenotype (4,7,12). Examples of disc induction test phenotypes are shown in Fig. 1.

Among the 230 *S. aureus* strains, 56 strains (24.3%) were found to exhibit the constitutive, and 18 strains (7.8%) the inducible MLS_B resistance phenotype, while there was no MS phenotype. Among CNS strains, 117 strains (40.2%) were found to have constitutive resistance, 43 strains (14.7%) to have inducible MLS_B resistance, and 53 strains (18.2%) to have the MS phenotype. On the other hand, in MRSA strains 43.7% had the constitutive and 5.4% the inducible MLS_B

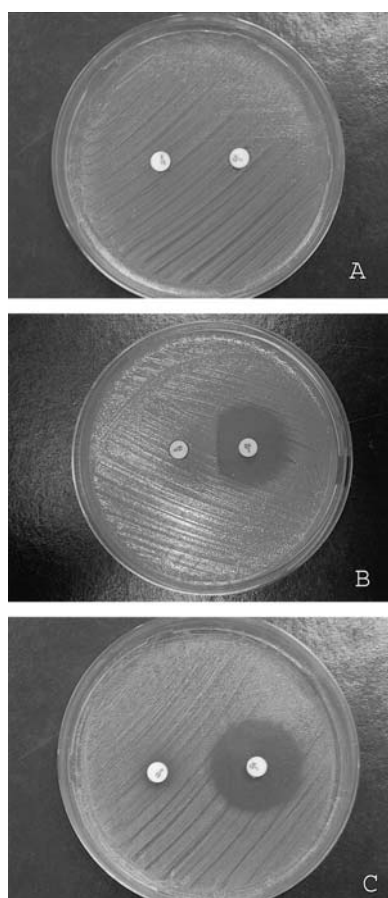


Fig. 1. (A) Constitutive MLS_B resistance by disc induction test of *S. aureus* strain. (B) Positive disc induction test indicating inducible clindamycin resistance of the *S. aureus* strain. (C) Negative disc induction test indicating the absence of inducible clindamycin resistance of the CNS strain (MS phenotype).

Table 2. MLS_B resistance phenotype of *S. aureus* and CNS isolates

	Constitutive MLS _B resistance <i>n</i> (%)	Inducible MLS _B resistance <i>n</i> (%)	MS phenotype <i>n</i> (%)
MSSA (102)	0 (0)	11 (10.7)	0 (0)
MRSA (128)	56 (43.7)	7 (5.4)	0 (0)
<i>S. aureus</i> (230)	56 (24.3)	18 (7.8)	0 (0)
MSCNS (111)	21 (18.9)	16 (14.4)	29 (26.1)
MRCNS (180)	96 (53.3)	27 (15.0)	24 (13.3)
CNS (291)	117 (40.2)	43 (14.7)	53 (18.2)

Abbreviations are in Table 1.

resistance, and no strains had the MS phenotype; and in MSSA strains no constitutive resistance and MS phenotype and 10.7% inducible MLS_B resistance were detected. In MRCNS strains 53.3% had constitutive and 15% had inducible MLS_B resistance and 13.3% had the MS phenotype; and in MSCNS strains 18.9% had constitutive and 14.4% had inducible MLS_B resistance and 26.1% had the MS phenotype (Table 2). Among the 74 erythromycin-resistant *S. aureus* strains, 56 strains (75.6%) were found to have constitutive and 18 strains (24.3%) to have inducible MLS_B resistance, and no strains showed the MS phenotype. Among the 213 erythromycin-resistant CNS strains, 117 strains (54.9%) were found to have constitutive and 43 strains (20.1%) to have inducible MLS_B resistance, and 53 strains (24.8%) exhibited the MS phenotype.

In our isolates, the susceptibilities to erythromycin and clindamycin were higher in MSSA and MSCNS than in MRSA and MRCNS isolates (Table 1). Schmitz et al. reported 61% susceptibility to erythromycin and 73% to clindamycin in *S. aureus* isolates and 37% susceptibility to erythromycin and 59% to clindamycin in CNS isolates. In their study, the percentage of MSSA isolates that showed susceptibility to erythromycin was 19.7 times higher than that of MRSA isolates. Whereas 90.3% of the MSSA isolates were susceptible to clindamycin, 10.6% of the MRSA isolates exhibited susceptibility. The percentage of MSCNS isolates showing susceptibility to erythromycin was 2.5 times higher and that showing susceptibility to clindamycin was 1.8 times higher compared with the values for MRCNS isolates. During therapy of MRSA infections, emergence of resistance to clindamycin is common, particularly if the isolate is resistant to erythromycin (9). Clindamycin has good tissue penetration (except for the central nervous system) and accumulation abscesses and no need to renal dosing adjustments. This is why; clindamycin is preferred, especially in staphylococcal infections of areas such as the skin and soft tissue, and as an alternative antibiotic in patients allergic to penicillin (3,4).

Fielbelkorn and colleagues determined the ability of disc induction tests to predict the resistance genotype by performing PCR for the *ermA*, *ermC*, and *msrA* genes with *S. aureus* and CNS isolates. They found that placement of erythromycin and clindamycin discs at a distance obtained with a standard disc dispenser allowed detection of 97% of *S. aureus* strains and 100% of CNS strains with inducible resistance, and the sensitivities of disc induction testing for detection inducible MLS_B strains containing *ermA* or *ermC* were 100% at 15-20 mm and 97% at 26-28 mm for *S. aureus*. The sensitivity was 100% at both 20 and 26 mm for CNS (4).

Lim et al. reported that inducible resistance was 14.6% in erythromycin resistant *S. aureus* isolates and 9.6% in erythromycin resistant CNS isolates by the double-disc test

in a Korean hospital (1). Hamilton-Miller et al. determined that in *S. aureus*, 12% had inducible, 2% had constitutive MLS resistance and 1% had the MS phenotype, in CNS strains 31% had inducible, 11% had constitutive MLS resistance and 13% had the MS phenotype, and reported that most common resistance phenotype was inducible MLS (12). Kisioglu et al. found that, in MRSA isolates, 62% had constitutive and 27.9% had inducible MLS_B resistance and 3.7% had the MS phenotype, in MSSA isolates, 6.8% had constitutive and 6.8% had inducible and 3.4% had the MS phenotype (13). In our study in MRSA strains 43.7% had constitutive and 5.4% had inducible the MLS_B resistance, and no MS phenotype, while in MSSA strains there was no constitutive resistance and MS phenotype and 10.7% had inducible MLS_B resistance. Thus, in all staphylococci isolates other than MSSA isolates, the level of constitutive MLS_B resistance was higher than the level of inducible MLS_B resistance.

Inducible clindamycin resistance in staphylococci can not be detected by using in vitro susceptibility tests such as broth dilution and agar dilution tests. In addition, disc diffusion testing may indicate false susceptibility when erythromycin and clindamycin discs are placed in nonadjacent positions. Accurate results of antibiotics susceptibility tests are important for deciding appropriate and effective therapy. By applying proper disc placement on a routine basis to detect inducible clindamycin resistance, clindamycin can be used effectively on staphylococcal infections. In addition, such testing can provide information about resistance phenotype MLS group antibiotics and can be useful for surveillance studies related to MLS resistance in staphylococci.

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