The Rate of Inducible MLS_B Resistance in the Methicillin-Resistant Staphylococci Isolated From Clinical Samples

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Background: Staphylococci are one of the most common pathogens in nasocomial and community-acquired infections. Methicillin-resistant staphylococci are known to be resistant against all beta-lactam antibiotics. Therefore, non-beta-lactam antibiotics such as macrolide and lincosamides can be used. Resistance to those antibiotics may lead to therapeutic failure. The purpose of the present study was to determine the prevalence of macrolide–lincosamide–streptogramin B (MLS_B) resistance by using *D*-test in staphylococcal isolates from various clinical samples. **Methods:** Seventy-one methicillin-resistant Staphylococcus isolates (six *S. aureus*, 65 coagulase negative staphylococci) were included in this study. Staphylococci were identified with conventional methods. According to Clinical Laboratory

Standards Institute (CLSI) criteria, susceptibility testing was performed by Kirby–Bauer disk diffusion method. **Results:** One of six (16.6%) methicillin-resistant *S. aureus* isolates and 19 of 65 (29.2%) methicillin-resistant coagulase-negative staphylococci (MR-CNS) were detected as *D*-test positive. Twenty of 71 (28.1%) staphylococcal isolates detected as *D*-test positive. Inducible clindamycin resistance was found at a higher rate in MR-CNS. **Conclusion:** Since the resistant community and hospital acquired staphylococcal infections have become a therapeutic problem, it is very important to detect MLS_B resistance routinely in microbiology laboratories. *D*-test is a cheap and reliable diagnostic method that can be performed in every laboratory. J. Clin. Lab. Anal. 30:490–493, 2016. © 2015 Wiley Periodicals, Inc.

Key words: *D*-test; MLS_B; MLS_B resistance; Staphylococcus

INTRODUCTION

Staphylococci are one of the most common pathogens isolated from nasocomial and community-acquired infections. They gain resistance to antibiotics and methicillin-resistant staphylococci are known to be resistant against all beta-lactam antibiotics. Therefore, non-beta-lactam antibiotics such as macrolide and lincosamides can be used to treat staphylococcal infections (1). Determination of effective antibiotics in the staphylococcal infections is closely related to the resistance status of bacteria. Increased resistance to methicillin and to some other antibiotics as well makes it difficult to treat and control the infections. In the last two decades, Gram-positive microorganisms with multiple resistance have been showing increased rate of infections all over the world (2). Macrolide–lincosamide–streptogramin B (MLS_B) group antibiotics are one of the alternatives in the treatment of staphylococcus infections. Although they have

different chemical structure, they show their effect in a similar mechanism. Therefore, genes that cause resistance to one of MLS_B antibiotics, also may cause development of the cross-resistance to others. Depending on the mechanism that causes resistance, MLS_B resistance may occur in the form of structural resistance or inducible resistance phenotypically. Clindamycin (CL) in the group of the MLS_B antibiotics is an appropriate option for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) induced skin and soft tissue infections (3). Low

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Received 23 October 2014; Accepted 25 July 2015

DOI 10.1002/jcla.21884

Published online in Wiley Online Library (wileyonlinelibrary.com).

cost, fewer severe side effects, the availability of oral and parenteral forms, the lack of a need for a renal adjustment, and good tissue penetration and ability to directly inhibit toxin production are its advantages. Moreover, CL is a useful choice in cases of penicillin allergy (4). However, MLS_B inducible resistance limits the effect of the drug (3).

Frequencies of the different resistance patterns vary by geographic location, bacterial species, and bacterial susceptibility profile. Incidence of MLS_B resistance varies between hospitals (5, 6). Kiziltepe is highly crowded county with different ethnicities and located in the southeast part of the Turkey. At the same time incidence of infections rate and use of antibiotics are high. There are no data about prevalence of MLS_B resistance in Kiziltepe region. The purpose of the present study was to determine the prevalence of MLS_B resistance by using *D*-test in staphylococcal isolates from various clinical samples.

METHODS

This study was conducted between October 2011 and March 2012. The number of patients with methicillin-resistant staphylococcal isolates during the study period was retrospectively obtained from the archive of the laboratory information system and these patients were followed up in Kiziltepe State Hospital. In this study, we included a total of 71 methicillin-resistant staphylococcal isolates, 6 *S. aureus* (SA) and 65 coagulase-negative staphylococci (CNS).

Staphylococci were identified with conventional methods. Mueller–Hinton agar were used for bacterial growth and for susceptibility tests. According to Clinical Laboratory Standards Institute (CLSI) criteria, susceptibility testing was performed by Kirby–Bauer disk diffusion method with erythromycin (ER; 2 µg) and CL (15 µg) disks. Inducible resistance to CL was detected by *D*-test method after 18 hr of incubation at 35°C. The erythromycin resistant (ER-R) showed resistance to ER (zone size < 13mm) and susceptibility to CL (CL-S) isolates (sensitivity to CL [zone size > 21mm]); and CL resistance in the form of *D* zone around the CL in the area between the two disks will be evaluated as positive for the presence of inducible resistance (*D*-test positive). Methicillin resistance of these isolates was determined by diffusion method with oxacillin disk (1 µg) (7). *Staphylococcus aureus* ATCC 25923 was used as the control strain.

Statistical Analysis

SPSS (version 15.0) was used for statistical evaluation of the data. Data analysis was performed using chi-square test and Fisher' exact test. *P* < 0.05 was assigned as statistically significant.

TABLE 1. *D*-Test Positivity

Strains (<i>n</i>)	<i>D</i> -test positivity %(<i>n</i>)
MRSA (6)	16.6% (1)
MR-CNS (65)	29.2% (19)
Total (71)	28.1% (20)

MRSA, methicillin-resistant *Staphylococcus aureus*; MR-CNS, methicillin-resistant coagulase-negative staphylococci.

Fig. 1. *D*-test indicating inducible CL resistance.

RESULTS

Results of disk induction testing for *S. aureus* and CNS are shown in Table 1. Examples of disk induction test phenotypes are shown in Figure 1.

One of six (16.6 %) MRSA isolates and 19 of 65 (29.2 %) methicillin-resistant CNS (MR-CNS) were detected as *D*-test positive. Twenty of 71 (28.1 %) staphylococcal isolates were detected as *D*-test positive. Inducible CL resistance was found at a higher rate in MR-CNS (Table 1). There was no significant difference between MRSA and MR-CNS isolates for inducible resistance to CL (*P* > 0.05).

DISCUSSION

The main findings of the present study are as follows: the rate of *D*-test was found as 16.6 % in MRSA, 29.2% in MR-CNS, and 28.1% in all staphylococcal isolates. Prior to the application of the antimicrobial therapy in infected individuals, the antimicrobial susceptibility testing is performed to avoid an indiscriminate usage of antibiotics and making an error. The empirical treatment of the staphylococcal infection is more important, because of the emergence of multidrug-resistant strains, especially MRSA. The increasing frequency of the staphylococcal infections among the patients and the changing patterns in antimicrobial resistance have led to a new interest in the use of the CL therapy in the treatment of such infections (4).

Macrolide antibiotic resistance in staphylococci may be due to an active efflux mechanism, or may be due to ribosomal target modification. Active efflux mechanism is encoded by *msrA* (conferring resistance to macrolides and type B streptogramins only). Ribosomal target modification is encoded by *erm* genes that affects macrolides, lincosamides, and type B streptogramins (MLS_B resistance). *erm* genes cause inducible or constitutive resistance to MLS_B agents. They reduce binding of MLS_B agents to the ribosome via methylation of the 23S rRNA which results in development of cross-resistance to macrolides, lincosamides, and type B streptogramins antibiotics (4, 5, 8). The MLS_B resistance may be either constitutive (cMLS_B) or the inducible (iMLS_B) type. The isolates with cMLS_B are readily detected by in vitro testing and are resistant to both ER and CL. The isolates with iMLS_B are resistant to ER, but they appear susceptible to CL in the routine susceptibility testing and are easily missed. Accurate susceptibility data are important for appropriate therapy decisions. In staphylococci, in vitro susceptibility testing or CL may indicate false susceptibility by the broth microdilution method and disk diffusion testing with ER and CL disks in nonadjacent positions (1, 3, 6, 7).

The incidence of inducible MLS_B resistance varies by geographic region and even from hospital to hospital, with some studies showing high local incidence of inducible MLS_B resistance in staphylococcal isolates (6, 9). For studies carried out in Europe, the incidence of inducible CL resistance was seen in 38% of community-acquired MRSA, in 6.7% of healthcare-associated MRSA, and in 63.6% of MSSA (10). Stewart et al. (11) reported that in MRSA strains, iMLS_B resistance was 38% and cMLS_B resistance was 30% in Atlanta. In a study performed in Japan, iMLS_B resistance was 40%. Researchers in the United States reported that the incidences of inducible CL resistance at two hospitals (an inner-city hospital and a suburban community hospital) were 7% and 12% for methicillin-resistant *S. aureus*, 20% and 19% for methicillin-susceptible *S. aureus*, and 14% and 35% for CNS (12). In India, Prabhu et al. (13) reported that iMLS_B resistance phenotype was reported as 10.52% among *S. aureus* strains.

Incidence of inducible resistance to ER and CL among staphylococcal isolates from Turkey was seen between 5.4% and 6.2% in MRSA, 15 and 30.8% in MR-CNS strains (1, 14–16). Saribas et al. (17) reported that iMLS_B resistance phenotypes were found 35.7% in MRSA strains and 22.4% in MR-CNS strains, respectively. Yilmaz et al. (18) reported the rates as 24.4% in MRSA and 25.7% in MR-CNS. Uzun et al. (19) reported that iMLS_B type in MRSA and MR-CNS was observed in 26% and 51%, respectively. Uyanik et al. (20) reported that iMLS_B resistance phenotypes were 30% in MRSA strains in Erzurum.

Our data show that the incidence of iMLS_B resistance phenotype was not different from the rates reported previously. Furthermore, the incidence of inducible resistance to CL varied between the MRSA and MR-CNS isolates tested in our hospitals, but have no statistical meaning ($P > 0.05$).

CONCLUSION

Since the resistant community and hospital-acquired staphylococcal infections have become a therapeutic problem, it is very important to detect MLS_B resistance routinely in microbiology laboratories. *D*-test is a cheap, easy, and reliable diagnostic method that can be performed in every laboratory to detect inducible resistance to CL in ER-R isolates of *S. aureus* and CNS.

In order to prevent treatment failure, *D*-test should be routinely used and the results should be reported to the clinician before starting a therapy with MLS_B group of antibiotics. To the best of our knowledge, the present study is the first report from Kiziltepe region on the incidence of inducible CL resistance among staphylococci. Thus, the prevalence should be determined in individual settings.

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