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Original Article

Macrolide-Lincosamide-Streptogramin B Resistance Phenotypes in Staphylococcus Aureus

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ABSTRACT

Staphylococcus aureus is one of the most frequently isolated pathogens in community and hospital-acquired infections. Macrolide-lincosamide-streptogramin B (MLSB) group antibiotics have frequently been preferred. In this study, it was aimed to determine MLSB group antibiotics resistance phenotypes observed in S. aureus strains. A total of 182 S. aureus strains were included in the study. Methicillin resistance was assessed using the cefoxitin (30µg) disc, MLSB resistance phenotypes were assessed using D zone test with erythromycin (15µg) and clindamycin (2µg) discs according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. Of the strains included in the study, 38 (20.9%) methicillin-resistant S.aureus (MRSA) and 144 (79.1%) methicillin-susceptible S.aureus (MSSA) were identified. MLSB resistance phenotype was found in 65 (35.7%) strains. MLSB resistance was found 84% in MRSA strains and 23% in MSSA strains: There was statistically significant between MRSA and MSSA strains. Constitutional MLSB resistance was found higher in MRSA strains (71%) and however, in MSSA strains was higher inducibleMLSB resistance (16.5%). It is suggested that, using the D test method in routine antibiotic susceptibility testing and determining resistance phenotypes in microbiology laboratories is the right approach and may play an important role in the prevention of treatment failure according to the substantial proportion of inducible resistance MLSB resistance observed.

Key words: *Staphylococcus aureus, MLSB resistance, D test, methicillin resistance*

Staphylococcus Aureus Suşlarında Makrolid-Linkozamid-Streptogramin B Direnç Fenotipleri

ÖZET

Staphylococcus aureus toplum ve hastane kaynaklı infeksiyonlarda en sık izole edilen etkenlerdendir. Makrolid-linkozamid-streptogramin B (MLSB) grubu antibiyotikler sıklıkla tercih edilmektedir. Bu çalışmada MLSB grubu antibiyotiklere S. aureus suşlarında görülen direnç fenotiplerinin belirlenmesi amaçlanmıştır. Çalışmaya toplam 182 S. aureus suşu dahil edildi. Suşların metisilin direnci sefoksitin (30µg) diski kullanılarak, MLSB direnç fenotipleri disk yakınlaştırma yöntemi ile eritromisin (15µg) ve klindamisin (2µg) diskleri kullanılarak Clinical and Laboratory Standards Institute (CLSI) önerileri doğrultusunda değerlendirildi. Çalışmaya alınan suşların 38'i (%20.9) metisiline dirençli S. aureus (MRSA), 144'ü (%79.1) metisiline duyarlı S. aureus (MSSA) olarak tespit edilmiştir. Tüm suşların 65'inde (%35.7) MLSB direnci bulunmuştur. MRSA suşlarında MLSB direnci % 84 iken, MSSA suşlarında %23 olarak tespit edilmiş ve bu değerler istatistiksel olarak anlamlı bulunmuştur. MRSA suşlarında yapısal MLSB (%71), MSSA suşlarında ise indüklenebilir MLSB(%16.5)direnci daha fazla bulunmuştur. İndüklenebilir MLSB direncinin azımsanmayacak oranlarda görülmesi nedeni ile mikrobiyoloji laboratuvarlarında D test yönteminin rutin olarak antibiyotik duyarlılık testlerinde kullanılması ve direnç fenotiplerinin belirlenmesinin tedavi başarısızlıklarını önlemede önemli ve doğru bir yaklaşım olduğunu düşündürmüştür.

Anahtar kelimeler: *Staphylococcus aureus, MLSB direnci, D test, metisilin direnci*

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INTRODUCTION

Staphylococcus aureus is one of the most frequently isolated pathogens in community and hospital-acquired infections. *S. aureus* is the most common cause of bacteremia due to gram-positive bacteria and causes severe infections in many different tissues such as skin, soft tissue and surgical site infections, necrotizing fasciitis, gastroenteritis and pneumonia(1). Macrolide-lincosamide-streptogramin B (MLSB) group antibiotics have frequently been preferred in the treatment of infections due to all gram positive bacteria, including staphylococci. Although being in different chemical structures, mechanisms of action are similar in MLSB group antibiotics. They have antibacterial effects via inhibiting protein synthesis due to connect bacterial 23S rRNA ribosomal subunit. Therefore, genes causing resistance to any of MLSB group antibiotics may lead to the development of cross-resistance to others (2-4).The most common mechanism of resistance to MLSB group antibiotics is carried out with methylase enzyms encoded by erm genes in the target zone. This kind of resistance is inducable by phenotypic expression of methylase enzyme (iMLSB) and may occur as constitutional resistance (cMLSB) (2,5). In both cases, resistance to all members of the group occurs and depending on the development of macrolide efflux pump, resistance to macrolide-streptogramin B (MSB) appears and remains susceptible to lincosamide (6,7). The resistance can be determined by using the D zone test in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations (8). It isrecommended to determine their own resistance rates of institutions due to the variations of incidence of inducible MLSB resistance between different countries, different geographic regions of countries and even health care centers (7,9).

In this study, it was aimed to investigate iMLSB, cMLSB and MSB resistance phenotypes observed in S. aureus strains that isolated from various clinical specimens in Erciyes University, Gevher Nesibe Faculty of Medicine.

MATERIALS AND METHODS

A total of 182 *S.aureus* strains included in the study that were isolated from various clinical specimens of outpatients and hospitalized patients and sent to microbiology laboratory in Erciyes University, Faculty of Medicine between April- July 2010. Strains were identified using routine microbiological methods (colony morphology in blood agar plate, catalase test, tube coagulase test, the effect of trehalose-mannitol, DNase test). Only one strain from each patient were included in the study. Methicillin resistance was assessed using the cefoxitin (30µg) disc, MLSB resistance phenotypes were assessed using D zone test with erythromycin (15µg) and clindamycin (2µg) discs in all strains according to the Clinical and Laboratory Standards Institute (CLSI) recommendations(8,10). For all strains, bacterial suspension adjusted in 0.5 McFarland were prepared and inoculated into Mueller-Hinton agar plates. All plates were incubated for 24 hours at 35 °C. Constitutional phenotype resistance (cMLSB) was defined as the absence of inhibition zones around both erythromycin and clindamycin discs during the evaluation of MLSB resistance phenotypes. InducibleMLSB resistance was defined as flattening at the edge of inhibition zone (D zone) around the clindamycin disc facing the erythromycin disc. MSB phenotype was defined as seen both of two inhibition zones circular and defined as erythromycin resistant, clindamycin sensitive (negative D test). S. aureus ATCC 29123 were used as the control strain. Chi-square test was performed to assess whether there were differences in terms of MLSB resistance phenotypes between MSSA and MRSA strains and p value ≤ 0.05 was considered as statistically significant.

RESULTS

A total of 182 strains included in the study, 38 strains were (20.9%) identified as MRSA and 144 (79.1%) strains were identified as MSSA. MLSB resistance phenotype was found in 65 (35.7%) strains. The MLSB resistance phenotype in MRSA and MSSA strains are summarized in Table 1. The MLSB resistance rate was found as 84% in MRSA and 23% in MSSA strains. Compared to MSSA strains, MLSB resistance phenotype was found higher in MRSA strains and this was statistically significant. Compared to MSSA strains, cMLSB resistance phenotype was found higher in MRSA strains and this was statistically significant. Compared in terms of iMLSB resistance phenotype, there was a higher rate in MSSA strains. The pump connected MSB resistance phenotype was observed only in MSSA strains.

DISCUSSION

Macrolide-lincosamide-streptogramin group antibiotics are often used in the treatment of staphylococcal infections. Clindamycin is used especially in soft tissue and

***Table 1**. The incidence of MLSB resistance phenotypes in MSSA and MRSA strains*

	MRSA (n:38)	*MSSA (n:144)*	*p value**
cMLSB n(%)	*27 (71)*	*5 (3.5)*	*<0.001*
iMLSB n(%)	*5 (13)*	*24 (16.5)*	*0.578*
MSB n(%)	*0 (0)*	*4 (2.8)*	*0.581*
Total n(%)	*32 (84)*	*33 (23)*	*<0.001*

MRSA: Methicillin-resistant S.aureus, MSSA: Methicillin-sensitive S.aureus, cMLSB: Constitutional macrolide-lincosamide-streptogramin B resistance phenotype, iMLSB: Inducible macrolide-lincosamide-streptogramin B resistance phenotype, MSB: Macrolide-lincosamide-streptogramin B resistance phenotype

** The chi-square test was performed statistically.*

skin infections and is oftenly preferred in infections of other regions in individuals with penicillin allergy(11,12). Constitutional and inducible MLSB resistance have been encountered at different rates in different geographical areas. Previous studies showed that, constitutional and inducible MLSB resistance rates may vary in concomitant multi-drug resistance strains such as methicillin-resistant *S.aureus* strains. In studies carried out in Europe, cMLSB phenotype in MRSA strains and iMLSB phenotype in MSSA strains were found higher (13,14). Fokas et al. found cMLSB resistance phenotype as 13%, iMLSB resistance phenotype as 20% in MSSA strains and 47% and 15% in MRSA strains, respectively(15). Researchers in the United States reported that iMLSB phenotypes were more prevalent than cMLSB phenotypes in MRSA strains(10). Stewart et al. reported that in MRSA strains, iMLSBresistance was 38% and cMLSB resistance was 30% in Atlanta(7). In a study performed in Japan, iMLSBresistancewas40% and cMLSB resistance was 61% (16). Another study in Southern Korea demonstrated that iMLSB and cMLSB was 4% and 79% in MRSA strains and 9% and 6% in MSSA strains, respectively(17). In India, Gadepelli et al. reported that cMLSB resistance phenotype was more prevalent in MRSA strains than MSSA strains(18).

In Turkey, Aktas et al. reported cMLSB and iMLSB resistance phenotypes were 63% and 18% in Istanbul, respectively(19). Uyanık et al. reported that cMLSB,iMLSB and-MSB resistance phenotypes were 21%, 30% and 5% in MRSA strains, respectively in Erzurum (20). They also found iMLSB resistance phenotype as 4% in MSSA strains but they could not determine other resistance phenotypes. In a study performed in Mersin, cMLSB and iMLSB resistance phenotypes were found as 43.7% and 5.4% respectively but MSB phenotype was not reported in MRSA strains (21). In MSSA strains, iMLSB resistance phenotype was found as 10.7% but MSB and cMLSBphenotypes were not reported . Sarıbas et al. reported that cMLSB and iMLSB resistance phenotypes were found 38% and 36% in MRSA strains and 2% and 20.5% in MSSA strains, respectively(22). They could not determine MSB resistance phenotype in all MRSA and MSSA strains in Ankara. In our study, MLSB resistance phenotype was found 84% and 23% in MRSA and MSSA strains, respectively and the difference was statistically significiant. In MRSA strains, cMLSB and iMLSB resistance phenotypes were found as 71% and 13%, respectively but MSB phenotype was not determined. In MSSA strains, cMLSB, iMLSB and MSB resistance phenotypes were determined as 3.5%, 16.5% and 2.8%, respectively. Constitutional MLSB resistance phenotype was higher in MRSA strains and inducable MLSB resistance phenotype was higher in MSSA strains. Constitutional resistance in MRSA strains was significiantly higher than in MSSA strains. Also, MSB resistance phenotype was only seen in MSSA strains and this is remarkable. Compared with other studies in Turkey and in other countries, MLSB resistance phenotype was higher in MRSA strains. According to these results, we have to review our policy on antibiotic usage.

Although inducable MLSB resistance phenotype was found as 16% and this is not a very high rate, automated systems that started to be widely usedin laboratories can't detect this phenotype and this may be a leading cause of strains incorrectlyreported as susceptible to lincosamide and treatment failure. The MLSB resistance phenotype was detected in a high rate in *S.aureus* strains. In conclusion, we think that performing the D test method in routine antibiotic susceptibility panels for these strains and being in touch with clinicians will be right approaches for preventing treatment failure.

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